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5.1 POSTSYNAPTIC LOCALIZATION OF RESERPINE-INDUCED α-ADRENERGIC RECEPTORS IN RAT SUBMAXILLARY GLAND. D.B. Bylund and J.R. Martinez*, Departments of Pharmacology and Child Health, University of Missouri School of Medicine, Columbia, Missouri 65212.

We recently reported that reserpine treatment results in the appearance of α_2-adrenergic receptor binding sites (AR) in the rat submaxillary gland (Nature, 284,in press). In control animals the amount of α_2 receptor binding was too low to be reliably quantitated ($\sim$0.3 pmol/g tissue). However, after one week of daily treatment with reserpine, the density of α_2-AR increased to 6 pmol/g. This system thus seemed ideally suited for the study of the synaptic localization of α_2-AR.

The following treatments were used: reserpine, 7 daily injections (0.5 mg/kg, i.p.); 6-hydroxydopamine (6-OHDA) two injections (34 mg/kg, i.v.) within 24 hours, followed a week later with an additional two injections (68 mg/kg); unilateral ligation of the main excretory duct. Rats were sacrificed two weeks after the first 6-OHDA treatment or the duct ligation, or one week after the start of reserpine treatment.

Treatment with 6-OHDA, which causes destruction of the presynaptic terminal and a severe depression in norepinephrine levels, resulted in only a slight increase in α_2-AR, while reserpine treatment resulted in the expected large increase. The combination of 6-OHDA and reserpine did not produce any further increase. Ligation of the excretory duct causes atrophy of the submaxillary gland, presumably due to a decrease in the size of acinar cells, while the innervation is not effected. The increase in α_2-AR induced by reserpine was markedly lower in the ligated gland as compared to the unligated gland of the same animal.

	Control	6-OHDA	Reserpine	6-OHDA+Res.
B_{max} (pmol/g)	0.4±0.1	1.4±0.2	4.6±0.3	4.7±0.3
	Control	Ligation	Reserpine	Lig.+Res.
B_{max} (pmol/g)	0.5±0.1	0.2±0.1	4.1±0.4	1.5±0.3

Previous attempts to localize the α_2-AR using either chemical or surgical denervation, have been hampered by the possibility that some presynaptic membrane may adhere to the postsynaptic membrane, even after degeneration of the nerve terminal. These 6-OHDA studies overcome this objection, and show a postsynaptic localization for the α_2-AR which are regulated by reserpine. The studies with ligated animals further support this conclusion. In addition, the results suggest that the mechanism whereby reserpine increases α-AR is not soley dependent on the depletion of norepinephrine.

(Supported in part by NIH grant AM18150 and NSF grant BNS7824715).

5.2 ALTERATIONS IN PRESYNAPTIC ALPHA-ADRENOCEPTOR ACTIVITY IN GUINEA PIG AND RAT VAS DEFERENS FOLLOWING GANGLIONIC BLOCKADE. Robert D. Sax* and Thomas C. Westfall. Departments of Pharmacology, University of Virginia, Charlottesville, VA 22908 and St. Louis University, St. Louis, MO 63104.

Surgical decentralization of the guinea pig vas deferens results in an increase in the release of ^{3}H-norepinephrine (^{3}H-NE) and dopamine-β-hydroxylase induced by electrical field stimulation (FS) of the isolated, superfused tissue (The Pharmacologist 18:208, 1976). This increase in release was in part due to a decrease in the activity of presynaptic α_2-adrenoceptors. Presently guinea pigs and rats were treated with the ganglionic nicotinic blocker chlorisondamine to induce a chemical decentralization of the postganglionic neurons of the vas deferens. The release of NE induced by FS from isolated, superfused tissues was determined in the presence and absence of α_2 agonists (clonidine and α-methyl-NE) and antagonist (yohimbine). FS-induced twitch contractions and ^{3}H-NE release were used as markers for the release of endogenous NE. After a 45 min preincubation in the presence or absence of l-^{3}H-NE (5×10^{-7}M) tissues from chlorisondamine (8mg/kg, b.i.d., i.p.) or saline treated animals were superfused at 2 ml/min with a Krebs-Henseleit solution with an extracellular Ca^{++} concentration of 1.8 or 1.0 mM. Blockers of $uptake_1$ and $uptake_2$ were present during the labelled-release experiments. Tissues were subsequently field stimulated at various frequencies and for various durations and the release of ^{3}H-NE or the twitch contractions was observed. In the absence of exogenous agents, FS induced a greater development of tension in tissues from chlorisondamine-treated animals than from the corresponding controls. Clonidine was observed to produce a concentration-dependent decrease in the FS-induced contractions and ^{3}H-NE release which was significantly attenuated in tissues from chlorisondamine-treated animals. In tissues from both species, α-methyl-NE was observed to be a weaker presynaptic agonist. Yohimbine was observed to produce a concentration-dependent increase in the FS-induced contractions and ^{3}H-NE release which was likewise attenuated by chlorisondamine pretreatment.

These results are consistent with our previous observations and extend to the rat the concept that a decrease in nerve impulse traffic in postganglionic sympathetic nerves as caused by chronic ganglionic blockade can cause a subsensitivity in the presynaptic α-adrenoceptor system of those nerves. This sensitivity change is modified by altering extracellular Ca^{++} concentration and thus implicates regulation of Ca^{++} metabolism as a controlling mechanism in the activity of the inhibitory α_2-adrenoceptors.

(Supported in part by USPHS-NINCDS 16215 and NIGMS 67055)

5.3 RECIPROCAL ALTERATIONS IN RAT BRAIN β- AND α_2-ADRENERGIC RECEPTOR SITES AFTER CHRONIC INTRAVENTRICULAR INFUSION OF ISOPROTERENOL. C.H. Wang* and D.C. U'Prichard (SPON: E. Silinsky) Dept. of Pharmacol., Northwestern Univ. Sch. Med., Chicago, IL 60611.

Decreases in β-receptor number can be achieved rapidly and reversibly in vitro by incubation of rat cortical slices with isoproterenol (ISO) (U'Prichard and Enna, Eur. J. Pharmacol. 59: 297, 1979), and in vivo following chronic tricyclic and atypical antidepressant treatment, over a much longer time-course with the loss being essentially irreversible in vitro (Wolfe et al., J. Pharmac. exp. Ther. 207: 446, 1978). While this in vivo treatment probably induces true β-receptor desensitization, it has not yet been shown that the antidepressant action is directly due to increased β-receptor activation; the loss of β-sites after in vitro ISO incubation may be mainly due to tight binding of agonist sequestered in membranes. Both treatments however induce an increase in the number of cortical α_2-receptor sites labeled by ^{3}H-clonidine (CLO) or ^{3}H-p-aminoclonidine (PAC), presumed agonist ligands. To resolve these issues, the effect of chronic infusion of a direct β-agonist on cortical adrenergic receptors was ascertained. Intracerebroventricular infusion in rats of 0.1, 1.0 and 10 mM ISO by Alzet minipump achieved dose-related steady-state brain ISO concentrations of approximately 0.1, 1.0 and 10 M after 2 days, which were maintained up to 7 days. ^{3}H-Dihydroalprenolol (DHA) β-receptor binding declined in a time- and ISO-concentration- dependent manner. DHA binding was decreased 0% by 0.1 mM ISO, and 20% by 1.0 or 10 mM ISO, after 2 days infusion, and 10% by 0.1 mM ISO and 26% by 10 mM ISO, after 7 days infusion. After 1 or 2 days infusion of 10 mM ISO, Scatchard analysis showed 30-40% reductions in the B_{max} of DHA (vehicle-infused control, 126 fmol/mg protein), with no change in K_D (0.9-1.1 nM). However 20% increases in the B_{max} of PAC (control, 105 fmol/mg protein) were observed, with a slight but not significant change in K_D (control 1.3 nM, ISO 2.0 nM). ^{3}H-Prazosin α_1-receptor binding was unaffected by ISO infusion. The results suggest that direct agonist-induced β-receptor desensitization in vivo induces reciprocal changes in functionally related cortical α_2-receptors which may reside on the same postsynaptic membranes.

Supported by USPHS grant NS 15595.

5.4 PHENOXYBENZAMINE DIFFERENTIATES DOPAMINE RECEPTOR SUBTYPES. M. Hamblin* and I. Creese. Department of Neurosciences, University of California, San Diego, 92093

Receptor binding studies with reversible ligands have been instrumental in establishing the existence of multiple dopamine receptor subtypes within the CNS. There still remains, however, considerable difficulty in equating the binding sites variously differentiated on the basis of their agonist/antagonist specificities, linkage to adenylate cyclase, guanine nucleotide sensitivity, and subcellular localization. The availability of a sufficiently selective irreversible dopaminergic ligand not only would offer an additional means of distinguishing multiple receptor subtypes, but, by inactivating certain subtypes of receptors while sparing others, would also allow the pharmacologic characterization of a relatively pure receptor population. We have investigated the effect of in vitro phenoxybenzamine (POB) treatment on dopaminergic ^{3}H-ligand binding to bovine striatal membrane fragments, and now report the utility of this agent as just such an irreversible dopaminergic antagonist.

POB treatment followed by thorough washing of membranes reduced subsequent binding of all dopaminergic ^{3}H-ligands investigated. Saturation studies showed this to be due to a decrease in receptor number without change in affinity. This effect was highly selective for certain receptor subtypes. Binding of ^{3}H-spiroperidol displayed high sensitivity and that of ^{3}H-dopamine low sensitivity, with respective IC_{50}'s of 1 and 100um POB (10 min, 37°C exposure). ^{3}H-Apomorphine binding showed intermediate sensitivity, and the dose response curve could be resolved into approximately equal high and low sensitivity components, paralleling the sensitivity of ^{3}H-spiroperidol and ^{3}H-dopamine binding sites respectively. That the parallel sensitivity of ^{3}H-spiroperidol sites and high sensitivity ^{3}H-apomorphine sites reflected alkylation of identical receptors was confirmed by spiroperidol displacement studies of bound ^{3}H-apomorphine. These showed that the half-maximal POB mediated decrease in ^{3}H-apomorphine sites resulted from complete loss of the ^{3}H-apomorphine sites with high spiroperidol affinity without effect on the sites with low affinity for spiroperidol. POB/receptor interaction is binding site-directed, as was shown by the ability of cold dopaminergic ligands to protect against POB mediated attack.

The number of high affinity binding sites for ^{3}H-spiroperidol, ^{3}H-apomorphine, and ^{3}H-dopamine are approximately equal in striatum. These studies therefore suggest that there are four striatal dopamine binding sites with respective high affinities for spiroperidol only, spiroperidol and apomorphine, apomorphine and dopamine, and dopamine only. M.H. supported by PHS GM07198. I.C. is an Alfred P. Sloan Fellow. Supported by PHS MH32990-01.

*Indicates nonmember of the Society for Neuroscience.

5.5 Butyrophenone Interactions with Distinct Monoaminergic Receptors in Human Prefrontal Cortex: Evidence for the Involvement of α_1-Adrenergic But Not $5HT_2$ or Dopaminergic Sites. A.C. Andorn*, J.C. Mitrius, and D.C. U'Prichard, (SPON: V. Rowland). Dept. Psychiat., Case Western Reserve Univ., Cleve., Oh. 44106, Dept. Pharmacol., Northwestern Univ. Schl. of Med., Chicago, Ill., 60611.

^{3}H-Spiroperidol binds specifically to an apparently heterogeneous set of sites in human prefrontal cortex. Competition of ^{3}H-spiroperidol binding in this region shows dopamine to be relatively weak (IC50:10^3nM) while α-adrenergic agents are more potent (IC50:100nM) with a rank order of potency consistent with an α_1-adrenergic receptor. These findings are in contradistinction to those in rat or human striatum, where spiroperidol appears to be binding at a dopamine receptor also, or the rat frontal cortex where such binding appears to occur at $5HT_2$ receptors. ^{3}H-Prazosine has been shown to label high affinity α_1-adrenergic sites in rat and calf frontal cortex ($K_D \simeq 0.1$nM, $B_{max} \simeq 100$ fmol/mg protein). We now report that ^{3}H-prazosine appears to specifically bind to a saturable and apparently homogeneous set of sites in human pre-frontal cortex ($K_D \simeq 0.1$nM, $B_{max} \simeq 150$ fmol/mg protein). Competition of ^{3}H-prazosine binding in this region parallels that observed in rat and calf: IC50 of α-antagonists ≃1nM, α-agonists ≃1μM and dopamine ineffective. Spiroperidol competes ^{3}H-prazosine binding with an IC50 of 0.1nM. The correlation coefficient for the competition of ^{3}H-prazosine binding versus ^{3}H-spiroperidol binding is 0.98 for the selective α-adrenergic agents. These findings suggest that ^{3}H-spiroperidol is possibly binding at an α_1-adrenergic receptor in human prefrontal cortex. Serotonin and tryptamine appear to have unusual interactions with ^{3}H-spiroperidol binding sites in this region which may not involve an interaction at the ^{3}H-spiroperidol binding site per se. ^{3}H-LSD was used to label 5HT sites in human prefrontal cortical homogenates. Unlike the rat frontal cortex where both $5HT_1$ and $5HT_2$ sites are present, we have observed 75% specific ^{3}H-LSD binding in this region less than 30% of which was competed by even μM spiroperidol. These findings suggest that $5HT_1$ receptor types predominate in human prefrontal cortex and that spiroperidol does not have a high affinity for these sites. Taken together these data suggest that the monoaminergic receptor systems involved in butyrophenone action in human prefrontal cortex are neither dopaminergic or serotonergic, but rather α_1-adrenergic. Whether these are the receptors involved in the antipsychotic action of these drugs remains unclear, but the neurochemical demonstration of α_1-adrenergic actions for ^{3}H-spiroperidol in the prefrontal cortex suggest that such properties rather than dopamine antagonist properties may have significance for the treatment of the perceptual and cognitive dysfunctions in the psychoses.

5.6 Butyrophenone Binding Sites in Human Amygdala: Heterogeneity of High Affinity Sites Without Apparent Dopaminergic Selectivity, M.E. Maguire, L.E. Weber*, A.C. Andorn*, (SPON: J. Foley), Depts. of Psychiat. and Pharmacol., Case Western Reserve Univ., Schl. of Med. Cleveland, Ohio 44106

Butyrophenone binding has been previously studied with the ligand ^{3}H-spiroperidol (^{3}HSP) in human prefrontal cortex and caudate. ^{3}HSP binds with high affinity and specificity at monoaminergic receptors other than dopaminergic in the prefrontal cortex, and to dopaminergic and α_1-adrenergic binding sites in the caudate. We now report that ^{3}HSP binds with high affinity to at least two sets of sites in human amygdala, neither of which appear to demonstrate selectivity for dopamine. Amygdalar tissue was obtained at autopsy from individuals with no known neurologic or psychiatric illness. Following preparation with extensive hypotonic washing, membrane fragments were used in standard filtration assays employing ^{3}HSP(0.5nM), and competitors with 10 min incubations at 25°C. Saturation isotherms were consistent with the presence of multiple sites ($K_D \simeq 0.4$ and 1.5 nM) with relatively low density (125 fmol/mg protein and 350 fmol/mg protein respectively). Specific binding, defined as that in the presence of 100μM fluphenazine subtracted from that in its absence was 70% of total binding and stereoselective with regard to the enatiomers of butaclamol by 2.5 orders of magnitude. Antipsychotic antagonists competed specific ^{3}HSP binding with IC50 ranging from 0.1nM(fluphenazine) to 1000nM(molindone); α-adrenergic agents also competed specific binding with IC50 ranging from 10nM(WB4101) to 10,000nM(clonidine) with a rank order of potency consistent with an α_1-adrenergic site. Dopamine was an ineffective competitor even at 0.1mM and +/-10μM pargyline. Serotonergic agonists inhibited only 60% of specific binding observed with IC50 of micromolar (for that binding that was inhibited) while the serotonergic antagonists inhibited 95% of specific binding with IC50 of 100 nM. Similarly, α-adrenergic agonists inhibited less than 50% of total specific ^{3}HSP binding observed. These results suggest that ^{3}HSP may be binding with high affinity at an heterogeneous set of sites in the amygdala. The differences in competition by agonists and antagonists may be reflective of differences in the selectivity of the sets of sites observed. The lack of apparent inhibition of specific ^{3}HSP binding by dopamine suggests that some actions of antipsychotic drugs may not be mediated through binding at dopaminergic receptors.

5.7

Withdrawn by Author

5.8 DISTRIBUTION OF CATECHOLAMINE RECEPTOR BINDING SITES IN DISCRETE HYPOTHALAMIC REGIONS AND THE INFLUENCE OF FOOD DEPRIVATION ON BINDING. M. Jhanwar-Uniyal*, B. Dvorkin*, M.H. Makman* and S.F. Leibowitz (SPON: E.B. Gardner). Rockefeller University, New York 10021 and Albert Einstein College of Medicine, New York 10461.

Studies of direct injections of catecholamine agonists and antagonists into rat hypothalamus indicate that feeding behavior is stimulated by α-adrenergic (α) receptor activation in the paraventricular nucleus (PVN), and is suppressed by dopaminergic (DA) and β-adrenergic (β) receptor activation in the perifornical lateral hypothalamus (PF-LH). The present study examines the distribution of α, β and DA binding in discrete hypothalamic and extrahypothalamic areas, compares binding of agonists and antagonists, and examines in a preliminary investigation the impact of food deprivation on binding. Brain areas of male Sprague-Dawley rats (350 g) were dissected using the micropunch technique. Binding studies were carried out: for α sites with 3nM ^{3}H-p-aminoclonidine (PAC) displaced by 10μM norepinephrine and 1nM ^{3}H-WB-4101 (WB) displaced by 10μM (+)butaclamol; for β sites with 3nM ^{3}H-dihydroalprenolol (DHA) displaced by 1μM l-propranolol; and for DA sites with 3nM ^{3}H-ADTN displaced by 10μM ADTN and with 1nM ^{3}H-spiroperidol (SP) in the presence of 1μM cinanserin and displaced by 20μM ADTN. The distribution pattern of α, β and DA binding sites is shown in the table. The ratio of agonist to antagonist sites for DA and α receptors varies in different regions, supporting the existence of receptor subclasses. This pattern of receptor binding for rats on ad libitum food is altered by 48 hrs of food deprivation. With deprivation, preliminary studies show that (1) binding of PAC to α receptors is specifically decreased in the PVN and VMH, while increased in other hypothalamic areas; (2) DHA (β) binding is selectively increased in the PF-LH; and (3) ADTN (DA) binding is generally increased throughout the hypothalamus, but most dramatically in the PF-LH.

^{3}H-RADIOLIGAND BOUND: fmoles/mg prot.

area	PAC	WB
PVN	106	47
DMN	147	55
VMH	184	52
AH	127	61
PH	66	61
PVS	134	47
SO	10	29
POM	82	44
ST	142	72
FC	91	69
OT	2	5

area	DHA
PF-LH	18
MFB	39
AH	25
POM	38
ST	32
FC	69
CP	95
OT	3

area	ADTN	SP
PF-LH	27	
DMN	45	
VMH	33	
MFB	28	57
ZI	50	
AR	30	18
ME	51	29
FC	50	22
CP	76	195
ACC	41	51
OT	5	13

Rats were fed ad libitum. Values are means of 2-7 experiments, each with pooled punches from 6 animals. AH, ant. hypothal.; PH, post. hypothal.; CP, striatum; ACC, n. accumbens; FC, frontal cortex; OT, optic tract; VMH, ventromed. hypothal.; PVS, periventric. n.; DMN, dorsomed. n.; ME, median eminance; MFB, med. forbr. bundle. (See also König & Klippel Atlas.)

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5.9 EFFECT OF COPPER DEFICIENCY ON DOPAMINERGIC, β-ADRENERGIC AND MUSCARINIC RECEPTOR BINDING. Daniel J. Feller, Boyd L. O'Dell* and David B. Bylund, Departments of Pharmacology and Biochemistry School of Medicine, University of Missouri, Columbia, Missouri 65212.

There is evidence that cerebrospinal copper levels are altered in schizophrenic patients which suggests an involvement of copper or copper dependent enzymes in this disorder. Copper deficient animals exhibit pathologies resembling Parkinsonian symptoms and may possibly provide a model for the study of the syndrome. Dietary copper deficiency significantly reduces the concentration of dopamine in both the whole brain and corpus striatum of the rat, while whole brain levels of norepinephrine are depleted without a change in either hypothalamic or cerebellar NE. The decline in whole brain levels of both dopamine and norepinephrine have been correlated with a reduction in tyrosine hydroxylase activity. Recently, it has been suggested that changes in the density of neurotransmitter receptors are inversely related to changes in the effective concentration of neurotransmitter at the synapse. Thus it was of interest to characterize the changes in several neurotransmitter receptors in the copper deficient animal. Female rats received a low (<1 ppm) copper diet throughout gestation and lactation. Their offsprings were fed the same diet after weaning. Controls received the same diet supplemented with 10 ppm copper. Animals were sacrificed at 5 to 9 weeks of age. ^{3}H-Spiroperidol, ^{3}H-quinuclidinyl benzilate and ^{3}H-dihydroalprenolol were used to assay the dopaminergic, muscarinic and β-adrenergic receptors respectively. B_{max} values were determined from saturation exeriments and expressed as pmole/g protein. In the corpus striatum from deficient rats the density of the high affinity and low affinity DA receptor binding sites were decreased 55% and 45%, respectively ($p<.05$), while a 27% ($p<.005$) drop was observed in muscarinic binding sites. Furthermore the number of muscarinic receptors in the cerebral cortex was reduced 19% ($p=.05$) compared to only 6.5% ($p>.1$) in the cerebellum of animals receiving the copper deficient treatment. β-receptors increased 10% ($p>.1$) in the cerebral cortex and 13% ($.05<p<.1$) in the cerebellum. No significent differences were found in K_D values between the experimental and control rats. We suggest that the observed changes in receptor density are due to effects of copper deficiency not directly related to changes in neurotransmitter levels. Supported by NSF Grant BNS 7824715.

5.10 KAINATE LESIONS DIFFERENTIATE STRIATAL DOPAMINERGIC AGONIST AND ANTAGONIST BINDING SITES. S. Leff*, L. Adams and I. Creese (SPON: C.E. Spooner). Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

The neurotoxin kainic acid (KA) causes degeneration of treated neurons but not of axon terminals of distal afferents or of glia. Thus KA can be used to study the neuronal distributions of specific ligand binding sites in CNS nuclei. Striatal KA injections have been previously shown to decrease ^{3}H-butyrophenone binding sites by 50% while producing virtually a total loss of dopamine (DA) sensitive adenylate cyclase activity. Further studies demonstrated a larger loss (70%) of high-affinity binding of the DA agonist ^{3}H-apomorphine. We now report, in direct comparisons a greater loss of high affinity ^{3}H-agonist and ^{3}H-flupenthixol (an antagonist believed to label the DA-sensitive cyclase linked receptor) binding as compared to the loss of ^{3}H-butyrophenone sites after unilateral striatal KA lesions.

KA (2ug in 1ul 0.9% NaCl) was slowly infused through a 30g needle into the left striatum of anaesthesized rats positioned in a stereotaxic apparatus. Radioreceptor assays were performed on single striata dissected from animals sacrificed 3 weeks post-operatively. Binding of four ligands: ^{3}H-N-norpropylapomorphine (NPA), ^{3}H-spiroperidol, ^{3}H-domperidone, and ^{3}H-cis-flupenthixol, was assessed in lesioned and control (contralateral) striata. Specific binding was measured as the excess over blank tubes containing 1uM (+)-butaclamol. Specific binding of all ^{3}H-ligands was assessed using a concentration equal to their apparent K_D as determined in saturation experiments.

In lesioned striata the butyrophenone ^{3}H-spiroperidol and the butyrophenone-like ^{3}H-domperidone showed 56% and 59% losses of specific binding respectively. In contrast significantly greater decreases in binding were observed with ^{3}H-NPA and ^{3}H-flupenthixol which showed 79% and 73% losses of high-affinity binding respectively. The difference in the loss of ^{3}H-NPA vs. ^{3}H-flupenthixol binding in lesioned striata, however, was not significant; nor was there a significant difference in the loss of binding between ^{3}H-spiroperidol and ^{3}H-domperidone.

These findings lend further support to the hypothesis that there are multiple DA receptors in the striatum. The relatively greater loss of binding of ^{3}H-NPA and ^{3}H-flupenthixol parallels the nearly total loss of DA-sensitive adenylate cyclase activity, and suggests that these markers may identify the same receptors. The similar pharmacological specificities and kainate induced losses of ^{3}H-spiroperidol and ^{3}H-domperidone binding suggest that they identify identical receptors in the striatum. S.L. is an NSF predoctoral fellow, I.C. is an Alfred P. Sloan Fellow. Supported by PHS MH32990-01.

5.11 GENETIC DIFFERENCES IN CATECHOLAMINE RECEPTORS AND DOPAMINE-MEDIATED BEHAVIOUR IN RATS. D.M. Helmeste*, P. Seeman and D.V. Coscina. (SPON. M. Burnham). Pharmacology Department, University of Toronto and Bio-Psychology Unit, Clarke Institute of Psychiatry, Toronto, Canada.

In order to examine the hypothesis that behaviour may be correlated with receptor density, we examined two rat strains (F344, Buffalo) with known biochemical and behavioural differences. Previous studies have shown that adult F344 rats have higher levels of striatal and mid-brain tyrosine hydroxylase than Buffalo (BUFF) rats (1). An inverse relation between tyrosine hydroxylase levels and locomotor activity in these two strains has brought speculation that the primary factor responsible for the behavioural differences might be receptor sensitivity (2).

We measured alpha-adrenergic receptor densities (Bmax) in the frontal cortex, hippocampus and hypothalamus of these two strains using ^{3}H-clonidine to label alpha-2 sites and ^{3}H-WB-4101 to label alpha-1 sites. ^{3}H-Spiperone was used to label D-2 dopamine sites in the striatum and olfactory tubercle (O.T.). Significant differences in Bmax between strains were only found in the following areas.

ligand	area	$\frac{\text{Bmax F344}}{\text{Bmax BUFF}}$ ± S.E.
^{3}H-clonidine	frontal ctx.	1.20 ± 0.06
^{3}H-spiperone	striatum	1.34 ± 0.03
	O.T.	1.50 ± 0.19

Receptor affinities (K_D values) were not different between strains.

Behavioural testing of apomorphine-induced stereotypy in F344 and BUFF rats showed that F344 rats achieved higher levels of stereotypy compared to BUFF rats across the three doses of apomorphine used (0.25, 1.0, 2.5 mg/kg, s.c.).

The results of this study support the hypothesis that the higher density of D-2 receptors in the F344 strain may be related to the more sensitive responses to apomorphine. The increased sensitivity of BUFF rats to norepinephrine-induced locomotor activity [as previously shown (2)], may also be related to alpha receptor density.

(Supported by Ontario Mental Health Foundation, MRC of Canada).

(1) Segal, D.S. et al., Behav. Biol. 7, 75-81, 1972.
(2) Segal, D.S. et al., Science 189, 301-303, 1975.

6.1 HYPOXIA DECREASES THE METABOLISM OF THE GLUCOSE-DERIVED NEUROTRANSMITTERS. G. E. Gibson, C. Peterson*, and J. Sansone* (SPON: F. Plum). Cornell Med. Coll., Burke Rehab. Center, White Plains, NY 10605.

The molecular basis of the brain's sensitivity to mild decreases in oxygen is unknown. One possible explanation is that the synthesis of the glucose-derived neurotransmitters whose formation depends upon mitochondrial oxidation may be reduced by hypoxia. These neurotransmitters include acetylcholine (ACh), glutamate, aspartate and γ-aminobutyrate (GABA). Previously, we showed that ACh synthesis is decreased by levels of chemical or hypoxic hypoxia that do not reduce cellular ATP levels (J. Neurochem. 27:37; Neurosci. Abstr. 5:285). We have now compared the reduced ACh production to the formation of the amino acids GABA, glutamate, aspartate, glutamine, serine and alanine during chemical hypoxia. Their synthesis was determined after a pulse injection of [U-^{14}C]glucose (3 μCi/gm).

Mild chemical (anemic) hypoxia was induced with $NaNO_2$ to give the following % methemoglobins (MetHb): 0.7 (control), 12.1, 30.9, 59.2 and 83.8. The incorporation of [U-^{14}C]glucose into ACh (specific activities) declined as % of control from 100±1% (n=8) to 66±9 (n=8), 43±4 (n=5), 33±4 (n=7) and 16±6 (n=4) with the respective MetHb. There was a parallel decrease in the flux of glucose into the amino acid neurotransmitters dependent upon mitochondrial oxidation and into the non-neurotransmitter, glutamine, whose synthesis also depends upon mitochondrial metabolism. This is illustrated by the high correlation between the decrease in the incorporation of [U-^{14}C]glucose into ACh and its incorporation into GABA (r=0.98), glutamate (r=0.99), aspartate (r=0.93) and glutamine (r=0.99). However, the synthesis of the amino acids whose formation is not dependent upon mitochondrial oxidation did not correlate well with changes in ACh metabolism: serine (r=0.68), alanine (r=-0.19).

A standardized string test (Trans. Amer. Soc. Neurochem. 11: 159) was used to monitor behavioral changes during chemical hypoxia. The control score (7.8±0.2, n=6) did not vary in mice with the 12.1% MetHb (8.0±0.0, n=6) but was significantly altered with 30.9% MetHb (5.0±0.2, n=7) or 59.2% MetHb (0.6±0.4, n=5).

Thus, anemic hypoxia causes a parallel decrease in the synthesis of ACh and in the amino acid neurotransmitters which require mitochondrial oxidation. Decreases in neurotransmitter formation occur before any detectable changes in string test behavior. The relative importance of these alterations in different neurotransmitters in the production of impaired brain function in hypoxia or other metabolic encephalopathies remains to be determined. (Supported by Grants NS03346 and NS15649).

6.2 DIFFERENCES IN THE STRUCTURE OF A AND B FORMS OF HUMAN MONOAMINE OXIDASE. *R.M. Cawthon, J.E. Pintar, F.P. Haseltine, M.S. Buchsbaum, D.L. Murphy, and X.O. Breakefield. Dept. Human Genetics, and Dept. Obstetrics and Gynecology, Yale Univ. Sch. Med., New Haven, CT 06510, Biological Psychiatry Branch and Clinical Neuropharmacology Branch, NIMH, Bethesda, MD 20014.

Monoamine oxidase (MAO) is a mitochondrial outer membrane enzyme which degrades amine transmitters in the nervous system. The irreversible inhibitor pargyline binds to the enzyme stoichiometrically, forming a stable adduct with the covalently bound flavin cofactor. Here sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to analyze ^{3}H-pargyline-labeled MAO from human tissues with A and B activity. These two types of activity differ in their substrate selectivity, drug sensitivity, and tissue distribution. It is not clear to what extent these differences in MAO result from intrinsic variation in its structure or from extrinsic modulation by its membrane microenvironment. Recently, ^{3}H-pargyline-labeled polypeptides of MAO A and B from rat tissues were shown to have different apparent MW's and different one dimensional peptide maps by electrophoresis under fully solubilizing and denaturing conditions in SDS-polyacrylamide gels (studies by ourselves and by B.H. Callingham and D. Parkinson of Cambridge University). In the current studies the ^{3}H-pargyline-labeled polypeptides of human MAO A and B were compared using tissues from two individuals. In one case ^{3}H-pargyline was bound to MAO B in blood platelets from an adult male and to MAO A and B in a crude mitochondrial fraction from fibroblasts of the same individual. Specific labeling of only the A or the B forms of the enzyme from fibroblast cells was achieved by preincubation with selective A and B inhibitors. In the other case, ^{3}H-pargyline was bound to MAO B in umbilical cord blood platelets from a male newborn and to MAO A in a crude mitochondrial fraction from the trophoblast portion of the newborn's placenta. SDS-PAGE of samples containing MAO A activity revealed a labeled protein band of MW 63,000, and analysis of samples containing MAO B activity revealed a band of 60,000. Similar MW's have been reported for MAO A and B in rat tissues.

These findings demonstrate that distinct enzyme molecules are associated with the A and B types of human MAO activity. Whether the observed differences in structure arise from differential post-translational modification(s) of a single enzyme precursor or from divergent amino acid sequences coded by separate structural genes is under current investigation.

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6.3 CHROMOSOMAL LOCATION OF GENES INVOLVED IN EXPRESSION OF MONOAMINE OXIDASE A AND B ACTIVITIES. John Pintar, James Barbosa, Uta Francke, Morris Hawkins Jr., Carmela Castiglione and Xandra Breakefield. Dept. Human Genetics, Yale Univ. Sch. Med., New Haven, Ct. 06510.

The chromosomal location of genes required for expression of type A and type B monoamine oxidase activities (MAO-A and MAO-B) has been studied in somatic cell hybrid lines. MAO-A activity was expressed in hybrid lines arising from fusion of a human fibroblast line (GM 316) that expressed both MAO-A and hypoxanthine phosphoribosyl transferase (HPRT) activities with a mouse neuroblastoma line (N1E-115TG2) that lacked both these activities. Subsequent growth of these lines in the presence of hypoxanthine, aminopterin, and thymidine (HAT) selected for hybrid cells that had retained the human X chromosome while randomly losing other human chromosomes; cell lines arising under these conditions continued to express MAO-A activity. Mouse and human MAO-A catalytic subunits were distinguished following specific and irreversible labelling with ^{3}H-pargyline and characterization by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Although these proteins have the same MW, they have unique one dimensional peptide maps following limited proteolysis. Hybrid lines expressed the human form of the catalytic subunit of MAO-A, indicating the presence of the human structural gene for this protein. Moreover, selection of these cell lines in the presence of 6-thioguanine (that only allows survival of cells without HPRT activity) led to coordinate loss of the human X chromosome, human forms of X-linked enzymes, and MAO activity. In one case, however, a line containing a putative translocation lost a recognizable human X chromosome and some, but not all, human X-linked markers; yet retained MAO activity and produced a human catalytic subunit. Based on these observations, we have assigned the structural gene locus for the human MAO-A catalytic subunit to the X chromosome.

Fusion of a rat hepatoma line that expressed MAO-A, MAO-B, and HPRT activities with N1E-115TG2 gave rise to hybrids that continued to express both MAO-A and MAO-B activities. Three out of three hybrids resistant to subsequent 6-thioguanine treatment lost both types of MAO activity, while four out of four clones arising from growth on HAT retained both types of activity. This observation provided preliminary support for the idea that both MAO-A and MAO-B catalytic subunits are X-linked in the rat. Processing of MAO is currently being investigated and should help determine whether the MAO-A and MAO-B catalytic subunits are coded for by the same gene locus.

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MH, Jr. currently at Dept. Microbiol. Howard U. Sch. Med., Wash, D.C.

6.4 DEXAMETHASONE SELECTIVELY INCREASES MONOAMINE OXIDASE TYPE A ACTIVITY IN HUMAN DIPLOID FIBROBLASTS. Susan B. Edelstein and Xandra O. Breakefield. Dept. Human Genetics, Yale Univ. Sch. of Med., New Haven, CT 06510.

Cultured human diploid fibroblasts express both the A and B types of monoamine oxidase (MAO) activity. Studies on the effects of culture conditions on MAO suggest that heat-sensitive and dialyzable factors present in serum can markedly affect levels of activity. The effects of several hormones known to affect MAO activity in vivo have been studied using cultured fibroblasts. Cells were grown in medium supplemented with serum until several days past confluency and then placed in serum-free medium for 24 hr prior to exposure to hormones for 6 or 24 hr. Estradiol, progesterone, testosterone and tri-iodothyronine increased MAO activity only 10-50%, whereas dexamethasone (DEX) consistently increased levels 200-300%.

Although DEX increases MAO activity over a broad range of concentrations (1nM to 1μM), the greatest increases are observed after exposure to physiologic concentrations of DEX with an optimum near 50 nM. MAO activity increases linearly with time at this concentration. No apparent lag is observed (even at times as short as 6 hr) and levels are elevated nearly 6-fold by 5 days and 10-12 fold after 8-9 days of exposure to DEX.

Preliminary studies in which cultures were exposed to 50nM DEX for 8-9 days indicate that increases in MAO activity result from an increase in the number of MAO molecules: 1) Kinetic analysis reveals that DEX does not alter the affinity of MAO for tryptamine but increases the maximum velocity about 10-fold. 2) Dose response curves with selective MAO inhibitors indicate that DEX changes the proportion of A and B activity in living cells--e.g. using clorgyline (a selective A inhibitor), the proportion changes from 50% A:50% B in control cultures to 90% A:10% B in cultures exposed to DEX. 3) When the irreversible inhibitor ^{3}H-pargyline is used to stoichiometrically radiolabel the active sites of MAO A and B in crude mitochondrial preparations prior to SDS polyacrylamide electrophoresis, it is found that DEX does not alter the electrophoretic mobility of the labeled bands corresponding to MAO A (63K) and MAO B (60K). However DEX increases the amount of ^{3}H-pargyline-labeled MAO A about 10-fold but only increases ^{3}H-pargyline-labeled MAO B about 2-fold.

Thus, the increase in MAO activity induced by DEX in cultured fibroblasts appears to be almost entirely accounted for by an increase in the number of MAO A molecules.

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6.5 CHARACTERISTICS OF RAT PHEOCHROMOCYTOMA TYROSINE HYDROXYLASE IN THE PRESENCE OF cAMP-DEPENDENT PHOSPHORYLATING CONDITIONS. J. Meligeni* and N. Weiner (Spon: J. Masserano). Dept. Pharmacology, Univ. Colorado Sch. Med., Denver, CO 80262

Tyrosine hydroxylase has been purified from rat pheochromocytoma tumor tissue by salt fractionation (28-42% ammonium sulfate, pH 7.8), ion exchange chromatography (phosphocellulose cation exchange, pH 6.2; DE-52 anion exchange, pH 7.8), and sucrose density gradient centrifugation (6-20% sucrose, pH 8.2). With this source of purified tyrosine hydroxylase, some physical characteristics of the enzyme, as well as alterations of those physical and functional characteristics consequent to exposure to protein phosphorylating conditions (ATP, Mg^{++}, purified catalytic subunit of cyclic AMP-dependent protein kinase), have been investigated in our laboratory. Tyrosine hydroxylase has a molecular weight of approximately 240,000 daltons as determined by polyacrylamide gradient gel electrophoresis, and probably exists as a tetramer with subunits of similar or identical size of approximately 60,000 daltons (Vulliet et al, PNAS, 77: 92-96, 1980). Two peaks of tyrosine hydroxylase activity at isoelectric points of approximately pH 5.4 and pH 5.2 can be detected by isoelectric focusing in flat-bed polyacrylamide gels (pH range 4-7). When purified tyrosine hydroxylase is exposed to ATP-γ-^{32}P and cAMP-dependent enzymatic phosphorylating conditions, ^{32}P is incorporated into both peaks of enzyme activity, and both peaks exhibit enhanced enzyme activity. Only minor shifts in the isoelectric points occur consequent to phosphorylation. Similarly, isoelectric focusing of crude pheochromocytoma supernatant reveals two peaks of tyrosine hydroxylase activity, and increases in enzyme activity and incorporation of ^{32}P into the regions of the gel which correspond to the sites of enzyme activity are observed when the crude tyrosine hydroxylase is incubated in the presence of ATP-γ-^{32}P, Mg^{++} and catalytic subunit of cAMP-dependent protein kinase. These data suggest that cAMP-dependent protein kinase can enhance tyrosine hydroxylase activity and that there may be more than one site at which the enzyme is phosphorylated in vitro. Rapid separation techniques, such as isoelectric focusing, may prove useful in further studies of the regulation of tyrosine hydroxylase both in situ and in vivo. Supported by USPHS grants NS 07927, NS 09199 and AA 03527.

6.6 DEBRISOQUIN, A PERIPHERAL MONOAMINE OXIDASE INHIBITOR AND ANTI-HYPERTENSIVE AGENT, REDUCES CORTICAL MHPG IN RATS. S. E. Hattox, A. C. Swann*, D. Jablonis* and J. W. Maas. Departments of Psychiatry and Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510

Debrisoquin sulfate is a monoamine oxidase inhibitor (MAOI) which does not cross the blood brain barrier. It has been clinically effective in lowering blood pressure. Although early reports confirm that debrisoquin has no effect on central aminergic neurons, more recent work has demonstrated a decrease in whole brain release of 3-methoxy-4-hydroxyphenethylene glycol (MHPG), a major metabolite of norepinephrine, in non-human primates treated chronically with debrisoquin (Maas et al., Biochem. Pharmacol. 28: 3153, 1979). In order to further examine the effect of debrisoquin on central monoaminergic systems, we have treated rats with various doses of debrisoquin and measured cortical MHPG at various times after treatment.

Rats were treated with 1, 5, 10 or 40 mg/kg debrisoquin, i.p., and sacrificed at 1 or 6 hours post injection. A second series of rats was treated with 10 mg/kg, b.i.d., for 24 hours and sacrificed 6 hours after the last injection. Conjugated plus free MHPG were measured in right cortical hemispheres by the mass spectrometric technique of selected ion monitoring employing deuterated internal standards, homogenization in sodium acetate buffer, ethyl acetate extraction and formation of the trifluoroacetyl derivative. Ions at m/e 358 and 360 were monitored, derived from endogenous and isotope-labelled MHPG, respectively. Caudate nucleus 5HIAA and HVA were also measured as indicators of central MAO inhibition or more generalized central effects. Again a selected ion monitoring procedure was used, employing deuterated internal standards, homogenization in 1 M formic acid, ethyl acetate extraction and formation of the pentafluoropropionyl derivatives. Ions at m/e 438 and 440 were monitored for 5HIAA while ions at m/e 460 and 462 were monitored for HVA.

Cortical MHPG was significantly lowered at 6 hr with 1, 5, and 10 mg/kg debrisoquin (N=5, $p < .01$, .02, .02) to 78, 78, and 74%, respectively of saline-treated control values. MHPG was not found to be significantly lowered, however, at 1 hr and had returned to control levels by 24 hours. Caudate nucleus 5HIAA and HVA were found to be unchanged at 6 hr regardless of dose. These data suggest that debrisoquin, in a time-dependent fashion, decreases central noradrenergic activity either indirectly via peripheral afferents or by a direct mode of action such as the formation of an active metabolite which can enter the brain. Further studies are underway to elucidate the mechanism by which debrisoquin decreases CNS MHPG. (Supported in part by grants from USPHS: Grants # MH-24393 and MH-25642.)

6.7 **DEPENDENCE OF BRAIN 3,4-DIHYDROXYPHENYLETHYLENEGLYCOL (DHPG) ON BRAIN NORADRENERGIC NEURONAL ACTIVITY. J. J. Warsh, P. P. Li*, D. D. Godse*, and S. W. Cheung*. Section of Biochemical Psychiatry, Clarke Institute of Psychiatry, University of Toronto, Toronto M5T 1R8, Canada.**

Using a direct gas chromatography-mass fragmentography (GC-MF) procedure, we have previously confirmed and extended earlier observations that in addition to 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), DHPG is also a major norepinephrine (NE) metabolite in rat and mouse brain (Soc. Neurosci. 9:575 1979). Moreover, GC-MF determination of significant although lower amounts of DHPG in human CSF (2.19 ± 0.57 ng/ml; N=6) compared to MHPG (9.92 ± 1.15 ng/ml; N=8) suggests that DHPG is also formed in human central nervous system (CNS). Although a variety of data suggest that brain MHPG production is dependent upon the functional status of CNS NE activity, the relationship of brain DHPG formation to NE neuronal activity is not as well established.

To characterize the functional significance and site of formation of brain DHPG, rat brain NE neuronal activity was modified pharmacologically followed by simultaneous determination of brain regional DHPG and MHPG levels by GC-MF. In rats given the α-adrenergic agonist clonidine (10 or 250 μg/kg, i.p.) 2-hr prior to sacrifice, cortical and spinal DHPG and MHPG concentrations were significantly decreased by 20 and 30%, respectively. On the other hand, the α-adrenergic antagonist yohimbine (1-10 mg/kg, i.p.), induced a parallel and dose dependent increase in cortical and spinal DHPG (130-200%) and MHPG (170-410%) concentrations compared to controls. Pretreatment with desipramine (10 mg/kg, i.p.) 30 min prior to yohimbine antagonized the yohimbine-induced elevation of DHPG levels in these same regions leading to a shift to the right of the log dose response curves. In contrast, desipramine augmented the effects of yohimbine on cortical and spinal MHPG levels resulting in a shift to the left of the dose response curves and a significant increase in the slopes. Desipramine alone significantly reduced cortical DHPG (23%) and not MHPG levels.

The parallel yohimbine and clonidine induced increments and decrements, respectively, of DHPG and MHPG indicate that DHPG is also sensitive to changes in brain NE neuronal impulse flow. Secondly, the differential effect of desipramine on the yohimbine-induced increments of brain DHPG and MHPG support the notion that brain DHPG and MHPG are formed in separate metabolic compartments, DHPG being formed in a presynaptic intraneuronal location and MHPG in a postsynaptic compartment.

6.8 Glucocorticoids increase β-adrenergic receptors in fetal rabbit lung in vivo and in vitro. James M. Roberts*, John B. Cheng*, Jeffrey Schwartz*, Robert Goldfien*, Philip L. Ballard*, Alan Goldfien*, (SPON: Cynthia L. Bethea). Depts. Ob. Gyn. & Reprod. Sci., Ped., & CVRI, U. CA at San Francisco, San Francisco, CA 94143.

Heterologous receptor regulation is an important mechanism of hormone action. We asked whether heterologous regulation of adrenergic receptors might have a role developmentally. Activation of pulmonary β-adrenergic receptors increases surfactant release and decreases lung water and is important in preparing the fetus for extrauterine existence. Glucocorticoid treatment accelerates lung maturation and also increases β-adrenergic receptors in adult lung. To determine if glucocorticoids might increase fetal lung β-receptors as one aspect of lung maturation, we used [^{3}H] dihydroalprenolol (DHA) to directly examine β-receptors in fetal lung. DHA binds to particulate preparations of fetal rabbit lung in a manner compatible with β_1-adrenergic interactions, and agonist affinities are similar to those in adult lung (isoproterenol K_I = .28±0.7μM; > epinephrine K_I = 4.1±1.7μM; = norepinephrine K_I = 6.6 ±2μM). We quantitated fetal pulmonary β-receptors throughout gestation and compared these to fetal free cortisol concentrations reported previously (Mulay, et al. Endoc. 93:1342, 1973). The concentration of DHA binding sites increased between 28 & 31 days gestation:

Gestational Age	[β-Receptors]	fmol/mg prot. ($\bar{x}$ ± SEM)
24 days	35.6± 6.5	
26 days	43.6± 7.4	
28 days	66.0± 7.0	($p < .001$)
31 days	138.7±17.7	
neonate	153.8±14.3	

This increase follows the increase in fetal free cortisol by 24 hours. To determine if the changes in glucocorticoid and β-receptors were causally related, we treated maternal rabbits at 25 days gestation with betamethasone (.17mg/kg) and found that glucocorticoid treatment increased β-receptors from 43.6±7.4 fmol/mg prot to 77.9±5.8 fmol/mg prot ($p<.001$) in lungs of fetuses removed 24 hours after treatment. We examined β-receptors in fetal lungs maintained in organ culture for 48 hours in the presence or absence of .1μM dexamethasone to determine if this increase was a direct effect of glucocorticoid and to provide a system for examining mechanisms of receptor increase. Lungs cultured with dexamethasone demonstrated an increase in β-receptor concentration (106 ±8fmol/mg prot) compared to lungs prepared prior to culture (36±9 fmol/mg prot), or lungs cultured without glucocorticoid (56±13fmol /mg prot) ($p<.05$). We conclude that heterologous regulation of pulmonary adrenergic receptors are an important developmental mechanism. Organ culture of fetal lung should provide a system in which mechanisms of receptor regulation can be evaluated

6.9 PUTRESCINE AS A METABOLIC SOURCE OF GABA. P.C. Caron*, L.J.Cote, L.T. Kremzner*. Depts. of Pathology, Neurology, and Rehabilitation Medicine. College of Physicians and Surgeons, Columbia University, New York, NY 10032.

Until recently, γ-aminobutyric acid (GABA) was considered to be formed solely by the enzymatic decarboxylation of glutamic acid. Although the concentration of GABA is highest in the central nervous system, low levels are found in non-neural tissues such as pancreas, adrenal, and kidney; (Gerber, J.C. and Hare, T.A. Diabetes 28: 1073, 1979) and glutamine acid decarboxylase activity has been found in these tissues. However, Seiler (Brain Res. 28: 371, 1971) found that putrescine was a precursor to GABA, as well as to spermidine and spermine. In the present communication the relative contribution of putrescine as a source of GABA in neural, as well as non-neural tissues, is assessed.

Female Sprague-Dawley rats (150-175 g) were injected with physiological levels of 1,4 ^{14}C-putrescine (S.A. 96.4 mCi/m mole) intraventricularly (0.4 μCi) or intraperitoneally (10 μCi). The animals were killed at 15, 30, 60, 120 and 300 min. The tissues were homogenized in 4 vol of 0.4 M PCA and the extracts were analyzed for radioactive GABA, putrescine, spermidine, spermine, and other GABA metabolites, using an amino acid analyzer. In addition, inhibitor studies using aminoguanidine (60 mg/kg), injected i.p. 6 hrs before putrescine administration, were performed.

Intraventricular injections of labelled putrescine showed little conversion (≤ 1%) to GABA. In contrast, more than 25% of the radioactivity was found in spermidine in the telencephalon and brain stem by 2 hrs. Low levels of ^{14}C-spermine were also found after 2 and 5 hrs.

Intraperitoneal injections of ^{14}C-putrescine showed marked differences in the uptake of radioactivity in neural and non-neural tissues. A significant conversion of putrescine to GABA occurred in the pancreas and adrenal gland, accounting for 5-10% of the total endogenous GABA in one hour. Smaller, but detectable levels of ^{14}C-GABA were also found in the brain, liver, heart, kidney, and skeletal muscle. After the administration of aminoguanidine, an inhibitor of diamine oxidase, as much as a 95% inhibition of GABA formation was found.

These studies show that a "significant" amount of GABA may have its origin in putrescine in some tissues. The role of GABA in non-neural tissue is unclear and needs further clarification. Perhaps GABA in the periphery plays a regulatory role by altering membrane permeability to anions. Alteration in GABA levels having its origin in the polyamines may provide clues to the function of GABA in neural and non-neural tissues.

6.10 EFFECT OF ANTIGANGLIOSIDE (ANTI-G_{M1}) ANTIBODY ON GABA RELEASE IN BRAIN SLICES. Brina Frieder* and Maurice M. Rapport., Div. of Neuroscience, N.Y. State Psychiatric Inst. and Depts. of Biochem. and Psychiatry, Columbia Univ., New York, N.Y. 10032.

Intracerebral injections of antibodies to G_{M1} ganglioside result in the perturbation of several CNS functions (induction of epileptiform spiking, inhibition of passive avoidance learning, blockade of morphine analgesia, etc.). Since the mechanism of action of these antibodies is not known, the present study was undertaken to determine the effect of such antibodies on transmitter release. Slices (0.7mm) of rat brain cortex were loaded with [^{3}H]-GABA (1 μCi/ml; 15 nM) for 15 min in 0.4 ml of aerated balanced salt medium. Slices were placed in fresh medium (containing 10 μM AOAA to block GABA metabolism), and medium was replaced at 5 min intervals. The effect of anti-G_{M1} ganglioside antibodies was studied using immunoglobulin (Ig) fractions from antiganglioside serum (in 10 μl PBS -- equivalent to the original volume of antiserum). As a control, Ig fractions were obtained from antiserum that had been absorbed with pure G_{M1} ganglioside to remove specific antibodies. Ig fractions were present in all replacement media. Spontaneous GABA release and release induced by K^+ (40 mM) and by veratrine (0.2 mM) were studied in the presence of GABA uptake inhibitors (50 μM 2,4-diaminobutyric acid plus 100 μM β-alanine). Four Ig fractions from four different antiganglioside sera (with anti-G_{M1} titers ranging from 350 to 750 using 4 C'50 units of complement) enhanced K^+-stimulated GABA release in the 1st (*) and 2nd (**) 5 min intervals (for which the K^+ effect on release is marked) by 82%* 82%**, 56%* 100%**, 37%* 60%** and 34%* 26%**. The two Ig fractions tested on veratrine-stimulated GABA release enhanced release by 32%* 12%** and 45%* 11%**. In contrast, spontaneous GABA release was inhibited (ca 25%) but only when uptake inhibitors were absent, suggesting that the reduction in spontaneous release of labeled GABA was the result of an increase in reuptake. It is concluded that antiganglioside antibodies can affect neurotransmitter release in the absence of complement since they enhanced depolarization-induced GABA release. The specificity of the action of antiganglioside antibodies warrants further study with respect to: 1) release of other transmitters, both spontaneous and depolarization-induced and 2) whether enhancement of release is a common effect.

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7.1 ACCUMULATION AND METABOLISM OF PIPECOLIC ACID IN THE BRAIN. H. Nishio*, J. Ortiz*, E. Giacobini, T. Schmidt-Glenewinkel*. Lab. of Neuropsychopharmacology, Dept. of Biobehavioral Siences, Univ. of Connecticut, Storrs, CT 06268.

Pipecolic acid (PA), an iminoacid related to lysine metabolism has been recently isolated in the mouse brain (Nomura, Y., Schmidt-Glenewinkel, T.,and Giacobini, E., Dev. Neurosci., 1:239-249, 1978). It occurs in appreciable amounts (18 + 4 pmoles/g), it is synthesized in vitro, it is taken up by a high affinity, Na^+ and temperature dependent mechanism into synaptosomes (K_m = 3.90 + 0.17 x 10^{-7}M) (Giacobini, E., Nomura, Y. and Schmidt-Glenewinkel, T., Cellular & Molecular Biology, 25:1-9, 1980). and it is released from rat brain slices by means of a Ca^{++} dependent, high potassium-induced release mechanism (Nomura, Y., Okuma, Y., Segawa, T., Schmidt-Glenewinkel, T. and Giacobini, E., J. Neurochem. 33:803-805, 1979). We have now studied the transport and accumulation of PA into the brain of the mouse-

About 1% of the (^{3}H)PA injected i.v. or i.p. (5.5-5500 nmole) in the mouse is rapidly accumulated in the brain from where it is slowly eliminated (T 1/2 = 6.7 hrs). In liver and kidney (^{3}H)PA is accumulated more rapidly than in the brain. Probenecid (200 mg/kg i.p.) produces a significant increase in brain accumulation. After 30 min. following i.v.injection approx 80% of (^{3}H) is found in the brain as (^{3}H)PA while the remaining part is constituted by metabolites. 80% of (^{3}H)PA injected i.v. is secreted in the urine as such after 30 min. The brain/plasma ratio following i.v. injection, is approximately 0.25 at 1 hr. and almost 1 at 6 hrs.

In summary we can say that PA is rapidly accumulated in the brain following i.p. or i.v. injection from where it is slowly eliminated. Probably, the brain is able to metabolize PA. The liver, on the contrary, does not seem to contribute substantially to PA metabolism. The existence of BBB for PA seems to be supported by our results. The transport of PA to the brain is similar to the one for its precursor aminoacid lysine. The BBB for PA seems to be more permeable than for 6-OH dopamine but less than for tyrosine.

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7.2 THE GLUTAMIC ACID DECARBOXYLASE OF LAMPREY CNS - DEVELOPMENTAL CHANGES AND EFFECTS OF CORDOTOMY. N.R. Krieger, U. Wald* and M.E. Selzer. Dept. of Pharmacology, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104.

The presence of glutamic acid decarboxylase (GAD) was demonstrated in the CNS of the sea lamprey (Petromyzon marinus). A microassay based on the release of $^{14}CO_2$ from (1-^{14}C) L-glutamic acid was used. The enzyme had similar characteristics in brain and spinal cord and in larvae and feeding stage adults. It had a sharp temperature optimum at 27-30°C. Optimum pH was 6.8. The Km for glutamic acid, measured at 27°C, pH 7.1 in the presence of 0.1 mM pyridoxal phosphate, was 5 mM.

In large larvae (3-5 years old) homogenates of brain had approximately 2.5 times the GAD specific activity of homogenates of cord (63 vs. 26 nmol. CO_2/mg protein/hr.). In feeding stage adults the brain showed approximately 8 times the specific activity of the cord (236 vs. 29 nmol. CO_2/mg protein/hr.). Thus between larval and adult stages the brain showed an almost 4-fold increase in GAD activity, while the spinal cord showed no statistically significant increase.

GAD activity was the same in rostral, middle and caudal thirds of the larval spinal cord. Following transection of the spinal cord of larvae at the level of the 6th gill, the GAD activities rose sharply in brain and in all three regions of spinal cord. The maximum increase was observed one week after transection, at which time activities in all regions were 5-6 times normal. By the fourth week GAD activities had returned to normal. GAD activity persisted in brain and cord at approximately control levels for at least seven weeks following cordotomy. Thus Wallerian degeneration, which is known to occur in the lamprey spinal cord, did not eliminate the GAD activity either rostral or caudal to the transection. We conclude that GAD activity does not originate exclusively in cells of either brain or spinal cord, but must be synthesized independently by cells of both structures.

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7.3 A unique action of 2-amino-4-phosphonobutyrate on the ON pathway in the mudpuppy retina. Malcolm M. Slaughter* and Robert F. Miller, Departments of Ophthalmology, Physiology, and Biophysics, Washington University School of Medicine, St. Louis, Missouri

Glutamate and aspartate have been proposed as photoreceptor synaptic transmitter candidates since Murakami, et al ('75) demonstrated that these excitatory amino acids depolarize OFF bipolar cells and hyperpolarize ON bipolar cells in fish. Recently, Wu and Dowling ('78) have reported that aspartate mimics the action of the photoreceptor transmitter on carp horizontal cells and both actions are blocked by DL α amino adipate. We have examined a number of glutamate and aspartate agonists and antagonists and report here on a selective action of 2-amino-4-phosphonobutyrate (APB), a glutamate analog, on the ON pathway in the mudpuppy retina. Intracellular recordings were obtained from all classes of retinal neurons in the superfused mudpuppy eyecup. In the outer retina, application of micromolar concentrations of APB blocked the light evoked response (LER) of the depolarizing bipolar cells while leaving unaffected the LER of receptors, horizontal cells, and hyperpolarizing bipolars. This blocking action was complete and reversible. In the inner retina, APB eliminated the LER of the ON amacrine and the ON component of the ON-OFF amacrine cells while the response of OFF amacrines persisted. APB blocked the ON but not the OFF IPSP of ganglion cells and also blocked the LER of ON ganglion cells. We have undertaken experiments to determine the mechanism of the APB action by superfusing with cobalt (3 mM) to clock synaptic transmission, a treatment which depolarized the ON bipolars. Application of APB superimposed on the cobalt block caused a hyperpolarization of the ON bipolar, consistent with the idea that APB is a transmitter agonist. A three carbon analog of APB, 2-amino-4-phosphonopropionate, did not block the ON pathway in concentrations up to 1 mM. Assuming that a single transmitter is released by photoreceptors, this selective effect of APB suggests that the transmitter receptors on depolarizing bipolar cells are unique and distinct from those on the two other types of second order neurons in the mudpuppy retina.

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7.4 LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) FOLLOWING SYSTEMIC ADMINISTRATION OF GABAERGIC AGONIST AND ANTAGONIST DRUGS. E.D. London, J.M. Palacios, S.I. Rapoport and M.J. Kuhar. Lab of Neurosciences, Gerontology Research Center, National Institute on Aging, Baltimore City Hospitals, Baltimore, MD 21224 and Departments of Pharmacology and Experimental Therapeutics and Psychiatry and the Behavioral Sciences, Johns Hopkins School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205.

Using radioligand binding and autoradiographic techniques, it has been observed that high affinity γ-aminobutyric acid (GABA) binding sites are heterogeneously distributed throughout the rat brain (Palacios et al., P.N.A.S. 77: 670, 1980). In order to assess the functional significance of these high affinity binding sites, we employed the autoradiographic ^{14}C-2-deoxy-D-glucose (DG) technique of Sokoloff et al. (J. Neurochem. 28:897, 1977) to study local cerebral glucose utilization (LCGU) in the conscious rat. Animals were injected i.v. with one of several doses of GABAergic agonist (muscimol, 1-7 mg/kg; 4,5,6,7,-tetrahydroisoxazolo[5,4-c] pyridin-3-ol,THIP, 12 and 24 mg/kg) or antagonist (bicuculline) drugs prior to the administration of 125 μCi/kg of DG. GABA agonists induced dose-dependent depressions in LCGU in most brain regions assayed. Decreases in LCGU were most dramatic in forebrain regions, especially cerebral cortex, caudate nucleus and thalamic nuclei. There were variable reductions in LCGU within the hippocampus. In contrast, LCGU in the red nucleus was increased by up to 100% with a high dose of muscimol (7 mg/kg). A subconvulsant dose of bicuculline (294 μg/kg) increased LCGU in several regions, notably the auditory cortex and inferior colliculus.

These observations support the proposed general inhibitory function of GABA in the central nervous system. Enhanced LCGU in the red nucleus may result from GABAergic inhibition in the cerebellum, thalamus and/or cerebral cortex; these areas have high densities of GABA receptors. There is no simple correlation between the magnitude of response in LCGU to GABAergic agonist and antagonist drugs and the density of high affinity GABA binding sites. Thus, LCGU following specific pharmacologic stimulation depends on the presence of relevant receptors for the administered drugs, but is influenced also by the neuronal circuitry of any region studied. Supported by MH00053, MH25951, DA00266, TW02583.

7.5 GLUTAMINE AND α-KETOGLUTARATE AS METABOLIC PRECURSORS OF AMINO ACID NEUROTRANSMITTERS. R. P. Shank and G. LeM. Campbell*, Franklin Research Center, Philadelphia, PA 19103.

As part of a project aimed at elucidating the mechanism by which the neurotransmitter pools of glutamate, GABA and aspartate are maintained and regulated we have examined the uptake and metabolism in vitro of glutamine, α-ketoglutarate and a variety of other compounds by cellular and subcellular fractions derived from the cerebellum of 1 to 3 week old mice. The cerebellar tissue was dissociated using a combination of enzymatic treatment (mild trypsinization and DNA ase) and mechanical agitation. Five cellular and subcellular fractions were obtained using a series of linear density gradients formed with Percoll. Four of the fractions were comprized almost entirely of cell bodies, one of which was enriched in granule cells. On fraction contained predominantly sub-cellular material. In this fraction nerve terminals were prevalent, but there were few, if any, free mitochondria.

Glutamine and α-ketoglutarate were both taken up comparatively fast by the fraction enriched in nerve terminals, but only glutamine was readily taken up by all five fractions. Both glutamine and α-ketoglutarate were rapidly converted to glutamate subsequent to being taken up by the nerve terminal enriched fraction. Much of the glutamate formed from these precursors was metabolized to GABA and aspartate. The transport of both glutamine and α-ketoglutarate appears to be mediated by high and low affinity systems; the K_t values for α-ketoglutarate (approximately 15 and 50 μM) being lower than those for glutamine (approximately 50 and 200 μM). The uptake of both glutamine and α-ketoglutarate by the fraction enriched in nerve terminals was inhibited by glutamate, aspartate, GABA and cGMP when this fraction was pre-incubated with those substances at concentrations of 0.2 to 2 mM. There was no apparent mutual interaction between glutamine and α-ketoglutarate transport.

Our observations support the concept that both glutamine and α-ketoglutarate derived from non-synaptic sources are instrumental in replenishing the molecules of glutamate, GABA and aspartate lost from nerve terminals during synaptic activation. Our data further suggest that the regulation of glutamine and α-ketoglutarate uptake is an important factor in the regulation of intracellular levels of glutamate, aspartate and perhaps GABA.

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7.6 RATES OF GABA ACCUMULATION IN VARIOUS BRAIN REGIONS AFTER IRREVERSIBLE INHIBITION OF GABA-TRANSAMINASE IN VIVO AND IN BRAIN SLICES. M.J. Iadarola, and K. Gale. Department of Pharmacology, Schools of Medicine and Dentistry, Georgetown University, Washington, D.C. 20007.

The production of GABA in different rat brain regions was evaluated in brain slices maintained in vitro. Fresh tissue slices (225u) from cerebellum, cerebral cortex, caudate nucleus, superior colliculus and substantia nigra were incubated at 37°C for various times (5-90min) in the presence of 10^{-4}M gamma-vinyl-GABA (GVG), a specific and irreversible inhibitor of GABA transaminase. In slices prepared and maintained in Krebs-Ringer phosphate (bubbled with 100% O_2 for 20min) GABA levels started to rise after a latency of 15min after addition of GVG. The rate of accumulation of GABA was linear between 20 and 70min; during this period we took time points at 10min intervals. Under these conditions the rate of accumulation of GABA was found to be in the range of 0.2-0.6nmoles/mg protein/min; these values were similar to those obtained in vivo after administration of GVG (600mg/kg i.p.). In general, brain regions with low initial steady-state levels of GABA (cerebellum, cortex and caudate) had lower rates of GABA accumulation than regions with high initial steady-state GABA (superior colliculus and substantia nigra). However, the rates of accumulation of GABA were not found to be proportional to the glutamic acid decarboxylase (GAD) activity measured in cell-free homogenates of the various regions. Similarly, in vivo accumulation of GABA in the various brain regions after GVG was not proportional to either control steady-state GABA levels or to GAD activity. These findings may be related to our previous observation that a large portion of the GABA increase after GVG occurs in a glial-perikarya-metabolic compartment of GABA (Gale and Iadarola, Science 208: 288, 1980). Consistent with this, we found that slices obtained from kainic acid-lesioned caudates generated GABA at a rate comparable to that obtained in intact tissue.

In addition, we found that the initial rate (5-30min) of GABA accumulation in the slices could be accelerated by addition of CO_2 or bicarbonate to the Krebs-Ringer buffer. Under these conditions, differences in rates between the various brain regions were accentuated.

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7.7 SPECIFIC BINDING OF [^{3}H]-GLUTAMATE AND [^{3}H]-KAINATE IN THE GRANULO-PRIVAL MOUSE CEREBELLUM. J. Slevin*, M. Johnston, K. Biziere and J.T. Coyle. Dept. of Pharmacol. and Expt. Therapeut., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

To examine the relationship between receptor binding sites for [^{3}H]-glutamic acid and [^{3}H]-kainic acid and cerebellar (Cb) circuitry, the effects of granule cell ablation on neurotransmitter receptors and neuronal presynaptic markers were examined in mice rendered granule cell deficient by treatment with methylazoxymethanol acetate (MAM). On the day of birth, Blu/HA mice received a single, subcutaneous injection of MAM (30 mg/kg), an alkylating agent that selectively kills mitotically active cells. In adult treated mice, the Cb mass was reduced by 47% as compared to saline treated litter-mates. Nissl-stained sections of the lesioned Cb revealed a profound loss of granule cells; the Purkinje cells, which did not appear reduced in number, had an abnormal distribution.

The specific activities of choline acetyltransferase and glutamate decarboxylase were increased significantly ($p<0.001$) by 46% and 59% respectively, resulting in a slight decrease in total activities per MAM Cb. The specific activities of the Na^+ dependent synaptosomal uptake for [^{3}H]-GABA was increased by 52% ($p<0.001$) whereas the uptakes of [^{3}H]-L-glutamic acid and of [^{3}H]-L-aspartic acid were reduced by 45% ($p<0.001$) and 28% ($p<0.05$) respectively. Congruent with these results, the concentration of GABA was elevated by 29% ($p<0.05$) whereas the concentration of glutamate was reduced by 29% ($p<0.001$); endogenous norepinephrine was increased by 67% ($p<0.001$).

In MAM Cb the specific binding of [^{3}H]-quinuclidinyl benzilate, a muscarinic receptor antagonist, and of [^{3}H]-dihydroalprenolol, a beta receptor antagonist, were unchanged. For contrast, the specific binding of ^{3}H-GABA was significantly reduced by 32% ($p<0.001$) per mg protein. The specific binding of [^{3}H]-kainic acid (50 nM; 2°C incubation) was not altered in the lesioned cerebellum per mg protein but total binding was significantly reduced by 47% ($p<0.001$). In contrast, the specific binding of [^{3}H]-L-glutamic acid (600 nM; 37°C incubation) was incrased by 106% ($p<0.001$) per mg tissue protein; as a result, the total amount of specific binding per MAM Cb was increased by 41%.

The data indicate that the MAM treatment results in a marked reduction in Cb granule cells and their pre-synaptic glutamatergic markers; the specific binding of ^{3}H-glutamate increased considerably, which suggests a postsynaptic localization and possible denervation supersensitivity. The binding site for [^{3}H]-kainate responds to the lesion differently than that of [^{3}H]-glutamate but similarly to the muscarinic and beta receptors.

7.8 EXCITATORY AMINO ACID RECEPTORS ARE ENRICHED IN SYNAPTIC JUNCTIONS. A. C. Foster*, E. E. Mena*& C. W. Cotman. Dept. of Psychobiology, Univ. of Calif., Irvine, CA. 92717.

Dicarboxylic amino acids have been shown to cause excitations when applied to mammalian neurons, and electrophysiological evidence suggests that multiple receptors for such amino acids exist in the CNS. The multiplicity of such receptors may reflect the presence of extra-junctional, as well as junctional receptors, as has been demonstrated for glutamate in the invertebrate nervous system. Binding studies with excitatory amino acids reported to date have used crude synaptic membranes, which would be expected to contain both junctional and extra-junctional receptors.

We have investigated the binding of L-[^{3}H]-glutamate, L-[^{3}H]-aspartate and [^{3}H]-kainic acid to purified synaptic junctions from rat brain. For all three ligands, specific binding (defined as displaceable binding in the presence of 10^{-4}M unlabelled ligand) was enriched as the purity of synaptic junctions increased i.e. homogenate < P_2 < synaptic plasma membranes < synaptic junctions. The highest specific binding was found in synaptic junctions, which for all three ligands was enriched approx. 10-15 fold over that in homogenate. Microsomal, mitochondrial and myelin fractions were found to have appreciably less binding than the synaptic fractions.

These results suggest that the use of synaptic junction preparations in the study of excitatory amino acid binding may allow differentiation between junctional and extra-junctional receptors for these compounds. (Supported by grant -- NIH no. NS 08597-11.)

7.9 BIOCHEMICAL PHARMACOLOGY OF KOJIC AMINE, A GABA RECEPTOR AGONIST. J.W. Ferkany,* M.F. Browner* and S.J. Enna. Depts. Pharmacol., Neurobiol. and Anat., Univ. Texas Medical School, Houston, Texas 77025

Kojic amine (2-aminomethyl-5-hydroxy-4H-pyran-4-one), a structural analogue of γ-aminobutyric acid (GABA), selectively inhibits ^{3}H-muscimol binding to GABA receptors in rat brain membranes (IC_{50}=4μM) and, when applied iontophoretically, hyperpolarizes cerebellar Purkinje cells, an action which is blocked by bicuculline (Arch. Int. Pharmacodyn. Therap. 241:266, 1979). These data suggest that this agent may be a direct-acting GABA receptor agonist. In the present study, experiments were undertaken to further explore and define the interactions of kojic amine with the GABA receptor and to determine whether this compound will activate this receptor system following systemic administration.

Like GABA, kojic amine enhances ^{3}H-diazepam binding to rat brain benzodiazepine receptors in vitro, though it is some 10 fold weaker (EC_{50}=64μM) than GABA in this regard. In addition, chronic administration of kojic amine (18 mg/kg b.i.d. for 15 days) induces a significant increase in dopamine receptor and a decrease in GABA receptor binding in the rat corpus striatum. Furthermore, acute administration of kojic amine (30 mg/kg) causes a significant increase in synaptosomal high affinity choline uptake in the corpus striatum. The results of these ex vivo experiments are qualitatively similar to those previously found after chronic administration of more established GABAergic agonists such as THIP and γ-acetylenic GABA.

These findings indicate that kojic amine is a GABA receptor agonist following systemic administration, suggesting that this compound may be a useful tool for defining the behavioral, biochemical and physiological effects of the GABAergic system. (Supported in part by USPHS grants NS-13803, NS-00335 and MH-07688).

7.10 RELEASE OF ENDOGENOUS AMINO ACIDS FROM NORMAL AND GRANULE AND STELLATE CELL DEPLETED RAT CEREBELLUM. R.S. Flint*, M.A. Rea* and W.J. McBride, Depts. Psychiatry & Biochemistry, Inst. Psychiatric Res., Indiana Univ. Sch. of Med., Indianapolis, IN 46223

The release of endogenous amino acids from rat cerebellum was studied using 300 x 300 μm diced tissue embedded in Sephadex G-50 columns. In preliminary studies, the whole cerebella from adult, male Wistar rats were used to ascertain the time course of release of glutamate (Glu), aspartate (Asp), GABA, taurine (Tau), glycine (Gly) and alanine (Ala). The tissue was perfused first for 30 minutes with a Ca^{++} free physiological saline medium containing 1 mM EGTA, then for 25 minutes with Ca^{++} free medium containing 55 mM K^+, and finally with medium containing 55 mM K^+ plus 2.5 mM Ca^{++}. After the initial 10-15 minute wash-out period, a small constant rate of spontaneous efflux was observed. Elevation of K^+ in the absence of Ca^{++} caused a slight increase in efflux of Glu and Asp without altering the efflux of GABA, Tau, Gly or Ala. Addition of 2.5 mM Ca^{++} to the medium containing 55 mM K^+ caused a peak release above baseline of 25-fold for Glu, 5-fold for Asp, 10-fold for GABA and 2-fold for Ala. The efflux of Tau and Gly was not increased by addition of Ca^{++} to the 55 mM K^+ medium. In a second study, male Long-Evans rats were used. One group (N=4) was exposed to x-irradiation treatment at 12-15 days following birth (12-15x group) which prevented the acquisition of late-forming granule cells; a second group (N=5) was exposed to a schedule of x-irradiation which prevented the acquisition of granule and stellate cells (8-15x group). Unirradiated littermates (N=11) were used as controls. Animals were killed at 90-120 days of age. The cerebellum was quickly removed; the vermis was dissected free, diced and perfused as described above. With respect to control values, there was a significant reduction in the K^+-stimulated Ca^{++}-dependent release from the 12-15x cerebellar vermis of (a) 50% for Glu; (b) 50% for Asp; and (c) 57% for Ala. With regard to control data, there was a significant reduction in the K^+-stimulated Ca^{++}-dependent release from the 8-15x group of (a) 77% for Glu; (b) 67% for Asp; (c) 55% for GABA; and (d) 92% for Ala. The reduction in the release of Glu from the granule cell deficient cerebella supports the hypothesis that it may be the excitatory transmitter released from these interneurons. The reduced release of GABA from the granule and stellate cell deficient cerebella supports the idea that GABA is the transmitter released from stellate cells. The fact that no increased release of Tau or Gly occurred in the elevated K^+ plus Ca^{++} medium could be taken as evidence against a neurotransmitter role for these two amino acids in the cerebellum. (Supported in part by NS 13925 and MH 00203).

7.11 ASPARTATE AS THE NEUROTRANSMITTER FOR THE PERFORANT PATHWAY OF THE HIPPOCAMPUS. A. Di Lauro* and J. L. Meek* (SPON: L. M. Neckers). Lab. Preclin. Pharmacol., NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

It has been reported that glutamate and/or aspartate could be the transmitter of the perforant pathway (PP), the pathway from the entorhinal cortex to the fascia dentata (FD) and to the hippocampus proper (Nadler et al., 1976, Nature 260:539). In order to determine the role of aminoacids in the PP we examined in the target areas of this pathway the changes in the concentration of various aminoacids (Tau, Gly, Ala, Glu, Asp, GABA) after unilateral entorhinal cortex lesion in rats.

At various survival time (1,3,7,15 days) after a radiofrequency lesion, the rats were killed by means of focused microwave irradiation. The hippocampus was excised from the lesioned side and from the contralateral side and the FD dissected. Aminoacids were separated on a cation exchanger by high pressure liquid chromatography and detected fluorimetrically. The lesion caused a decrease in the aspartate content of the homolateral FD. This decrease reached its maximum (70% of control; $P<0.001$) after three days; at longer survival times (7-15 days after the lesion) we observed a slow recovery toward control values. This recovery is probably due to the known sprouting process that starts early after this lesion (West et al., 1975, Brain Res. 97:215). In the contralateral FD and in the hippocampus proper we observed only slight variations in the aspartate concentration. We were unable to detect any significant difference between control and lesioned rats in Glu or in the other aminoacids tested. In order to determine whether this decrease of aspartate is presynaptic or postsynaptic we injected kainic acid in the hippocampus of 10 rats at a dose (0.75 μg) able to destroy granule cells; the following day half of rats received entorhinal cortex lesion while the remaining rats (KA alone) were used as controls. Three days after the PP lesion we observed a significant decrease in aspartate content of FD when compared to rats treated with KA alone. The decrease in FD aspartate after PP lesion was not, therefore, due to granule cells but to degenerating presynaptic terminals of PP. In conclusion though our experiments do not prove that aspartate is the neurotransmitter of the PP, they strongly support this hypothesis.

8.1 ELECTROGENESIS OF THE EVENT-RELATED SLOW POTENTIAL DETERMINED BY INTRACELLULAR RECORDING IN THE CONSCIOUS CAT BRAIN, G. L. King*, and J. E. Skinner.

Frontocortical neurons in the conscious cat brain manifest slow membrane potential and input resistance shifts in parallel with an extracellular event-related slow potential, all of which are evoked by a tone that forewarns a cutaneous shock. The biophysical responses of each cell were recorded with a new "floating-micropipette tip" technique that enabled intracellular impale ments to be maintained when the animal was presented the previous ly conditioned stimuli. Two classes of cells were recorded, those with large resting membrane potentials and action potentials that manifested overshoot of the extracellular zero potential (overshoot cells) and those with small resting potentials and small action potentials that did not overshoot the extracellular zero potential (undershoot-cells).These two classes of cells are easily distinguished by their resting input resistances and other cable properties. Both the cable properties and the location of inject ed dye suggest that the undershoot cell records are from dendritic impalements. Individual cells of both cell classes manifested different patterns of responses to the tone and to the shock. The predominant response patterns of the undershoot-cells were input resistance decreases accompanied by depolarizations (rD-re sponses) and input resistance increases accompanied by hyperpolar izations (RH-responses). One other group of undershoot-cells showed responses following the shock that were different from those following the tone (MM-responses). The overshoot cells showed similar response patterns, although no RH-responses were recorded. These input resistance and membrane potential responses of the cells were not correlated in time; they showed different latencies to onset and peak amplitude. Some cells of both class es were recorded for a long enough time to permit the recording of the responses of the cells to repeated conditioning trials. It was observed that the cell responses during the repeated trial were not always the same response as during the first trial.

All of these data have been interpreted to suggest that a neuronal mechanism underlies the electrogenesis of the event-related slow potential. Furthermore, the response patterns of these neurons, which manifest themselves only in the conscious state, may be the basis for a type of neural integration that is characteristic of information processing in a higher cerebral brain system.

8.2 TWO CLASSES OF SPONTANEOUS MINIATURE SYNAPTIC POTENTIALS IN CA3 HIPPOCAMPAL NEURONS. Thomas H. Brown and Daniel Johnston, Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010, and Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

Spontaneous miniature synaptic potentials were recently detected in CA3 neurons of the hippocampus (Brown et al., Brain Res. 177, 194, 1979). In the present study, we have examined the amplitude and polarity of miniature potentials in these cells, at membrane potentials ranging from -150 to +50 mV, to address the following two questions: Can inhibitory miniature potentials be detected when the cells are depolarized? What is the approximate reversal potential of the excitatory miniature potentials?

Recordings were made in the in vitro slice preparation which was continuously perfused with oxygenated normal saline containing 1 μg/ml tetrodotoxin (TTX) and 2 mM $MnCl_2$. Current was injected and voltage recorded through a single micropipet using a 3 KHz time-share system. Pipets were filled with either 3 M KCl, 4 M KAc or 1 M $CsSO_4$. At membrane potentials between -150 and -50 mV, only depolarizing events were observed, their amplitudes being greater at more hyperpolarized potentials. In many cells, at about -40 mV, both hyperpolarizing and depolarizing events were present. At membrane potentials between -30 and 0 mV, the events were always hyperpolarizing. These hyperpolarizing events appeared to be spontaneous inhibitory miniature potentials, since they were abolished by adding to the bath putative inhibitory blockers (10 μM bicuculline, 10 μM picrotoxin, or 2 mM penicillin). With the blockers in the bath, the usual depolarizing events were still present at membrane potentials between -150 and -50 mV. In the presence of these agents, hyperpolarizing events were not observed unless the membrane was depolarized beyond +30 mV. The amplitude of these hyperpolarizing events increased as the cells were further depolarized. These events appeared to be reversed excitatory miniature potentials. Their apparent reversal potential was near 0 mV, but the value could not be determined accurately because the events could not be resolved above the background noise when the membrane was within ±30 mV of the estimated reversal potential. All of the above results were duplicated in parallel studies in which TTX and $MnCl_2$ were omitted from the bath. In these studies, the only apparent difference was that the synaptic events were larger, presumably because they consisted of both spontaneous and evoked release.

The ability to resolve both inhibitory and excitatory miniature potentials in these cells should be useful in neuropharmacological studies as well as in testing hypotheses concerning the mechanism of long-term potentiation in these cells. (Supported by NIH grants NS15772 and NS11535.)

8.3 REVERSAL POTENTIAL FOR EXCITATORY SYNAPTIC EVENTS IN HIPPOCAMPAL CA3 PYRAMIDAL NEURONS. D. Johnston, T. H. Brown, J. J. Hablitz and F. J. Lebeda. Dept. of Neurology, Baylor Coll. of Med., Houston, TX 77030 and Div. of Neurosciences, City of Hope Research Inst., Duarte CA 91010.

Reversing the direction of excitatory postsynaptic potentials (EPSPs) with an accurate measure of the membrane potential at the reversal point has proved a difficult task in mammalian CNS neurons. In this study we have attempted to demonstrate a reversal of mossy-fiber EPSPs in CA3 pyramidal neurons from in vitro hippocampal slices in order to address the following questions: Is the mossy-fiber EPSP entirely chemical in origin? If a reversal is obtained, what is the apparent reversal potential, and therefore what ions might be carrying the synaptic current? Are the synaptic endings electrically distributed on the postsynaptic neuron? Does concomitant inhibition play a role in the mossy-fiber EPSP?

Intracellular recordings were made using pipettes filled with 4M KAc or 2M Cs_2SO_4. Current and voltage clamping were performed using a single microelectrode system. Several procedures (including high Mg and low stimulus strengths) were used to reduce the recurrent inhibition obtained with mossy-fiber stimulation. The traditional recurrent IPSP was still occasionally present, but was distinguishable from the EPSP on the basis of a slower time course. However, the mossy-fiber EPSP was found to reverse direction completely at a single and very negative membrane potential (about -50 mV). Moreover, under voltage clamp conditions all reversal potentials and extrapolated reversal potentials of the inward current were only 10 to 30 mV depolarized from the resting potential. Since these reversal potentials seemed unreasonable for an EPSP, putative inhibitory blockers (10 μM bicuculline, 10 μM picrotoxin, or 2 mM penicillin) were added to the bath in an attempt to separate possible occult inhibitory components in the normal EPSP. With one of these agents in the bath, the mossy-fiber EPSP reversed direction completely at or near 0 mV. If the mossy-fiber terminals are electrotonically remote from the recording site, then this apparent reversal potential may be an overestimate of the true synaptic equilibrium potential.

The results suggest, however, that the mossy-fiber ending is a chemical synapse producing a conductance increase, that the terminals are not electrotonically distributed on the postsynaptic neuron, and that the normal EPSP is contaminated by a large occult inhibitory component of similar time course. The data suggest also that a measured reversal potential is a better test of a pure EPSP than the separation of events on the basis of time course. (Supported by NIH Grants NS15772, NS11535 and the Epilepsy Foundation of America.)

8.4 SPONTANEOUS IPSPs IN HIPPOCAMPAL PYRAMIDAL CELLS. R. A. Nicoll and B. E. Alger, Depts. of Pharmacology and Physiology, Univ. of Calif., San Francisco, CA 94143.

In CA1 pyramidal cells of rat in vitro hippocampal slices, intracellular recording with 3 M KCl-filled electrodes reveals many small (1 - 10 mV) spontaneous depolarizing potentials on the baseline. These potentials vary in frequency from one to about 20 Hz and appear to be chloride (Cl^-) dependent, as they are not seen in CA1 cells with electrodes filled with potassium salts of impermeant anions. Since Cl^- dependence is a property of hippocampal inhibitory post-synaptic potentials (IPSPs), and not EPSPs, we have considered the hypothesis that these small depolarizations are spontaneous, reversed IPSPs.

Because the major hippocampal inhibitory transmitter is probably γ-aminobutyric acid (GABA) we have tested the sensitivity of these spontaneous depolarizations to various drugs known to affect GABA-mediated events. Experiments were done on single cells (24) impaled with KCl electrodes in a normal bathing medium and then switched, while the impalement was held, to different experimental media. GABA antagonists bicuculline, picrotoxin and penicillin abolish the spontaneous events. Excitatory transmission is not blocked with these drugs. When GABA antagonists are applied to cells recorded with KCl electrodes the membrane hyperpolarizes and the input impedance increases, as expected when a spontaneous inhibitory input is blocked. Pentobarbital, an anesthetic which in low doses (10^{-4} M) has a very selective effect of enhancing GABA-mediated events, markedly prolongs the spontaneous depolarizations. Pentobarbital has a depressant effect, if any, on EPSPs. From their Cl^- dependence and responses to drugs which affect both GABA actions and synaptic inhibition, we conclude that the small spontaneous depolarizations are reversed, GABA-mediated IPSPs.

Spontaneous IPSPs are also blocked by tetrodotoxin and calcium antagonists. Since true "quantal" release processes are ordinarily immune to such treatment, it appears that "spontaneous" IPSPs in CA1 pyramidal cells are actually activity-dependent, and result from spontaneous firing of inhibitory interneurons. Consistent with this interpretation is the finding that analysis of the distribution of these potentials does not agree with predictions based on a random release process.

Besides its intrinsic interest, study of spontanous IPSPs has practical usefulness for investigating modulation of inhibitory synaptic transmission. For example, we have recently found that these small spontaneous IPSPs and the evoked IPSP are blocked by the opiate peptide d-ala^2-met^5-enkephalinamide. Since responses to iontophoretically applied GABA are not blocked by enkephalin, we conclude that the site of IPSP blockade by enkephalin must be presynaptic on the inhibitory interneurons.

8.5 INHIBITORY ACTION OF GABA IN RAT HIPPOCAMPUS. K. Krnjević, Y. Ben-Ari and W. Reinhardt*. Anaesthesia Research and Physiology Depts., McGill University, Montreal, Canada H3G 1Y6.

Although there is plenty of evidence from extracellular studies that GABA inhibits hippocampal cells and that it is probably synthesized in (and released from) inhibitory interneurons such as basket cells (Storm-Mathisen, Prog.Neurobiol. 8, 119, 1977), there has been no systematic comparison between IPSPs and the effects of GABA.

In rats under urethane, we have recorded intracellularly from cells in the CA1 and CA3 area, usually with KCl (but also some K citrate) -containing microelectrodes. IPSPs were evoked by stereotactic stimulation of the ipsilateral fimbria or entorhinal cortex. GABA and other agents were tested by microiontophoretic release from a second, closely adjacent extracellular microelectrode. In addition to the membrane potential and IPSPs, we also monitored continually the neurons' input resistance at "rest" and during IPSPs, by injecting regularly repeated series of known current pulses through the recording electrode.

In spite of quite variable conditions of intracellular recording, in most cases a marked conductance increase was readily detected near the peak of IPSPs (mean of +155% for 84 different cells) evoked by 10 V, 0.5 ms stimulating pulses. In a selected group of 11 cells, having resting potentials of at least -55 mV (mean -65.0), the mean input resistance was 10.3 MΩ and the mean fall in resistance near the peak of the IPSP -72.7%. There was no significant difference in time course or in reversal potential between IPSPs evoked by entorhinal or fimbrial stimulation. Iontophoretic applications of GABA (mean dose 108 nA) caused a large increase in conductance (mean +245%, for $n = 55$), as well as a change in potential that followed the direction of IPSPs. Indeed, a comparison of the calculated IPSP reversal potential (E_i) for the period immediately preceding the release of GABA and the reversal potential for the maximum potential change evoked by GABA (E_G) revealed a very high positive correlation ($r = 0.893$, for $n = 44$) and a mean difference between corresponding values of E_i and E_G of only 0.78 mV (SE = 1.25, for $n = 44$). By contrast glycine was almost totally ineffective in evoking either inhibition of unit firing or any significant conductance increase. As in the cerebral cortex and elsewhere, the action of GABA showed a pronounced "fading", the conductance increase diminishing within a few seconds to a sharply lower plateau. This change was often associated with a parallel diminution in the conductance increase that accompanies EPSPs. These effects of GABA are fully in keeping with its probable role as the transmitter responsible for the most prominent IPSPs observed in the hippocampus.

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8.6 INCREASE IN MUSCARINIC BINDING SITES AT PRIMARY AND SECONDARY FOCI AFTER HIPPOCAMPAL KINDLING. F. Morrell, A. Hitri*, T. J. Hoeppner*, D. Bergen*, E. Kessler*, and S. Fleming*. Dept. of Neurological Sciences, Rush Medical College, Chicago, Il 60612.

Male Sprague-Dawley rats were implanted with stimulating electrodes in the left perforant path and recording electrodes in right and left CA_3 region of the hippocampus. Animals were stimulated twice daily with 60 Hz for 2 sec at a current which produced a brief after-discharge (6-12 sec). Stimulation was continued until the development of generalized seizures. Animals were sacrificed 18 hrs. after the last convulsion, the brain quickly removed, rapidly frozen and the hippocampi dissected out at 4°C. Muscarinic cholinergic receptors were measured with a radioligand binding assay using tritium labeled quinuclidinyl benzilate (QNB) according to the method of Yamamura and Snyder (Proc. Natl. Acad. Sci. 71:1725-1729, 1974). Specific binding was defined as the difference in total QNB binding obtained in the absence and presence of 1 μM atropine. Results indicated more than two-fold increase on the stimulated and more than three-fold increase on the unstimulated side compared to the intact controls. Both of these differences were significant ($p < 0.01$). There were no significant differences in the dissociation constants; thus, no change in receptor affinity was demonstrated.

Our findings imply that hippocampal kindling may depend upon an increase in muscarinic cholinergic receptors. These observations are consistent with the iontophoretic studies of Burchfiel et al. (Science 204:1096-1098, 1979) and with the chemical stimulation experiments of Wasterlain et al. (Neurology 28: 346, 1978) both of which also imply an augmentation of muscarinic cholinergic responsiveness in kindling. Differences between these data and the report of McNamara (Brain Res. 154: 415-420, 1978) may reflect the substantial variations in both the kindling technique and the method of measuring QNB binding.

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8.7 THE EPILEPTIFORM BURST DISCHARGE IS ASSOCIATED WITH A CALCIUM-DEPENDENT AFTERHYPERPOLARIZATION. B. E. Alger and R. A. Nicoll, Depts. of Pharmacology and Physiology, Univ. of Calif., San Francisco, CA 94143.

Intracellular recording from cells in experimentally produced epileptic foci reveals an interictal spike ("burst") potential and an afterhyperpolarization (AHP). We have found the burst and AHP are produced by synaptic activation of pyramidal cells in rat in vitro hippocampal slices during blockade of synaptic inhibition by γ-aminobutyric acid (GABA) antagonists picrotoxin and bicuculline, in concentrations which block responses to iontophoretically applied GABA. GABA is the major hippocampal inhibitory transmitter candidate. The synaptically evoked AHP is 8.6 ± 3.4 mV in amplitude and lasts 2.9 ± 1.2 sec ($n = 16$); it is associated with a conductance increase of 36 ± 12 %, and is functionally inhibitory. Previous work has suggested the interictal spike AHP is either an unusual form of inhibitory post-synaptic potential (IPSP) or a calcium (Ca)-dependent potassium potential. However, there is as yet little direct evidence on this point. Our data favor the latter hypothesis.

In our experiments the AHP usually appears as an all-or-none potential following an epileptiform burst. In addition we find the AHP is independent of chloride (Cl^-) movements, as it can be elicited in cells in which the Cl^- gradient has been reversed. These properties are not expected of IPSPs. Indeed we have compared the synaptically activated AHP (AHP_s) seen with GABA antagonists, with the AHP which follows a train of action potentials produced by direct depolarizing current injection into a cell (AHP_d). The AHP_d has been shown by Hotson and Prince (1980) to be an intrinsic, Ca-dependent potential. We have found both AHP_s and AHP_d are similarly depressed by barium, which is known to depress Ca-dependent potassium potentials in several other systems. Finally, in many cases we have been able to reverse both AHP_s and AHP_d by hyperpolarizing the membrane potential. Both potentials reverse at about -86 mV. These reversal potentials are, moreover, dependent on the extracellular potassium concentration $[K^+]_o$, there being a -50 mV shift in reversal potential per decade change in $[K^+]_o$. This is very close to the -60 mV shift predicted by the Nernst equation for a purely potassium dependent potential.

Our evidence therefore supports the hypothesis that the AHP associated with a synaptically elicited epileptiform discharge in the presence of GABA antagonists is a Ca-dependent potassium potential. This demonstration has direct implications for understanding the neurophysiology of epilepsy.

8.8 EGTA REDUCES THE AFTERHYPERPOLARIZATION (AHP) FOLLOWING CURRENT-INDUCED AND SPONTANEOUS BURSTING BUT NOT AFTER EPILEPTIFORM DISCHARGES IN HIPPOCAMPAL NEURONS. C. E. Stafstrom and P. A. Schwartzkroin. Depts. of Physiol. & Biophysics, and of Neurol. Surgery, Univ. of Wash. School of Medicine, Seattle, WA 98195.

Calcium has been shown to increase membrane conductance to potassium ions and to activate a long-lasting hyperpolarization in both vertebrate and invertebrate neurons. This Ca^{++}-mediated K^+ flux may serve to limit prolonged depolarization. The present experiments, employing intracellular injection of EGTA, investigate the role of Ca^{++} in this conductance mechanism in vertebrate cortical neurons. Transverse slices of guinea pig hippocampus (400 μm) were maintained in vitro using standard techniques. Control recordings were made using 4M K-acetate electrodes. Pyramidal cells from the CA3 region exhibited spontaneous bursting behavior consisting of 2-20 spikes riding on a slow wave of depolarization; similar burst discharges could be elicited by current injection. Membrane conductance increased during the AHP following both types of bursts as well as during IPSPs.

When impaled with 0.2M K-EGTA electrodes, CA3 pyramidal cells showed prolonged discharges with broader spikes both spontaneously and in response to a depolarizing current pulse. These effects were presumably due to the chelation of free Ca^{++} by the diffusing EGTA. The AHP after both bursts was reduced, and the conductance change under both conditions was decreased. The prolonged bursting in CA3 cells could be reset with a hyperpolarizing pulse. Conductance during the IPSP did not differ from the control penetration using K-acetate electrodes.

Addition of 2mM Na-penicillin to the bathing medium led to spontaneous epileptiform bursting. These bursts could be distinguished from spontaneous or EGTA-bursts on the basis of their rhythmicity (the same as that of epileptiform field potentials), and their resistance to blockage with hyperpolarizing current. The AHP following epileptic bursts was not affected by EGTA, and the conductance increase was maintained. These results are inconsistent with the hypothesis that AHPs following epileptiform bursts are mediated by the same mechanisms as those following spontaneous or current-induced bursts.

8.9 SPONTANEOUS EPILEPTIFORM DISCHARGES AND CLINICAL SEIZURES OCCUR EARLY IN HIPPOCAMPAL KINDLING. L. deToledo-Morrell* and F. Morrell (SPON: M. M. Cohen). Dept. of Neurological Sciences, Rush Medical College, Chicago, IL 60612.

Three and twenty-seven mos. old Fisher 344 strain rats were subjected to hippocampal kindling as part of a project concerned with alterations in neuronal plasticity during ageing. Stimulating electrodes were placed in the perforant path and recording electrodes in the ipsilateral dentate gyrus and on the cortical surface bilaterally. Depth electrode placement was accomplished using stereotaxic coordinates initially and then correcting until single pulse stimulation of the perforant path yielded a "population spike" (Lømo, Exp. Brain Res., 12: 18-45, 1971) of dentate gyrus granule cells. Animals were kindled with either once daily or twice daily stimulations with parameters (60 Hz, 2 sec.) and current levels sufficient for production of an after-discharge (AD) under 10 sec. In addition to the usual augmentation of AD duration, we observed the development of spontaneous epileptiform potentials arising primarily in the dentate electrode and unaccompanied by clinical signs. Such observations confirm the findings of many other workers.

We now report a further, previously undescribed phenomenon. Beginning usually after the seventh stimulation, often after a delay of ten or more min. following the AD, electrographic seizures were seen having an origin in the region of pyriform or entorhinal cortex. Within 2-3days, these electrical discharges were associated with clinical attacks which usually consisted of behavioral arrest, rhythmic head shaking, and clonic jaw movement. The electrographic seizure grew in frequency and duration as stimulations continued. Eventually, these discharges occurred throughout the day, were unrelated to stimulation times, and were often seen prior to the first stimulation of the day. All the criteria of true spontaneous epilepsy were fulfilled. Records obtained simultaneously from the stimulating electrodes revealed frequent triggering by cortical ictal spikes. The time relationships were such as to raise the possibility that antidromic activation of cortical neurons may play a role in this form of epileptogenesis.

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8.10 Are the Epileptogenic Effects of γ-Hydroxybutyrate Secondary to its Dopaminergic Actions? O. Carter Snead, III. Dept. Pediatrics and The Neuroscience Program, Univ. Alabama in Birmingham Sch. Med. B'ham.

γ-hydroxybutyrate (GHB) is a naturally occurring substance which produces profound EEG and behavioral changes as well as elevation of striatal dopamine when administered to animals. The evidence for a cause-effect relationship between the dopaminergic and the EEG and behavioral actions of GHB is contradictory (Snead, Neurology 28:1179, 1973). The GHB-induced EEG changes represent a model for petit mal epilepsy, hence such a cause-effect relationship would have important ramifications for this disorder.

Using γ-butyrolactone (GBL) the pro-drug of GHB, we investigated the relationship of the dopaminergic to the EEG effects of this compound in a number of ways. Rats were implanted with permanent epidural cortical electrodes to permit recording of the electroencephalogram (EEG), which was quantitated by a computerized frequency analysis system. A flurometric assay was used to determine striatal dopamine concentration. Dose response curves for the dopaminergic and EEG effects of GBL were compared as was the time course of each action of the drug. Animals were also sacrificed at various time points during evolution of specific changes induced in the EEG by GBL and striatal dopamine determined. In addition the effect of ethosuximide, trimethadione, and sodium valproate, drugs known to abort GHB-induced EEG abnormalities (Snead et al, Neuropharmacology 19:47, 1980), on the GBL-induced elevation of striatal dopamine was determined.

The GBL-induced EEG changes occurred at lower doses, had an earlier onset and lasted longer than the GBL-induced elevation in striatal dopamine concentration. There were marked sequential changes in EEG and behavior in the face of normal striatal dopamine concentrations in animals administered 400 mg/kg GBL. Ethsuximide, sodium valproate and trimethadione were all effective in aborting or decreasing the rise in striatal dopamine concentration produced by GBL. We conclude that the elevation in brain dopamine produced by GBL is not responsible for the EEG or behavioral effects seen with this substance.

8.11 EXTRACELLULAR SEROTONIN INHIBITS ELECTRICALLY STIMULATED SEROTONIN RELEASE FROM MOUSE CEREBELLUM SLICES. H.R. Figueroa*, P.B. Yurgens* and T.R. Hall*. (SPON: D. Czech). Dept. of Biology, Marquette University, Milwaukee, WI 53233.

Mouse cerebellum slices, preincubated with ^{3}H-5 hydroxytryptamine (5HT, serotonin), were superfused in a lucite chamber with a physiological solution of Krebs-Ringer-Phosphate (KRP) at a flow rate of 4 ml/min. Fractions of 1 or 4 min of the superfusate were collected throughout the whole experiment. After the first 22 minutes of superfusion the slices received a first electrical stimulus (S_1) with square monophasic pulses of 2 ms duration, 50 HZ and 100mA, for 1 minute. After S_1 the slices were superfused for a further 22 minutes with either KRP alone or with KRP containing 1 μM 5HT, 1 μM 5HT plus 20 μM Methysergide or 1 μM 5HT plus 10 μM Methiothepin. Then the slices received a second stimulus S_2 identical to S_1. At the end of the superfusion the slices were solubilized and the radioactivity in the slices and superfusate samples was determined by liquid scintillation counting. Fractional ^{3}H release was calculated for S_1 and S_2. S_2/S_1 fractional release ratio was found to decrease significantly ($p<.001$) in the presence of 1 μM extracellular 5HT, with respect to the control group. The presence of 20 μM methysergide did not alter the effect of 1 μM 5HT on tritium release. However the simultaneous administration of 10 μM Methiothepin antagonized the inhibitory effect of 5HT, ($p<0.05$). A first order kinetic analysis of basal tritium efflux from slices showed that the efflux rate constant was significantly more negative ($p<0.02$) during the superfusion with 1 μM 5HT compared with that obtained with KRP alone, indicating that extracellular 5HT increases the basal, unstimulated cellular 5HT efflux. These results suggest that 5HT modulates its own release from central serotoninergic neurons via a negative feedback mechanism, probably mediated through the activation of presynaptic 5HT autoreceptors.
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8.12 NERVE FIBER DEGENERATION IN THE BRAIN OF RATS FOLLOWING A SINGLE CEREBRAL CONCUSSION. L. C. Parsons, M. Skinner*, B. Fitzgerald*, and M. Guthrie. Physiology Lab., Univ. of Va., School(s) of Nursing and Medicine, Charlottesville, VA 22903.

Adult laboratory rats of both sexes, between the ages of 40 and 75 days and weighing between 235 and 375g. were used for the purpose of initiating an acceleration cerebral concussion (ACC). Single measured blows varying between 26 and 40 PSI were delivered to the reinforced calvarium of each rat by use of a calibrated, rigidly fixed spring coil concussion gun. The severity and duration of each ACC were measured using the behavioral manifestations of length of convulsive seizure(s), loss of corneal reflex and loss of voluntary motor reflexes as criteria. These data were then correlated with morphological changes as demonstrated through use of the silver staining technique of Fink-Heimer II, a method used to show nerve fiber and terminal degeneration in the cerebrum, cerebellum and brainstem. The extent of whole brain injury as analyzed in a rostral to caudal manner provided evidence and verification for three sites of injury resulting from a single ACC. Nerve fiber and terminal degeneration were closely correlated with injuries likely to have been received at coup, contrecoup and craniocervical junctional sites in this rat model. The extent of nerve fiber degeneration associated with coup injuries involved structures of the corticospinal tract, and intrinsic nerve fibers of the cerebellum, while injury at the contrecoup site showed nerve fiber degeneration in the optic nerve pathways and selected forebrain structures. Widespread nerve fiber degeneration was shown in fiber tracts traversing the medullary, pontine and mesencephalic brainstem and suggested an injury occurring at the craniocervical junction which resulted from the rapid and extreme flexion of the neck as a consequence of the ACC. The severity and duration of the ACC were correlated positively with the extent of injury as measured by number of neuroanatomical structures showing nerve fiber and terminal degeneration.

8.13 HIPPOCAMPAL POTENTIATION IN VITRO: EFFECTS OF HIGH Mg-LOW Ca MEDIA AND OF DURATION OF INCUBATION. Philip W. Landfield, Dept. Physiol. Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103

Substantial data indicate that facilitation (potentiation) in peripheral systems is due to increased transmitter release, and depression to transmitter depletion. Elevating the extracellular $(Mg^{++})/(Ca^{++})$ ratio can increase facilitation during or after repetitive stimulation (stim.) by retarding transmitter depletion. Ca^{++} appears necessary both for facilitation in peripheral systems and for hippocampal long-term potentiation (LTP). However, it has been questioned whether hippocampal frequency potentiation (FP) and LTP depend on mechanisms similar to those in the periphery. This study examines whether Mg/Ca balance affects hippocampal potentiation in a manner analogous to its effects on peripheral facilitation. Data from 37 hippocampal slices from young-mature rats are described for CA1 population spike amplitudes during and after stimulation of stratum radiatum afferents. Slices were simultaneously incubated in 2 recording chambers containing either "normal" medium (Mg/Ca ratio of 1.3/2.4 (mM)) or high Mg/Ca ratio media (ranging from 2.7/1.0 to 4.0/2.4 (mM); osmotic balance maintained by altering NaCl). Paradigms for each slice were as follows: Continuous stim. at 6 Hz for 4 min (FP); 100 Hz stim. for 5 sec; 0.2 Hz stim. for 15 min (LTP assessment); 6 Hz stim. for 4 min. During 6 Hz stim. spike amplitude was measured every 15 sec. In slices studied 1-3 hrs after cutting, high Mg/Ca ratios moderately increased the percent of FP and sometimes of LTP, in comparison to slices from the same animal in normal medium. Threshold was elevated and control responses at 125% of threshold were reduced, so that absolute FP or LTP were not increased. LTP was sometimes completely blocked. However, in many slices incubated in elevated Mg and low Ca for 4 hr or more, striking increases in FP and LTP were seen (in comparison to normal medium slices), both in terms of absolute (e.g., 6-10 mV vs 2-3 mV) and percentage (e.g., 400-500% vs 50-100%) values. Persistence of FP was also increased. Fiber spike potentials were largely unchanged. Field EPSPs are still under study. Incubation for 4-6 hrs in all media lowered thresholds and increased control spike amplitudes, but this effect was not correlated with enhanced potentiation.

Dependence of the effect of elevated Mg/Ca ratios on duration of incubation may be due to several factors, including the intracellular accumulation of transmitter or of a minimum Ca level. Nevertheless, dramatic absolute value increases in potentiation were induced by an ionic manipulation which presumably increases transmitter stores. This suggests that enhanced transmitter release is an important, but not necessarily exclusive, factor in both FP and LTP in hippocampus. (Supported by AG01737).

9.1 SACCADES SERVE AS MARKERS FOR VISUAL INFORMATION. C.Torda
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Visual stimuli activate specific tunable photoreceptors located in the retina. Through automatically ongoing parallel processing the photic energy is transformed to spatial frequency codes. Coding is a gradual process whereby the chunks of photic information are substituted by parsimonious and efficient representations that permit to eliminate redundancies. During the passage from retina to higher CNS centers, the visual information undergoes repeated phase-coding (an analytical process) performed through generation of graded slow potentials. The prime function of the dendritic trees is weighted summation in the wave mode. The waves generate pulses at the dendritic and/or somatic trigger zones. The neuron is an unidirectional integrator and transmitter.It transmits the pulses to higher CNS centers where further analysis and synthesis occur under the organizing influence of decision making control systems and regulators (i.e. the hippocampus) before the information becomes part of the long-term memory system.--Concurrently with this coding process the visual stimuli also initiate a saccade (rapid eye movement) with a few msec delay. This saccadic system is a sampled data positional servomechanism. The activity necessary to produce saccades is coded in the firing rates of phasic units located in the rostral zone of the paramedian pontine reticular formation(PPRF). By integration of the phasic pulses from the PPRF,the periabducens neurons generate the tonic component of the saccades.

The here presented research addressed the possibility that the saccades contain a stimulus specific marker system that serves to facilitate allocation of the codes of the original visual stimulus during the process of coding, transformation, elaboration,storage and recall.Saccades seem to be excellently suited to serve as markers:(1) they are generated concurrently with the onset of the visual process;(2)the codes for saccades are simpler and the process is faster;(3)because of simplicity and speed they are suited fortracing and allocating codes; (4)the dual type of processing and transmission to higher CNS centers has already been demonstrated for the rapid eye movement contained in the PGO waves(Torda,Int.J.Neurosciences, 9:455 & 10:230,1979;Psychobiology of Memory & Dreams,Walters,1980). Bioelectric processes were extra-and intracellularly recorded from cat and rat from the rostral PPRF,the III & VI nuclei, the hippocampus,the visual-and infratemporal cortices.A technique has been developped that permits statistically valid comparison of the recall of a specifically designed visual stimulus. Comparison of results obtained from sham-operated animals and those with surgically and/or chemically eliminated rostral PPRF zone led to the conclusion that the saccade contains a marker of the original visual stimulus. In absence of this marker storage and recall is significantly delayed and is defective. By application of some lateralization techniques,human clinical data were obtained that supported the conslusions.

9.3 PULSE WIDTH NOT PULSE HEIGHT ERRORS CAUSE GLISSADES. A. Terry Bahill. Department of Electrical Engineering, Carnegie-Mellon University, Pittsburgh, PA 15213.

Saccadic eye movements with glissadic overshoot can be simulated by increasing either the pulse width (PW), or the pulse height (PH) of the motoneuronal controller signal. However, increasing the pulse height increases the peak velocity, whereas, increasing the pulse width does not. Human saccadic eye movements with glissadic overshoot have normal or less than normal peak velocities, suggesting that glissadic overshoots are caused by pulse width errors (Bahill, Hsu, and Stark 1978).

Our sensitivity analysis explains why this is so by showing that eye position is not sensitive to pulse width variations until near the end of the saccade, after the point of peak velocity. So, pulse width changes affect the size of the saccade, but not its peak velocity. On the other hand, eye position is sensitive to pulse height variations throughout the saccade. Therefore, pulse height changes do affect the peak velocity.

Our function minimization programs show that there must be variations in the pulse width rather than in pulse height in order to minimize the mean squared error between human and model saccadic eye movements with glissadic undershoot or glissadic overshoot. This was true for normal subjects as well as for patients with multiple sclerosis. When the parameter estimation routine was allowed to vary the pulse width and the pulse height, the only two physiological parameters that are likely to change between saccades, the best fit for a typical movement with glissadic undershoot, was obtained by decreasing the pulse width 16% below its nominal value while decreasing the pulse height 2.5% below its nominal value. Making these changes from the nominal values reduced the mean squared error by a factor of ten. When the estimation program was allowed to vary pulse width, pulse height and the four time constants, the mean squared error decreased by only 3%.

This means that the central nervous system makes more pulse width errors than pulse height errors. It must be harder to control the duration of a motoneuronal burst than the firing frequency and number of motoneurons recruited.

9.2 SACCADIC EYE MOVEMENT CHARACTERISTICS - AN AUTOMATED, LARGE DATABASE STUDY. L. A. ABEL* and B. TODD TROOST. Neurol. Control Systems Lab., Univ. Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Most studies of the characteristics of normal human saccadic eye movements, have been performed on relatively restricted groups (1 - 25 subjects), have taken limited data from each subject (< 100 refixations), or have neglected the effects of age (subjects < 50). The present report describes the use of a microcomputer-based system for real time stimulus presentation and data collection. Large amounts of eye movement information can be immediately analyzed with results graphically displayed. We have attempted to cover a broad age range to delineate normal saccadic characteristics for the general population.

Fifty normal subjects ranging in age from 18 to 75 years viewed an array of light-emitting diodes driven by Digital Equipment Corp. MINC laboratory computer. Saccades of 3 deg to 30 deg were elicited by programmed LED switching. Target amplitude and direction were randomized to minimize anticipation. A total of 280 saccades were obtained from each subject. Eye movements were recorded using infrared reflectance with a vertical EOG channel to detect eye blinks. The two eye movement position channels and the blink trace were digitized at 200 Hz; if a blink occurred during the saccade, it was not stored and the trial was automatically repeated. Verbal encouragement was used to maintain subject alertness and the analog records were visually monitored for signs or fatigue or loss of calibration in which case the data was rejected. Thus, only noncontaminated data was stored for analysis. The entire process, including calibration, took less than 30 minutes per subject.

Data were stored on floppy disk and analyzed off-line by the microprocessor. Saccadic amplitude, velocity, duration, and latency for each eye in both horizontal directions were calculated and stored in disk files for each subject. This enabled us to plot results for individual subjects and to produce cumulative results for each age cohort and for the overall population. The relationship between saccadic amplitude and velocity showed considerable intersubject variability, even within a given age group. Intrasubject variability followed characteristic patterns for individual subjects: some showed little data scatter, others much more. Repeat trials with several subjects showed these characteristics to be reproducible. Results shown include examples of individual subject's saccadic characteristics and the cumulative results for different age cohorts. The study is continuing for further definition of normal characteristics.

9.4 THE VELOCITY OF SACCADIC EYE MOVEMENTS IN REM SLEEP AND THE AWAKE STATE. John H. Herman, Howard P. Roffwarg, Dept. of Psychiatry, David R. Barker*, Medical Computing, Southwestern Medical School, University of Texas Health Science Center, Dallas, Texas 75235.

The characteristics of saccadic eye movements have been reported to be vastly different in REM sleep as compared to the awake state in terms of velocity (Fuchs and Ron, 1968), patterning (Aserinsky, 1971), and prevalence of rotatory or oblique components (Jacobs, Feldman, and Bender, 1971). None of these reports included optimal awake state controls for REM sleep eye movements.

We studied the properties of saccades in three subjects in an auditory orienting task under the following conditions: (1) normal lighting, eyes open; (2) total darkness, eyes open; and (3) total darkness, eyes closed. These conditions were performed both when the head was free and when it was rigidly immobilized. Condition order was counterbalanced. Eye movements during awake state reenactments of dream scenes were compared to REM state saccadic activity. On-line computer analysis of horizontal electroculographic (EOG) signals, utilizing a 5-msec sampling rate and a visually validated 8-sample algorithm, provided peak velocity-amplitude relationships. Calibrations of the EOG were performed in the light and in the dark to permit compensation for the changing amplitude of the corneo-retinal potential.

Analysis of variance of the equality of slopes demonstrated that awake state eye movements in normal lighting were of a significantly greater velocity than those of similar amplitude performed with eyes open in the dark. Saccades in the dark with eyes open were, in turn, significantly more rapid than those executed awake with eyes closed. Head restraint increased all velocities. The velocity of REM sleep saccades was not significantly different from awake state, closed eye saccades, though their variability was significantly greater. Slow, rolling, oscillatory changes in eye position were continually present in REM sleep, but were observed only infrequently in head unrestrained, awake state conditions. The so-called "torsional" and oblique eye movements seen in REM sleep were observed in the awake state when the head was unrestrained. Visual reenactment of shifts of gaze in REM sleep dream scenes, while the subjects lay recumbent with eyes closed, yielded eye movement patterns that were similar to the REM sleep EOG.

The attenuated velocity of saccades in the absence of a visual target indicates that actual visual factors play an important role in oculomotor control. Secondly, the serial decrement whereby the velocities of waking eye movements, when eyes are closed, become similar to those of REM sleep suggests that the physical characteristics of the closed eyelid, or its effect upon neuromuscular mechanisms, plays a more important role in affecting saccadic velocities than does state of consciousness.

9.5 CEREBELLAR MODULATION OF AN INTRINSIC SACCADIC COUPLER DURING VISUAL FIXATION. J. R. Hotson, M.D. Department of Neurology, Stanford Univ. Sch. of Med., Palo Alto, CA 95070.

During visual fixation on a small target it is well known that small saccades and slow drift eye movements occur continuously. These fixation eye movements are often smaller than 10 min arc. Recently it has been shown in normal subjects that horizontal, but not vertical, fixation saccades larger than 10 to 15 min arc may couple spontaneously and form square waves or saccadic oscillations (J. Hotson, Neurosci. Abstr. #1249, 1979). The mean duration between these coupled saccades is less than 200 msec and can be as brief as 50 msec. Horizontially coupled saccades with no intersaccade interval also occur in normal subjects and are called flutter movements.

These observations led to the proposal that an intrinsic saccade coupling mechanism functions in normal subjects during visual fixation and that it is activated by particularly large fixation saccades. Therefore if a disorder of supranuclear oculomotor control systems, such as the cerebellum, causes an enlargement of fixation saccades, it should also increase the frequency of saccade coupling. To test this postulate fixation eye movements as small as 1 min arc were recorded with a purkinje image eyetracker in four human subjects with pan-cerebellar disorders and compared to control subjects.

In subjects with cerebellar disorders the mean amplitude of fixation saccades and velocity of slow drift was significantly increased ($p<0.008$) compared to control subjects. However the frequency of horizontal, but not vertical, fixation saccades was less ($p<0.03$) in the cerebellar group. In contrast, both the amplitude ($p<0.009$) and frequency ($p<0.04$) of square waves and the frequency of saccadic oscillation ($p<0.04$) were increased in cerebellar subjects when compared to controls. Vertical square waves and saccadic oscillations did not occur in cerebellar subjects and flutter movements did not happen with greater frequency.

These results indicate that disorders of the cerebellum may limit the ability to reduce the size of fixation saccades below 10 min arc and thereby increase the activation of a horizontal saccade coupler. A decreased frequency of uncoupled saccades and an increased frequency of square waves and saccadic oscillations results. The frequency of flutter, however, was not increased by the loss of cerebellar control. Flutter eye movements, therefore, appear to be activated by a different mechanism than square waves.

9.6 DISCHARGE OF SUBSTANTIA NIGRA NEURONS DECREASES BEFORE VISUALLY-GUIDED SACCADES. O. Hikosaka* and R. H. Wurtz. Lab. of Sensorimotor Research, NEI, NIH, Bethesda, MD 20205.

Recent anatomical evidence has shown that the pars reticulata of the substantia nigra projects to the layers of the superior colliculus where cells discharge before saccadic eye movements. In order to determine the relation of pars reticulata neurons to the initiation of saccadic eye movements, we recorded the discharge of single cells as rhesus monkeys made saccades to visual targets. The location of cells was histologically verified.

Pars reticulata neurons had a steady rapid discharge rate usually averaging between 40 and 100 spikes per second. We found at least two types of cells related to saccades both of which decreased their discharge rate before onset of saccades.

In the first type of cell, a decrease in the discharge rate occurred in closer temporal relation to the onset of the saccade than to the onset of the visual target. The discharge rate usually decreased about 100 msec before and continued for 100 msec after a saccade. These cells have limited movement fields with the most pronounced decrease in activity usually associated with saccades to the contralateral visual field.

The second type of cell showed a decrease in discharge rate better correlated to the onset of the visual target than the saccade. They showed a decrease in discharge rate to the spot of light even when the monkey did not make a saccade to the target. These cells have visual receptive fields usually also primarily on the contralateral side and have a latency of 70-140 msec.

Some of these visual cells showed a more pronounced suppression of their discharge rate when the visual stimulus was the target for a saccadic eye movement. This enhancement effect has been reported for cells in superior colliculus and frontal eye fields, but these pars reticulata neurons showed a modulation not seen in other structures: when the monkey made a saccade to a target outside of the visual receptive field, the suppression effect of the stimulus in the receptive field was reduced.

The anatomical connections and temporal relation of cell discharge to saccades suggest that some cells in pars reticulata of the substantia nigra are possible precursors of the saccade-related cells in the superior colliculus and are involved in the initiation of visually-guided saccadic eye movements.

9.7 INFORMATION IN THE MESENCEPHALIC RETICULAR FORMATION (MRF) RESPONSIBLE FOR HORIZONTAL VISUALLY GUIDED OR VISUALLY INDUCED RAPID EYE MOVEMENTS. V. Matsuo*, B. Cohen, T. Raphan, J. Fradin* and D. Dennett*. Dept. of Neurology, Mount Sinai School of Medicine, CUNY, New York, New York 10029.

The MRF appears to be an important region for processing activity that originates in the visual system and reaches the paramedian zone of the pontine reticular formation (PPRF) to induce horizontal saccades and quick phases of nystagmus. Microstimulation of the MRF in alert monkeys elicited only rapid eye movements. This indicates that this region is associated with production of saccades and quick phases of nystagmus, not slow phases or pursuit movements. If stimulating currents and frequencies were above threshold (8-10 μA and 100-125 Hz), the size of the induced eye movements was dependent only on the region of the MRF that was stimulated. Fixed-amplitude saccades were elicited from dorsal portions of the MRF. Deeper in the MRF saccades or quick phases were evoked whose size depended on initial eye position, being larger from positions on the side of stimulation. At dorsal loci, a constant number of pulses was required to trigger saccades, causing the latency of the induced movements to be longer at lower frequencies of stimulation. Stimulation at frequencies below threshold affected the excitability of the mechanism that generated rapid eye movements in the PPRF. OKN quick phases were either facilitated or suppressed by MRF stimulation, depending on the direction of the quick phases. The frequencies necessary for quick phase suppression increased with slow phase velocity. This indicates that there are neurons in the MRF whose activity is related to the velocity of the eyes during slow phases. The data suggest that neurons in the MRF act to trigger saccade generating networks in the pons. Above threshold the amplitude of the rapid movements that are induced is dependent on the region of the MRF that is active rather than on the parameters of stimulation. A similar dependence of size and direction of eye movement on locus of stimulation is found in the superior colliculus and frontal eye fields. This is in contrast to the PPRF where there is a direct relationship between parameters of unit activity or of stimulation frequency, and size of rapid eye movements that are induced. It suggests that activity in descending visual-oculomotor areas is organized into channels that trigger saccades of specific sizes. Moreover it would appear that a transformation from a spatial code to a frequency code characteristically takes place between suprapontine pathways and the PPRF to induce rapid eye movements in the horizontal plane.

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9.8 INHIBITORY BURST NEURONS IN THE ALERT CAT. C.R.S. Kaneko and A.F. Fuchs. Regional Primate Research Center and Dept. Physiol. and Biophys., Sch. Med., Univ. Washington, Seattle, WA 98195.

Neurons in the pontine and medullary reticular formations of the cat exhibit a burst of action potentials for saccadic eye movements. Recent studies have suggested that many of the pontine burst neurons (EBNs) provide monosynaptic excitatory connections to abducens motoneurons while the medullary burst neurons (IBNs) provide monosynaptic inhibition. To investigate whether EBNs and IBNs play different functional roles in the generation of saccades, we have recorded from and stimulated in the region containing IBNs in alert cats trained in visual tracking tasks. The quantitative and correlative analyses of IBN discharge characteristics in relation to saccadic eye movements and eye movements during electrical stimulation are reported here.

In most of their discharge properties, IBNs are similar to EBNs. IBNs discharge only with saccades and are otherwise silent. Saccades in a preferred ("on") direction are accompanied by the greatest maximum average discharge frequnecy although IBNs discharge over a broad range of saccade directions. On-directions are always ipsilateral to the recording site. Latencies from the burst onset to the onset of an on-direction saccade are typically 10 to 20 msec. In contrast to EBNs, during on-direction saccades, IBNs discharge at higher peak and average firing rates, have bursts of longer duration and consequently also have a greater number of action potentials within a burst than do EBNs. For example, IBNs have maximum on-direction average discharge rates of about 190 spikes/sec compared with 120 spikes/sec for EBNs.

Preliminary statistical analyses suggest that IBNs, like EBNs, show the highest correlations between average discharge frequency and number of action potentials in the burst on the one hand and the size and maximum velocity of the on-direction component of the saccade on the other. However, unlike EBNs, IBNs have high correlations between burst duration and the duration of the on-direction component of the saccade.

Stimulation through the recording microelectrode results in an assymetric saccade interruption. For the contralateral eye, saccades in the contralateral direction are most profoundly effected. Within as little as 10-15 msec after a stimulus train (30 msec,30μA),contralateral saccades of the contralateral eye show a decrease in velocity which often reaches zero. On average, contralateral saccades during stimulation exhibit lower peak velocities and longer durations. During ipsilateral saccades of the contralateral eye stimulation produces only a modest (if any) decrease in velocity at a much longer latency (20msec). During stimulation, ipsilateral saccades of the ipsilateral eye are relatively normal whereas contralateral saccades are interrupted. The most likely pathway for mediating this effect is via internuclear neurons. Thus IBNs probably inhibit both contralateral abducens motoneurons and internuclear neurons.

9.9 INTRACELLULAR INJECTION OF HRP INTO OMNIPAUSE NEURONS IN THE ALERT CAT. C. Evinger, R.A. McCrea & R. Baker, Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016

Extracellular recordings from alert cats have shown that omnipause neurons are tonically active cells which cease firing for all saccades regardless of either direction or amplitude for a period proportional to saccade duration. Their frequency is not modulated during optokinetic and/or vestibular induced eye movement. Since microstimulation in the omnipause area of the brainstem prevents the occurrence of saccades, but does not interrupt smooth eye movements, it was suggested that omnipause neurons inhibit neurons that exhibit a burst of spikes during saccades. In spite of the wealth of physiological data concerning the activity of these neurons little is known about their axonal trajectory and termination areas. To examine this question we identified omnipause neurons according to the above physiological criterion in the alert cat and then intracellularly injected HRP. The cell bodies of these neurons (30-40 μm) were located within .2 mm of the midline rostral to the abducens nucleus even though the dendrites extended a considerable distance from the soma (up to 1 mm). The parent axons bifurcated near the soma and sent branches to both sides of the brainstem. These branches gave off many smaller collaterals with en passant and terminal boutons in the pontine reticular formation. These branches further gave rise to large collaterals which ascended and descended throughout the brainstem. The caudal-going branches passed medial to the VIth nerve rootlets and terminated in the medullary reticular formation between the abducens nucleus and MLF as well as more posteriorly in the dorso-medial reticular region. The axons could be followed caudally to the level of the inferior olive. The rostral ascending branches gave rise to terminal collaterals in the pontine reticular formation up to the level of the Nucleus reticularis tegmenti pontis and continued rostrally to the level of the trochlear nucleus where they could no longer be traced. In the pontine and medullary reticular formation the termination zones were in those areas known to contain excitatory and inhibitory burst neurons which had been identified by HRP staining in other alert cats. The combined anatomy and physiology of omnipause neurons strongly supports prior suggestions that these neurons specifically gate burst neurons involved in both horizontal and vertical saccades. It is interesting to note the lack of evidence for omnipause neuron collateralization in the vestibular and perihypoglossal nuclei or any brainstem area not linked in some fashion with eye movement. Therefore, we suggest that omnipause neurons are not only specific for oculomotor function, but also are uniquely involved with saccadic eye movement. Supported by NS-13742 and EY-02007.

9.10 ACTIVITY OF IDENTIFIED MOTONEURONS IN THE ABDUCENS AND ACCESSORY ABDUCENS NUCLEUS OF THE ALERT CAT DURING EYE MOVEMENT AND RETRACTION. J. Delgado-Garcia*, C. Evinger and R. Baker. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York 10016.

In the cat, motoneurons from both the abducens (Abd) and accessory (Ac) Abd nucleus innervate the retractor bulbi muscle which retracts the eye into the orbit causing the nictitating membrane to sweep up over the globe. Retraction most often occurs reflexly in response to mechanical stimuli applied directly to either the eye or the periorbital region. Prior electrophysiological studies suggested that all motoneurons in the Ac Abd nucleus received strong disynaptic excitation from trigeminal activation, especially the cornea, but little or no response to vestibular nerve stimulation. Exactly the opposite synaptic effects were found in motoneurons of the Abd nucleus. We investigated the hypothesis that motoneurons of the Ac Abd nucleus would only be active during retraction and exhibit little, if any, activity during rotational eye movement and that motoneurons in the Abd nucleus would respond in the opposite fashion. Extracellular recordings were made from antidromically identified motoneurons in the Abd and Ac Abd nucleus of alert cats equipped with two scleral eye coils, one measuring rotational eye movement and the other retraction. Bursts of spikes were produced in every Ac Abd motoneuron from 5-10 msec after onset of the air puff and approximately 6 msec prior to eye retraction. Ac Abd motoneurons were silent during all classes of rotational eye movement (saccadic, vestibular, etc.). Although tonic activity has not yet been observed in any Ac Abd motoneuron, repetitive dendritic spike activity as well as a remarkable enhancement in active dendritic responses coincident with subthreshold adequate stimulation has been observed. In contrast, all antidromically identified Abd motoneurons (and internuclear neurons in the Abd nucleus) exhibited a burst and/or pause in activity both preceding and during saccades as well as a tonic activity proportional to eye position. When the air puff stimulus was tested in motoneurons and internuclear neurons of the Abd nucleus, both exhibited weak, but consistent, responses; yet they did not occur until at least 5 msec after the onset of retraction. Given the latency of the responses and ability to condition its occurrence with an auditory stimulus, we conclude that all neurons in the Abd and Ac Abd nuclei may participate in general 'alerting' responses associated with eye retraction and blinking during withdrawal behavior; however, Ac Abd motoneurons are active prior to, and during, 'reflex' and 'alerting' retractions. Since Ac Abd motoneurons were not modulated during any rotational eye movements, we also conclude that eye movement responses described in retractor bulbi muscle must result from innervation by motoneurons in the Abd nucleus. (Supported by NS13742 and EY02007).

9.11 DIFFERENTIAL LOCALIZATION OF ACETYLCHOLINESTERASE IN CAT ABDUCENS MOTONEURONES AND INTERNUCLEAR NEURONES. R.F. Spencer (SPON: W.I. Rosenblum). Dept. of Anat., Med. Coll. of Virginia, Richmond, VA 23298.

The cat abducens (Abd) nucleus contains coextensive populations of lateral rectus (LR) and retractor bulbi (RB) motoneurones (MNs) and internuclear neurones (INs) that project to the medial rectus (MR) subdivision of the oculomotor (Oc) nucleus. Although the morphological features of Abd INs are quite different from the MNs, both populations of neurones receive the same types of afferent synaptic connections and exhibit qualitatively similar behaviors during eye movement. The extent to which the different ultrastructural features of ABd MNs and INs is related to differences in neurotransmitters utilized by these neurones is unknown.

Cat Abd MNs and INs were labelled by retrograde transport of horseradish peroxidase (HRP). Vibratome sections through the Abd nucleus were incubated either separately or sequentially for the histochemical localization of acetylcholinesterase (AChE) and/or HRP. Electron microscope examination revealed dense AChE reaction product associated the granular endoplasmic reticulum (GER) of MNs. AChE reaction product also was observed extracellularly on the MN soma-dendritic membrane in association with all (e.g., ipsi- and contralateral vestibular) synaptic endings, regardless of synaptic vesicle morphology. In each case, AChE reaction product was absent from sites of presumed synaptic contact indicated by pre- and/or postsynaptic membrane specializations and accumulations of synaptic vesicles along the presynaptic membrane, and from sites of neuroglial appositions and capillary basal laminae.

By contrast, no AChE reaction product was observed within the GER or any other cytoplasmic organelle within Abd INs. AChE reaction product furthermore was absent extracellularly between the IN soma-dendritic membrane and synaptic endings whose morphology was similar to those contacting the MN soma-dendritic membrane.

The intracellular localization of AChE in Abd MNs is consistent with the synthesis of this enzyme within the MN soma with subsequent axonal transport to the neuromuscular junction for the hydrolysis of acetylcholine (ACh) released from the motor end-plate. The absence of AChE from Abd INs thus suggests that ACh is not the neurotransmitter involved in mediating the excitatory IN synaptic effects upon contralateral MR MNs. The extracellular localization of AChE on the MN soma-dendritic membrane, by contrast, may subserve a trophic function upon presynaptic supranuclear neurones. Given the different soma-dendritic architectures of Abd MNs and INs, this may represent one mechanism by which afferents during development selectively establish synaptic connections with the MNs and INs. A similar function could be postulated for the re-establishment of synaptic connections with regenerating MNs following axotomy. (Supported by U.S.P.H.S. Grant EY 02191).

9.12 LOCALIZATION OF THE MOTONEURONS WHICH INNERVATE THE RETRACTOR BULBI MUSCLE IN THE RABBIT. T. S. Gray*, S. E. McMaster*, J. A. Harvey and I. Gormezano. Dept. of Psychol., Univ. of Iowa, Iowa City, IA 52242

Recent studies employing the horseradish peroxidase (HRP) method have suggested that the retractor bulbi muscle (RBM) of the cat may be innervated by the paraabducens nucleus (PAbN) rather than the abducens nucleus (AbN). Since the rabbit's nictitating membrane reflex, a response mediated by contraction of the RBM, has been used extensively in neurobehavioral research, it was important to determine the precise location of the motoneurons which supply this muscle.

Adult, New Zealand, albino rabbits (n=24) were injected with a 50% HRP and 2% dimethyl sulfoxide solution (5μl per muscle slip) into the following extraoccular muscles: one or four slips of RBM, lateral rectus (LRM), medial rectus, superior rectus, or superior oblique. After 48 hour survival times, 60μm sections of the brainstem were processed by the tetramethyl benzadine method.

Injections of HRP into all four slips of the RBM resulted in consistent labeling of neurons throughout the rostrocaudal extent of the ipsilateral AbN. Inconsistent labeling was seen in the occulomotor, trochlear and facial nuclei. However, a group of labeled neurons were observed ventrolateral to and extending rostral to the ipsilateral AbN. Since the axons of these neurons were seen to course dorsomedially to join the VI nerve, we concluded that this group of motoneurons is the homologue of the PAbN reported in the cat. The mean number of labeled RBM motoneurons in the PAbN was 100 as compared with 290 in the AbN. Reactive neurons within the PAbN and AbN were of the same size and shape averaging 30μm in diameter. Similar results to above were seen after single medial or superior slip RBM HRP injections. After LRM injections neurons were labeled throughout the rostrocaudal extent of the ipsilateral AbN. Labeled neurons were never observed in the PAbN; however, reactive cells were sometimes present in the occulomotor, trochlear or facial nuclei. The per cent of AbN neurons labeled after LRM injections was 33 as compared with 70 after four slip, 59 after medial slip and 35 after superior slip RBM injections. HRP placements into the medial rectus, superior rectus or superior oblique muscles resulted in reliable labeling of areas in the occulomotor-trochlear complex as previously described for the rabbit, but the PAbN and AbN were free of reaction product.

The results demonstrate that the retractor bulbi muscle in the rabbit is innervated by motoneurons located in both the paraabducens and abducens nuclei. Thus, the PAbN projects only to the RBM while the AbN sends axons to the RBM and LRM.

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10.1 BUFFERING OF INTRACELLULAR CALCIUM IN MOLLUSCAN NEURONS. Z. Ahmed*, F. Jung* and J. A. Connor. Dept. of Physiology and Biophysics, Univ. of IL, Urbana, IL 61801.

The relationship between calcium influx and increase in cytoplasmic free calcium, $\Delta[Ca_I^{2+}]$, has been examined using the intracellular Ca-indicator Arsenazo III (Ar) and voltage clamp membrane current. Comparisons of the incoming Ca during voltage pulses estimated from the absorbance change of Ar with the amount of entering calcium computed from I_{Ca}^{2+} indicate that only 15 - 30% of the incoming Ca is sensed by Ar. The remaining fraction is presumably bound extremely rapidly by cytoplasmic buffers. Cytoplasmic Ca-buffer capacity (B C_{cell}) during a voltage clamp pulse was measured by injecting calibrated amounts of EGTA and using the relation B C_{cell} = [B C_E/($\frac{\Delta pCa'}{\Delta pCa''}$ - 1)] - B C_{Ar}, where B C_E and B C_{Ar} are buffer capacities of measured amounts of EGTA and Ar respectively. $\Delta pCa'$ and $\Delta pCa''$ were calculated from the absorbance changes of Ar before and after injection of EGTA. The rate of recovery of Ca^{2+} (rate of Ar absorbance decrease) following the termination of a voltage pulse was examined with different Ca loads induced by changing either the duration or amplitude of the voltage pulse. For larger loads of Ca (within physiological limits) the initial rate of Ca^{2+}-recovery increased, suggesting a recruitment of slower buffering or sequestering processes. Injecting small quantities of EGTA accelerated the rate of absorbance recovery in addition to reducing peak absorbance change for a given calcium load. A computational model based upon calcium diffusion and experimental measurements of Ca-buffer capacity was developed. This model reproduces several important aspects of the Ar absorbance time course and suggests that diffusional movement of Ca within the strongly buffering cytoplasm may play a significant role in the regulation of Ca^{2+} levels. Supported by PHS-NS15186.

10.2 VOLTAGE DEPENDENT, EARLY OUTWARD CURRENT IN A PHOTORECEPTOR OF HERMISSENDA CRASSICORNIS. J.J. Shoukimas* and D.L. Alkon. Lab. of Biophysics, NINCDS, NIH, Marine Biological Laboratory, Woods Hole, MA 02543.

The response of Type B photoreceptors of Hermissenda crassicornis to paired light and rotation stimuli has been implicated in associative training of this animal. A voltage clamp study of the Type B photoreceptor is in progress with the intent of fully characterizing light and voltage dependent conductances. Step clamp commands from a holding potential of -60 mV elicit an early, transient outward current that appears to be carried predominantly by K+ ions. Both steady state activation (peak value) and inactivation of the conductance are voltage dependent. Activation shows a maximum, "e"-fold change/12.5 mV over the range of -20 to +10 mV. The outward current shows inactivation from a relative value of 1 at -70 mV to zero at about -10 mV. -30 mV is the voltage at which the current is half inactivated at steady state. Unlike some other gastropod neurons in which similar currents have been reported, such as Anisodoris (Connor, J.A. and Stevens, C.F., 1971, J. Physiol. 213:21), the kinetics of inactivation, like the steady state value, show voltage dependence. The decline of the outward current during sustained depolarization can be fitted by two exponentials, one with a comparatively short time constant which declines from about 130 msec at -20 mV to about 50 msec at +10 mV. The longer time constant (> 1 sec) may reflect the behavior of another outward conductance.

The early outward current is strongly activated at the time that the light induced sodium current is rising (Alkon, D.L., 1979, Science 205:810). The temporal position of these two currents suggests that possible extrinsic control of resting potential by paired stimulus presentations could influence the rising phase of the generator potential by setting the degree of early outward current inactivation.

10.3 VARIABILITY IN MOLLUSCAN NEURON SOMA CURRENTS. E.E. Serrano* and P. A. Getting. Department of Biological Sciences, Stanford University, Stanford, CA 94305.

This study focuses on the characterization of variability in neuron soma currents between and within identifiable cell types. The transient outward potassium current (A-current) was studied under voltage clamp. This current is particularly suitable for these measurements because it can be activated, by using the appropriate voltage paradigms, in isolation from other membrane currents without the need for pharmacological agents.

Somata from identifiable neurons of Archidoris montereyensis and Anisdoris nobilis were isolated and voltage clamped at 10°C with a two microelectrode voltage clamp. Variability was examined in:

a) magnitude of A-current
b) voltage dependence of steady state activation and inactivation
c) time constant for onset of inactivation (T_{BA})
d) time constant for removal of inactivation (T_{REM})
e) membrane capacitance

Variability in A-current parameters was measured by comparing data from the same identifiable cell in different animals. Comparison between cells within and across species was then possible.

The magnitude of A-current obtained from a given cell in different animals shows a variation of 30% about the mean. Membrane capacitance may vary by ten-fold between cells. The normalized steady state voltage dependence of activation and inactivation is highly conserved. This implies that the steady state voltage dependence of the A-current channel is relatively invariant between cells. The time course of inactivation (as characterized by T_{BA} and T_{REM}) shows considerable variation.

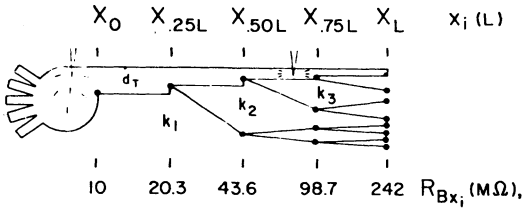
In conclusion, the major variability in A-current appears to reside in the time course of inactivation while the steady state voltage dependence of activation and inactivation is relatively invariant. Current values normalized to input capacitance show as much as eight-fold variation between cells. If the specific capacitance of the cells is assumed to be similar, the data suggests variation in channel density and/or unit channel conductance. Across species, the most striking difference is in the magnitude of A-current which can be from four to eight times larger in Anisdoris than in Archidoris.

10.4 SLOW POTENTIALS IN THE SECONDARY CELLS OF THE APLYSIA EYE. K.J. Markowitz* and J.W. Jacklet. (SPON: J. Schmidt). Dept. of Biol. Sci. and Neurobiology Res. Ctr., SUNY Albany, Albany, N.Y. 12222.

The isolated eye of Aplysia expresses a circadian rhythm of compound action potentials (CAP) frequency recorded in the optic nerve. The CAP's are produced by ~ 1,000 secondary cells which are electrically coupled to one another as well as to the primary photoreceptors. The axons of the secondary cells, photoreceptors and other cell types comprise the optic nerve. To better understand how the circadian clock controls this membrane activity, further studies of the electrophysiological properties of the secondary cells were undertaken. CAP's as well as graded potentials were recorded from the optic nerve with a sucrose gap. A suction electrode on the cornea was used to electrically stimulate the eye and record the electroretinogram (ERG). The ERG closely mirrors light evoked potential changes in the photoreceptors. In the dark spontaneous activity recorded with the sucrose gap consists of bursts of CAP's and underlying slow wave potentials similar to those recorded from identified bursting neurons in the central nervous system. The CAP burst terminates with a depolarizing hump similar to a DAP followed by a post-burst hyperpolarization. The burst duration and the interburst interval are considerably longer than those recorded from single central bursting cells. Electrical stimulation of the cornea evokes a CAP followed by a depolarizing after potential (DAP) in the gap recording. The DAP is not blocked by high Mg^{++} low Ca^{++} indicating that chemical synapses are not involved in its production. The amplitude of the DAP increases as the size of the electrically evoked secondary cell CAP increases. When only the low threshold H cell axons are stimulated, the DAP is absent. This indicates that the DAP is produced in the secondary cells. Similar DAP's were described for central bursting neurons in Aplysia and other gastropods (Thompson and Smith, J. Neurophysiol. 39: 153-161, 1976), and appear to be absent from other cell types. A potential similar to the DAP is triggered by depolarizing input from the receptors. The light evoked slow potential recorded by the sucrose gap is biphasic. An early depolarization is correlated with the ERG and represents receptor depolarization. A latter phase associated with the CAP has no correlate in the ERG and closely resembles the electrically evoked DAP. These results show that the secondary cells have many of the electrophysiological properties exhibited by bursting neurons, including a voltage sensitive slow depolarization. These membrane properties may be modulated by the circadian clock. Supported by NSF BNS 11154.

10.5 EVIDENCE SUPPORTING DENDRITIC IMPALEMENT OF CORTICAL NEURONS IN THE CONSCIOUS CAT BRAIN: CABLE PROPERTIES AND DYE INJECTION. J. E. Skinner and G. L. King*. Neurophysiology Section, Neurology Department and Neuroscience Program Baylor College of Medicine, Houston, Tx 77030.

Frontocortical neurons in the conscious cat brain manifest slow membrane potential and input resistance shifts, in parallel with an extracellular event-related slow potential, in response to a tone that forewarns cutaneous shock. The biophysical responses of each cell were recorded with a new "floating-micropipette tip" technique that enabled intracellular impalements to be maintained when the animal was presented the previously conditioned stimuli. Two classes of cells were recorded, those with large resting mem brane potentials and action potentials that manifested overshoot of the extracellular zero potential (overshoot-cells) and those with small resting potentials and small action potentials that did not overshoot the extracellular zero potential (undershoot-cells) These two classes of cells were also easily distinguished by their resting input resistances. The undershoot-cells are suggested to represent dendritic impalements based on two observations. The first is that electrophoretic injection of small amounts of Lucifer Yellow dye into cells with undershoot-cell properties always results in dye located in a dendritic process near the termination of the microelectrode track in the tissue. The second observation is that 1) input resistance, 2) action potential amplitude, 3) voltage-time integral of the action potential and 4) reduction in action potential amplitude during steady-state current injections through the recording electrode, all are observed in the undershoot-cells to be within the range of values predicted by Rall's mathematical model of the neuron dendrites. The figure below shows the range of values of input resistance predicted by the model that is identical to the range actually recorded. The average input resistance for the overshoot-cells was 10MΩ; the range for the overshoot-cells was 15-98 MΩ.



10.6 SPONTANEOUS HYPERPOLARIZATIONS AT THE MEMBRANE OF MOUSE DORSAL ROOT GANGLION CELLS IN TISSUE CULTURE. David A. Mathers* and Jeffery L. Barker (Sponsor: H.G. Wagner) Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, 20205.

We report here the occurrence of spontaneous hyperpolarizations at the membrane of mouse dorsal root ganglion (DRG) neurons grown in tissue culture.Cells were obtained from 13 day old mouse embryos and maintained for 8-12 weeks in modified Eagle's culture medium.DRG cells were distinguished from co-cultured spinal cord cells by established morphological and electrophysiological criteria. Intracellular recordings were made from DRG cells (18-30μ M in diameter) bathed in Hank's Balanced Salt Solution containing 5.3mM KCL, 142mM NaCl, 1mM $CaCl_2$ and 1mM $MgCl_2$ at pH 7.4 (temperature 23-27°C). DRG cells were invariably electrically excitable but showed no spontaneous action potential activity. Spontaneous hyperpolarizing potentials of amplitude 0.5-6mV and frequency 1-20Hz were observed at the resting potential (-42 ± 6.7 mV) of 34/69 cells studied. In the remaining cells, no resolvable discrete potential events were seen.Voltage clamp recordings showed that the spontaneous outward currents underlying the potential fluctuations were reduced in amplitude and apparent frequency at more negative membrane potentials and were not detectable at potentials in the range -75 to -85mV.These current events were reversibly abolished by 5mM TEA but not by 1μM tetrodotoxin or 10mM Mg^{++} ions. Power density spectra of membrane current fluctuations were compared for DRG cells lacking and possessing the spontaneous outward currents.In cells of the former population, spectral intensity S(f) was generally proportional to 1/f (frequency) over the potential range -40 to -70mV. In cells showing spontaneous activity the power density spectrum was frequently better approximated by a Lorentzian term ($S(f) \propto 1/f^2$) when measured at the normal resting potential (-30 to-50mV). Hyperpolarization of the membrane of these cells led to the suppression of this Lorentzian component concomitant with the loss of the spontaneous outward currents.It remains unclear whether these currents reflect processes exogenous to the DRG cell(e.g. quantal release of a transmitter) or are an endogenous property of some DRG cell membranes.Spontaneous hyperpolarizations have been observed in motoneurons of the mudpuppy cardiac ganglion while spontaneous depolarizations occur at the membrane of several types of photoreceptors.The relationship of the events seen in DRG cells to these phenomena is unknown.

10.7 CALCIUM-DEPENDENT ACTION POTENTIALS IN MAMMALIAN SPINAL CORD AND DORSAL ROOT GANGLION NEURONS IN PRIMARY DISSOCIATED CELL CULTURES. E.J. Heyer, R.L. Macdonald, G.K. Bergey and P.G. Nelson. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48109 and LDN, NICHD, NIH, Bethesda, Maryland 20014.

Action potentials (APs) dependent on sodium and potassium conductance changes are elicited in normal medium in mouse spinal cord (SC) and dorsal root ganglion (DRG) neurons in primary dissociated cell (PDC) cultures. DRG neurons have, in addition, a prominent calcium conductance that contributes to their longer AP duration. No calcium conductance has been described in APs from SC neurons. We have demonstrated a calcium conductance in SC neurons that can produce calcium-dependent action potentials.

PDC cultures of dissected spinal cord from 12-14 day old fetal mice were grown by conventional techniques. After 4-6 weeks, intracellular recordings with 4M KAc-microelectrodes were made in neurons bathed in Tris buffered saline with 5 mM calcium (TBS). Addition of tetraethylammonium (TEA) was compensated by an equal molar decrease in the sodium concentration.

Short duration APs were evoked by intracellular stimulation in SC and DRG neurons in normal TBS. Addition of 3 μM tetrodotoxin (TTX), a sodium conductance blocker, eliminated APs in most SC neurons but did not alter APs in DRG neurons. In bathing medium containing 100 mM TEA, a potassium conductance blocker, long-duration APs lasting many hundreds of milliseconds were generated in both SC and DRG neurons. These APs had three phases: a rapidly rising depolarization; a long duration plateau; and an afterpotential that might be depolarizing, hyperpolarizing, or depolarizing followed by hyperpolarizing. Calcium dependency was proven by the following results: (1) AP overshoot varied linearly as a function of the extracellular calcium concentration $[Ca^{+2}]_0$ (a ten-fold change in $[Ca^{+2}]_0$ produced a 27.5 mV change in the overshoot). (2) Maximum rate of depolarization varied as a function of $[Ca^{+2}]_0$. (3) Pressure ejection of 10 mM calcium onto neurons in low calcium containing bathing medium raised the AP plateau and prolonged its duration. (4) Application of 5 mM manganese, a calcium conductance blocker, lowered the AP plateau and decreased its duration. Supported by the Grass Foundation (R.S. Morison Fellowship) (E.J.H.), NINCDS grant (NS 15225), and NINCDS Research Career Development Award (NS 00408) (R.L.M.).

10.8 DEVELOPMENT OF CALCIUM SPIKES IN MOUSE HIPPOCAMPAL CELL CULTURES. J.H. Peacock and C.R. Walker. Dept. of Neurology, Stanford University Medical School, Stanford, CA 94305.

Intracellular recordings from mature hippocampal cultures in media containing Na^+ (140 mM) and Ca^{++} (8 mM) show well-developed actions potentials (APs) and bursts of APs frequently superimposed on depolarizing events lasting up to 500 msec. After the addition of tetrodotoxin (TTX; 1 μg/ml), intracellular stimulation elicits slow, graded APs in up to 84% of the cells tested despite marked variability in different culture series. This variability is eliminated by recording from cells in defined media containing Na^+, 14 mM, and Ca^{++}, 8 mM, with TTX as above and tetraethylammonium chloride, 5-20 mM. Cultures prepared from 18 day fetuses were examined between 18 hours and 43 days in culture. The incidence of cells with calcium AP activity is as follows: 46% (n = 35) from 18 hours to 2 days; 89% (n = 38) from 3 to 7 days; 83% (n = 53) from 10 to 16 days; and 83% (n = 6) at 43 days. These data suggest that the incidence of calcium mediated APs remains relatively constant after two days in culture. The lower incidence of calcium potentials recorded from cultures less than two days old could indicate either that these cells have not yet acquired calcium spikes or that this property has been temporarily lost in culture preparation. The latter possibility seems most likely because 91% (n = 22) of cultured neurons equivalent in age to intact neurons in 16-18 day fetuses have calcium APs.

Special features include the following. (1) Calcium APs are optimally developed when the membrane potential is depolarized to about -40 mV by steady electrical current. (2) AP morphology suggests multiple generator sites may exist in some cells. (3) Up to 25% of the cells in selected cultures have rhythmic pacemaker-like, calcium APs with a frequency which increases linearly from about 1 to 7 Hz with increasing steady current injection.

These calcium dependent APs are reversibly blocked by cobalt, 10 mM, and are absent in defined medium containing up to 2 mM calcium and equimolar amounts of nickel. Manganese does not block and may even enhance these APs suggesting that manganese may traverse the same channel as calcium in these cells.

Sodium dependent APs can be demonstrated in cells which have calcium APs by use of local perfusion of medium containing Na^+ (140 mM). These dual AP mechanisms persist within the same cell throughout the time span of the cultures studied here.

In summary, our data show that cultured hippocampal neurons have the capability for calcium electrogenesis which can be recorded within a day of plating and persists in high incidence for at least six weeks in culture.

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10.9 EFFECTS OF BLOCKERS OF POTASSIUM CONDUCTANCE ON SPINAL NEURONS OF A VERTEBRATE. R. J. Bookman* and M.E. Selzer. Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The effects of 40 mM tetraethylammonium chloride (TEA), 1 mM 4-aminopyridine (4-AP), and 1 mM 3,4-diaminopyridine (3,4-DAP) were tested on spinal neurons of the sea lamprey (Petromyzon marinus). On dorsal cells (DCs) and giant interneurons (GIs) these drugs produced a dramatic broadening of the action potential. The prolonged plateau could be permanently interrupted by a brief pulse of hyperpolarizing current. The current threshold for this all-or-none repolarization decreased as the pulse was applied later in the plateau. This long lasting potential could be produced in tetrodotoxin but was blocked by 0 Ca^{++} or 5 mM $MnCl_2$. It was associated with an increased input conductance, which lessened progressively during the plateau. In 3,4-DAP, the potential was clearly regenerative since the threshold voltage for activation was well below the peak depolarization of about +10 mV. Thus in the presence of these drugs DCs and GIs appear to develop voltage-dependent, regenerative Ca^{++} conductances.

The giant Müller's and Mauthner's axons never developed these spike prolongations. This is striking because these unbranched axons form their synapses en passant and require Ca^{++} for transmitter release. Smaller axons also did not show Ca^{++} spikes in TEA. In one case we recorded simultaneously from the cell body of a GI and its axon. The soma showed TEA-induced spike prolongation while the axon did not.

Stimulation of DCs and GIs at frequencies greater than 0.02 Hz produced progressive shortening of the action potential until a steady spike duration was reached. This duration decreased with increasing stimulus frequency up to 0.2 Hz. Frequencies faster than this did not usually shorten the duration further. However, when pairs of action potentials were elicited in GIs at very short intervals (40-100 msec.), the second spike was prolonged relative to the first. This facilitating effect was not seen in DCs. It is interesting that the EPSP produced in one GI by stimulation of a second GI shows facilitation at short interstimulus intervals. However, the EPSP of a GI, seen in response to stimulation of a DC, does not facilitate. Therefore, it is possible, though still speculative, that increased Ca^{++} conductance plays a role in synaptic facilitation.

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10.10 REGIONAL DIFFERENCES IN THE TEA SENSITIVITY OF A VERTEBRATE INTERNEURON. C. Kaars* and D.S. Faber. Div. Neurobiology, Dept. Physiol., SUNY at Buffalo, Buffalo, NY 14214.

Recent voltage clamp data (Chiu et al., J. Physiol., 298: 171-184, 1980; Brismar, J. Physiol., 298:171-184, 1980) indicate that axons of two mammalian species, unlike those of amphibian axons (Hille, J. gen. Physiol., 50:1287-1302, 1969), lack voltage sensitive K^+ conductance. TEA and 3-AP are potent blockers of voltage sensitive K^+ conductance. We have examined the distribution of TEA and 3-AP sensitivity in a vertebrate interneurone, the goldfish Mauthner cell. Our results indicate that TEA and 3-AP sensitivity is restricted to the axon hillock and initial segment while the axon is insensitive to TEA. Intrasomatic iontophoresis of TEA results in a marked broadening of the antidromic action potential recorded intracellularly in the soma and extracellularly in the axon cap surrounding the axon hillock-initial segment. The prolonged spike often evokes a second action potential of short duration, presumably generated in the axon. Similar effects are observed following intra- or extracellular application of 3-AP.

In order to determine if axonal and axon hillock-initial segment membranes are differentially sensitive to TEA, we examined its effects on the action potentials generated in these two regions. The axon spike, which can be isolated by blocking antidromic invasion of the axon hillock-initial segment with hyperpolarizing current, is unaffected by intra-axonal TEA iontophoresis at sites from 0.5 to 2.0 mm distal to the axon hillock. The axon hillock-initial segment action potential can be isolated by axonal hyperpolarization during orthodromic activation. Intra-axonal iontophoresis up to 2 mm distal to the axon hillock typically increases the duration of the axon hillock-initial segment spike by ≥ 2, without altering its rise time. Increased duration is associated with prolongation of the slow phase of repolarization, resulting in a spike with a pronounced shoulder. Topical application of 5% $CoCl_2$ partially reverses the effect of TEA on the axon hillock-initial segment spike. This suggests an inward calcium current may contribute to the prolonged axon hillock-initial segment action potential.

In summary, our findings suggest voltage sensitive K^+ channels contributing significantly to the repolarizing phase of the action potential are limited to the axon hillock-initial segment of the Mauthner cell. Thus, rapid repolarization of axonal membrane in the absence of voltage sensitive potassium currents may not be limited to the nodes of mammalian peripheral axons. (Supported in part by NIH Grant No. 1 R01 NS15335.)

10.11 ELECTROPHYSIOLOGICAL LOCALIZATION OF ACTIVE MEMBRANE SITES IN THE GOLDFISH MAUTHNER AXON. Paul G. Funch and Donald S. Faber, Div. Neurobiology; Dept. Physiology; SUNY at Buffalo, Buffalo, NY 14214.

The myelinated axon of the goldfish Mauthner cell makes synaptic contact every 0.2 to 0.3 mm with motoneurons and interneurons. No interruptions in the axon's myelin sheath have been observed, other than at the terminations of the short collaterals where the synaptic contacts are made. The lack of typical nodes of Ranvier has led to an hypothesis that the collateral terminals are the active membrane sites which support impulse propagation. However, we have analyzed the components of the axonal action potential, and our results indicate that distinguishable active sites are spaced only about every 2½ mm along the rostral portion of the axon.

During simultaneous intracellular recordings from the Mauthner axon, hyperpolarizing currents applied through the distalmost electrode (fixed locus) were used to block all-or-nothing components of the orthodromically initiated axonal impulse. Spike component amplitudes at the critical blocking current levels were measured at proximal sites with the second electrode (variable locus). In one experiment, detailed spatial profiles of the voltage output from individual active sites were obtained. These exhibit sharp maxima separated by roughly 2 mm; decays to either side of the maxima are consistent with the passive electrical characteristics of the axon. Furthermore, in eight experiments, the critical hyperpolarizing currents required to block the apparent active sites were (nA; mean ± S.E.): 77.7 ± 5.2, 124.7 ± 8.7, and 223.0 ± 31.0. The average ratio of these currents at adjacent active sites is thus 1.70, indicating an electrotonic decrement, in a semi-infinite cable, over a distance of 0.53 space constants (λ). The λ of the Mauthner axon is estimated to be 4.8 ± 0.7 mm. Therefore the average spacing between the apparent active sites is roughly 2.5 mm.

In summary, the active sites in the Mauthner axon are spaced at distances of about 0.53 λ, which is comparable to nodal spacing in peripheral fibers; in contrast, collateral spacing is on the order of 0.05 λ. Therefore either specific clusters of collateral terminals function collectively as an active membrane site, or the morphological correlate of a discrete active site remains to be identified. (Supported in part by NIH Grant No. 1 R01 NS15335.)

10.12 SITE OF IMPULSE INITIATION IN NEURONS. J.W. Moore, N. Stockbridge*, M. Westerfield*†. Dept. of Physiology, Duke University, Durham, NC 27710.

Computer simulations have been carried out in order to determine how a neuron's shape and membrane excitability affect the locus of apparent impulse initiation. We simulated a neuron as a 10 µm diameter unbranched axon attached to a 50 µm diameter soma and lumped equivalent cylinder dendrite 2800 µm long. Any segment of membrane could be made passive or excitable (Hodgkin-Huxley description) and synaptic current could be injected at any location.

We found that, when the whole neuron including the dentritic tree was made active, an impulse was always initiated at the site of the synaptic stimulation. However, when excitability was restricted to the axon and soma, the apparent site of initiation moved out beyond the soma and into the initial segment of the axon. This finding was true for synaptic locations throughout the soma-dendrite and results from the fact that the very heavy electrical load of the passive dendritic tree restricts the voltage excursions in the active cell body. Therefore the full-size impulse occurs first in the less heavily loaded axon.

Thus we have shown that uniform channel density and membrane properties in the soma and axon are sufficient to give impulse initiation in the axon initial segment.

Current efforts are under way to extend these conclusions to neurons with myelinated axons.

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11.1 CELLULAR IMMUNE RESPONSES (CMI) TO CNS MYELIN BASIC PROTEIN (MBP) FOLLOWING STROKE IN THE GUINEA PIG, B. Gran*, Z. Latif, W. Sheremata. Depts. of Neurology and Microbiology, Univ. of Miami Sch. of Med., Miami, FL 33101.

Immune responses to damaged tissue are a recognized factor in the induction of autoimmune disease. Immune responses following nervous system injury might theoretically contribute to the morbidity of such injury, but experimental evidence for or against this is lacking. We have therefore studied CMI 2 weeks after experimental embolic stroke in Hartley guinea pigs. The direct macrophage migration inhibition factor (MIF) assay was used with 1 μg-25 μg/ml MBP or 10 μg/ml peripheral nerve myelin P_2 protein as antigens. Eighteen of 24 guinea pigs survived operation and were assayed concomitantly with 19 normals. Mean normal % migration of peritoneal exudate macrophages from 12 animals (in 10% guinea pig serum supplemented TC199) was 102 ± 5.40 for MBP 10 μg/ml and 104 ± 3.62 for P_2 10 μg/ml. Stroke animals gave % means of 98 ± 4.21 for MBP, and 99 ± 3.62 for P_2. Two of 10 animals showed significant responses ($p < 0.01$) to MBP only. Additionally 8 stroked animals and 7 normals were studied utilizing 10% fetal calf serum supplemented TC199. Mean % migrations was 100 ± 3.93 in normals, and 102 ± 6.27 in stroke animals at 1 μg/ml MBP, 104 ± 6.19% in controls and 103 ± 4.12% at 10 μg/ml MBP, and 103 ± 3.48% in controls and 102 ± 4% stroke animals at 25 ug/ml MBP. Three of these 8 stroke animals gave a significant response to MBP but only one to P_2. Positives in both groups occurred in the smallest animals suggesting age may be a factor in determining CMI to encephalitogenic MBP and other neural antigens. A factor in normal guinea pig serum may protect against such a response. However, the clinical significance of such in vitro responses remains to be determined.

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11.2 T-CELL BLASTOGENIC RESPONSES TO MYELIN BASIC PROTEIN (MBP) IN MULTIPLE SCLEROSIS (MS), William Sheremata, Diana Lopez*, and Mario A. Moscarello. Dept. of Neurology and Microbiology, U. Miami Sch. of Medicine, Miami, FL 33101, and Neurological Research Unit, Univ. of Toronto, Toronto, ONT.

Preliminary results from our laboratory revealed that T-cells from MS patients in acute attacks underwent marked blastogenic responses to low MBP concentrations. Unseparated peripheral venous blood mononuclear and T-cell responses to MBP and the mitogens Con-A, and PHA have now been studied in 10 MS patients in acute attacks, 24 during recovery and in 35 controls. Based on the preliminary data, concentrations of 2.5 and 5 μg/ml MBP, 4 μg/ml Con-A, and 10 μg PHA were utilized. Normals gave values (X 1000) of -0.2 ± 0.06 for unseparated cells, 0.2 ± 0.6 for T-cells, and 0.1 ± 0.3 for non T-cells. Acute MS patients results were 9.0, 15.3, and 1.0; other MS patients 0.5, 0.3, and 0.2; and other neurological diseases (OND) 0.1, 1.2, and -2.0 respectively. Con-A results were lower in MS exacerbation but PHA results were not. T-cells from 7 of the 10 acute MS patients gave marked responses not seen in any normals or OND. Alterations in B cell function may impair effective suppressor T function. However, these results probably indicate that, in addition to suppressor T- cells, non E-rosetting cells - probably B cells - play an important part in regulating immune responses to neural antigens.

Supported by the National MS Society.

11.3 EFFECTS OF TUNICAMYCIN ON GLYCOPROTEIN SYNTHESIS IN PNS MYELIN. M. E. Smith. Dept. of Neurology, Veterans Administration Medical Center, Palo Alto, and Stanford University School of Medicine, Stanford, CA 94305

PNS myelin contains at least four glycoproteins in molecular weights of 100,000 (MAG), 27,000 (P_0), 23,000, and 19,000 daltons. Biosynthesis of these glycoproteins in PNS myelin was studied in vitro using radioactive precursors. Spinal roots or sciatic nerves from 21 day rats were incubated with a [^{3}H] or [^{14}C] amino acid mixture or with [^{3}H] fucose, in Krebs Ringer bicarbonate buffer, then purified myelin was prepared from the incubated tissues. The myelin was delipidated, and the proteins separated on SDS polyacrylamide gel electrophoresis. The gels were stained with fast green, scanned, and cut into slices which were counted for their radioactivity. The separated stained myelin proteins on the gels corresponded to definitive radioactive peaks showing good incorporation of labeled amino acids and fucose. Tunicamycin, an inhibitor of protein glycosylation, when incubated with the spinal roots or sciatic nerves, inhibited the uptake of [^{3}H] fucose into all the glycoproteins. Tunicamycin did not inhibit uptake of [^{3}H] amino acids into protein to any great extent, but the radioactive peak corresponding to the main structural PNS myelin protein, P_0, was displaced to a position corresponding to about 25,000 daltons. When tissue was incubated with tunicamycin, [^{14}C] amino acids and [^{3}H] fucose, the new protein was labeled with [^{14}C] but not with [^{3}H]. We propose that this 25,000 dalton protein is the P_0 protein which has remained unglycosylated. No new forms of the other glycoproteins were apparent on the gels. Although glycoprotein function is usually associated with "recognition," it appears that the association of P_0 with the PNS myelin membrane can proceed in the absence of the carbohydrate side chain. Supported by the Veterans Administration and by Grant #NS-02785 from the NIH.

11.4 THE ALTERATION OF FUCOSE/LEUCINE INCORPORATION INTO PNS MYELIN BY ISONIAZID NEUROPATHY. M. P. Colip*, S. Baughman*, and R. G. Peterson. Anatomy Department, Indiana University School of Medicine, Indianapolis, IN 46223.

The ratio of [^{3}H]fucose to [1-^{14}C]leucine incorporation into rat sciatic nerve during in vitro incubation has been previously studied in Wallerian degeneration and in diabetic neuropathy by this lab (submitted for publication). This model was applied to a clinically relevant and morphologically well studied experimental toxic neuropathy produced by isoniazid (INH) to further quantify altered protein-glycoprotein metabolism. Male Sprague-Dawley rats, weighing 200g, were injected daily I.P. with INH 150mg/kg (10% INH in 0.9% NaCl) and controls with a comparable volume of 0.9% NaCl. After 1, 2, 4, and 7 days, sciatic nerves were harvested for metabolic studies and posterior tibial nerves for morphological comparison. Sciatic nerves were stripped of their epineurium and individually incubated at 37°C in 0.5ml of a solution composed of: Krebs-Ringer bicarbonate (KRB); complete amino acid mixture (Tolman et al., J. biol. Chem. 248: 4552, 1973); 3% BSA; 0.2% glucose; 50uCi/ml [^{3}H]fucose; and 5uCi/ml [1-^{14}C]leucine (NEN). After 3hr the nerves were washed in iced KRB, glycerinated, and frozen in liquid nitrogen. Myelin was purified according to Wiggins (Brain Res. 89: 99, 1975). Delipidated myelin protein was solubilized in SDS by sonication. Aliquots of the supernatant were taken for protein assay (Lowry) and liquid scintillation counting. Both DPM/ug protein of each incorporated precursor and the DPM ratio (fucose/leucine) were compared to control values. At 7d the ratio for the four INH treated rats was 2.23 ± 0.14 (S.D.), statistically significant ($p < 0.001$) from the four controls at 3.03 ± 0.17. The decreased ratio reflected a concomitant decrease in fucose and increase in leucine incorporation into myelin of about equal specific activity. 14.3 ± 7.4% of fibers teased from tibial nerves of the 7d INH rats showed ovoid formation as the criterion of degeneration (Lubinska, Brain Res. 130: 49, 1977) as compared to no degeneration in controls. This 7d data along with earlier time periods, especially at 4d, suggest that lower DPM ratios correlated with higher percent degeneration. This model thus appears to be a quantitative indicator of modified protein-glycoprotein synthesis and of nerve fibers undergoing degeneration induced by INH. Supported by NSF BNS 78-00616.

11.5 POSTNATAL SYNTHESIS OF MYELIN AND BRAIN SUBCELLULAR MEMBRANE FRACTIONS FOLLOWING POSTNATAL PHENYTOIN ADMINISTRATION. Philip N. Patsalos* & Richard C. Wiggins. Dept. of Neurobiol. & Anat., Univ. Texas Med. Sch., Houston, Texas 77025

Long Evans rats were administered intraperitoneally with various concentrations of phenytoin (PHT) during the period from birth through 17 days postnatal age. Synthesis of brain subcellular membrane proteins was examined by double isotope methodology as we have previously described in detail.

At age 21-24 days ^{3}H-leucine and ^{14}C-leucine were injected intraperitoneally into pairs of test and control rats respectively. Brains of experimental and control pups were combined and subcellular fractions prepared. For each subfraction $^{3}H/^{14}C$ (test/control) ratios were determined and normalized to corresponding microsomal value for that double brain homogenate. The resultant percentage values obtained are a measure of relative synthesis for each subfraction of the experimental pup.

Relative Synthesis (percentage; Mean±SEM)

	PHT dose (mg/kg)					
Subcellular Fraction	5.0		15.0		45.0	
Myelin	94±1[c]	(N=6)	82±3[b]	(N=11)	91±2[d]	(N=5)
Nuclear	81±4[c]	(N=6)	80±2[a]	(N=11)	91±4	(N=5)
Synaptosomal	97±5	(N=6)	96±1	(N=11)	97±4	(N=5)
Mitochondrial	97±2	(N=6)	95±1	(N=11)	97±2	(N=5)
Microsomal	100	(N=6)	100	(N=11)	100	(N=5)

a,b,c, and d represent $p<0.001$, $p<0.005$, $p<0.01$ and $p<0.025$ respectively.

The myelin ratio was consistantly low at 20-24 days postnatal age, the period of most rapid myelin synthesis, reflecting a 10-20 percent relative reduction in synthesis. Synthesis of nuclear membrane protein was also decreased. Myelin and nuclear membrane protein synthesis deficits occurred at all PHT concentrations studied. No significant change in relative synthesis of the synaptosomal and mitochondrial protein fractions was observed.

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11.6 INCREASED MYELINATION IN CULTURES OF MYELIN-DEFICIENT CEREBELLUM WITH ADDED NORMAL OPTIC NERVE GLIA: MORPHOLOGICAL STUDIES. Susan Billings-Gagliardi, Lori H. Adcock*, Gail B. Schwing* and Merrill K. Wolf. Department of Anatomy, University of Massachusetts Medical Center, Worcester, MA 01605

CNS myelination is severely reduced in mice with the single-gene mutation myelin synthesis deficiency (jp^{msd}) (Billings-Gagliardi et al., '80a). The jp^{msd} abnormality is reproduced in organotypic cultures of cerebellum (Billings-Gagliardi et al., '80b). The defect could be the result of primary abnormalities in the axon, oligodendrocyte, and/or some third cellular element. To test these ideas, cerebellum from 0-day jp^{msd} mice was co-cultured with 0-14 day normal mouse optic nerve to permit normal optic nerve glia to invade jp^{msd} cerebellum. Each cerebellum was cut into 8 parasagittal slabs which were cultured in pairs. Optic nerve was added to the lateral and medial cultures from one half of each cerebellum. The corresponding cultures from the other half served as controls. After 21-30 days in vitro, when cultures of normal cerebellum would be fully myelinated, cultures were fixed and studied by light and electron microscopy.

Quantitative study of ten jp^{msd} cultures with added optic nerve demonstrates that myelination is substantially greater than in the corresponding control cultures without optic nerve. The proportionate increase in myelination ranges from 1.5x to 25x, with an average approximating 10x (Wolf et al., '80). In jp^{msd} cultures with increased myelination, the optic nerve and cerebellar explants are fused. Separation of optic nerve and cerebellum by as little as 0.2 mm prevents increase of myelination, presumably by preventing invasion of optic nerve glia. Myelinated axons in jp^{msd} cultures with added optic nerve usually occur in long, broad tracts close to the optic nerve, whereas myelin segments in control jp^{msd} cultures form small, randomly distributed clusters. Oligodendrocytes are more numerous and myelin sheaths are usually thicker than in controls. Thus myelin in cultures with added optic nerve looks like normal rather than like jp^{msd} myelin.

These findings are difficult to explain on the basis of a diffusible factor made by normal optic nerve, but are simply explained by assuming that the additional myelin is directly made by the added normal oligodendrocytes. This suggests (1) that oligodendrocytes from normal optic nerve are competent to myelinate axons of jp^{msd} cerebellum, and (2) that the mutant axons are receptive to myelination by normal oligodendrocytes. Supported by NIH Grant NS-11425.

11.7 MYELINOGENIC GENE EXPRESSION IN LONG-TERM CULTURES OF PURIFIED RAT OLIGODENDROCYTES. C. Shama Bhat*, E. Barbarese*, and S.E. Pfeiffer. Dept. of Microbiology, Univ. of Connecticut Health Center, Farmington, CT 06032.

Oligodendrocytes are isolated from 11 day old primary cultures prepared from 19-21 day fetal or 1-2 day neonatal rat brain cerebral hemispheres by shaking them from the primary cultures followed by lysis of other contaminating cells in Hepes buffered balanced salt solution at pH7.2. More than 99% of the isolated cells are viable and can be cultured as monolayers for over 60 days. At least initially the oligodendrocytes synthesize DNA and divide. The isolated oligodendrocytes are characterized by: (a) morphological parameters (phase contrast, Nomarski, and transmission electron microscopy); (b) immunofluorence labelling with antisera to myelin basic protein and galactosyl-ceramide; and (c) biochemical analyses for myelin basic protein, 2',3'-cyclic nucleotide 3'-phosphohydrolase, and sulfogalactocerebroside synthesis (450 pmoles/mg protein; 6 umoles/min/mg protein; and 6 nmoles/24h/mg protein, respectively, at 32 total days in culture). Since these values are similar to the peak activities of these parameters observed in mixed primary cultures of the same age, in which the cellular "specific activity" of oligodendrocytes is estimated to be about 1/10 that in the purified cultures (by staining with anti-myelin basic protein antisera), the data suggest that either the maximal levels or the rates of development of the expression of these parameters requires non-oligodendrocyte positive signals for optimal expression of myelin-related differentiated function by the oligodendrocytes.

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11.8 PARALLEL RIDGES IN FREEZE FRACTURED CNS MYELIN FROM A MYELIN DEFICIENT MOUSE R.G. Miller and J.-M. Matthieu* 20 Rue Ecole de Médecine, 1211 Geneva and Service Pédiatrie, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland.

The myelin deficient mouse, "MLD", is a mutant which, in the homozygote, contains a reduced amount of total myelin and only 3-4% (on the basis of total protein) of the normal amount of myelin basic protein (MBP). Although such myelin appears normal in the PNS, in the CNS it lacks a compact major dense line which is normally derived from the cytoplasmic surfaces of the oligodendrocyte and the number of lamellae are less than 8. (Matthieu, Ginalski, Friede, Cohen, and Doolittle, Brain Res. v.190, in press, 1980). Since the depletion of a major myelin protein can be expected to alter the ultrastructure of myelin membranes, we have used freeze fracture to determine the fine structural differences between the myelin from the MLD mice and their heterozygote littermates which contain an approximately normal amount of MBP.

Optic nerves from 30-38 day mice were removed under pentobarbital and either immediately quenched in Freon on a specimen stub, or were fixed in glutaraldehyde (2% in PBS at RT) and infiltrated in 20% glycerin before quenching. The description which follows applies equally to the freshly frozen and fixed/cryoprotected tissue.

The most striking observation is that there is a hitherto unreported membrane configuration on MLD myelin, consisting of shallow (less than 4nm), parallel ridges. Their low profile made their detection very labile to shadow angle and, thus, it is not certain whether all or just some portions of MLD myelin contained these ridges. Ridges are propagated across the interperiod line to the adjacent fracture plane. Typically, they appeared roughly parallel to the major axis of the axon. These ridges resemble neither the usual paranodal junction nor other specializations which are present in other tissues. Another difference between the MLD and Normal myelin is an abundance in MLD of patches (ca .1 micron in diameter) of "paranodal" specializations which were not associated with paranodal regions and were not organized into regular arrays as they are in normal tissue.

11.9 MYELINATION-DEPENDENT AND INDEPENDENT MEMBRANE SPECIALIZATIONS AT THE NODE OF RANVIER, DEMONSTRATED IN INSUFFICIENTLY MYELINATED NERVES OF THE DYSTROPHIC MOUSE. Clayton A. Wiley-Livingston and Mark H. Ellisman, Dept. of Neuroscience, U. Calif. at San Diego, La Jolla, CA 92093

"Dystrophic" mice of the 129/ReJ-Dy strain have a genetic defect affecting Schwann cell proliferation. Spinal nerve roots of these animals contain myelinated and unmyelinated axons in addition to groups of large "amyelinated" axons. In affected regions of the spinal roots, myelinated axons are missing their myelin sheaths. Where the myelination terminates or begins, half-nodes are created. Freeze-fracture analysis of these half-nodes shows a patch of particles in the nodal fracture faces similar to the annulus of particles around the axon of normal nodes. Rows of dimeric-particles (putative Na^+ channel) in the P-face of the paranode are seen only on the myelinated side. The P-face on the amyelinated side contains a dense, even distribution of particles, many of which are the size of dimeric-particle subunits, but only a few of which are arranged in rows.

The E-face of the completely amyelinated axons are comparatively particle-free except for infrequent patches of particles. These patches are very similar to the patches present at half-nodes, and are similar to, but smaller than, annuli of particles at the node of Ranvier. We conclude that the paranodal rows of dimeric-particles are dependent upon myelination for their organization. In contrast, the positioning of the nodal patches of particles is independent of myelination.

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11.10 A CROSS SPECIES COMPARISON OF 2',3'-CYCLIC NUCLEOTIDE 3'-PHOSPHODIESTERASE. T. J. Sprinkle* and M. R. Wells. Neurochem. Lab. V. A. Med. Ctr. Res. Service (151) Augusta, GA 30904 and Lab. of CNS Injury and Regeneration. V. A. Med. Ctr. Washington, D.C. 20042.

The enzyme 2',3'-cyclic nucleotide 3'-phosphodiesterase (EC 3.1.4.37, CNP) has been purified from human and bovine white matter and from myelin in the rat, guinea pig, and rabbit. Purifications were accomplished primarily by column chromatography (ionic-exchange, hydrophobic, gel filtration and affinity). In all of the species examined, purified CNP appeared on polyacrylamide SDS slab gels as 2 major protein bands in the region of 46-50,000 daltons separated by a distance of 2-3,000 daltons. There was a tendency for CNP to appear in higher molecular weight forms in all species to a varying degree. The relative intensities of the two major protein bands of CNP varied between species in a manner consistent with the contention (Sprinkle and Wells, Trans. Am. Soc. Neurochem. Abstr. 11: 214, 1980) that the enzyme is at least a major component of the Wolfgram proteins W1 and W2 described in myelin (Waehneldt and Malotka, Brain Res. 189: 582, 1980).

11.11 DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR BOVINE WHITE MATTER PROTEOLIPID APOPROTEIN. Wendy B. Macklin* (Spon: Marjorie B. Lees) E.K. Shriver Center, Waltham, Ma 02154 and Harvard Med. School, Boston, Ma 02115

An indirect microtiter plate ELISA has been developed to quantitate anti-proteolipid antibodies produced upon immunization of rabbits with the bovine white matter proteolipid apoprotein (APL). APL is a hydrophobic membrane protein which tends to aggregate and precipitate in aqueous solution. This microtiter plate procedure reduces the problem of APL aggregation since the APL need not be radioiodinated or chemically treated. APL binds avidly to many surfaces, and is thus well suited for microtiter immunoassay. The protein is prepared as a chloroform-methanol soluble protein free of complex lipids which can be converted to a water soluble form. Antibodies to the water soluble form of APL were produced by immunization of rabbits with APL in the presence of bovine serum albumin (BSA). The presence of anti-BSA antibodies in these animals precluded the addition of BSA or calf serum to the microtiter plates to reduce non-specific binding of immune serum or second antibody to the wells. The only protein tested which reduced the non-specific background without binding the anti-BSA antibodies in the immune serum was ovalbumin. In this assay, water soluble APL was bound to the microtiter plate wells, followed by ovalbumin. After washing the plates with phosphate buffered saline, pH 7.6, containing Tween 20, immune and control sera were added in the presence of ovalbumin plus Tween 20. After washing, second antibody, goat anti-rabbit IgG antibody conjugated to horseradish peroxidase (HRP), was added in the presence of BSA plus Tween. The sensitivity of enzyme-conjugated second antibody was compared to that of ^{125}I protein A.

This assay has permitted quantitation of anti-APL antibodies produced by several different immunization protocols. A comparison of the crossreactivity of the water soluble and chloroform-methanol soluble forms of APL was made using this assay. Preliminary experiments suggest that the assay can be adapted to quantitate the apoprotein rather than antibody. Since APL is the major myelin protein, comprising 50% of the total myelin protein, a quantitative assay for both the apoprotein and its antibody would be useful in screening sera and CSF of animals and/or patients with various neurological disorders, particularly demyelinating disorders.

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12.1 MOTONEURON POOLS OF PRIMATE FORELIMB MUSCLES LABELED BY HORSERADISH PEROXIDASE R.F. Martin and E.E. Fetz, Dept of Physiology and Biophysics and Regional Primate Research Center, Univ of Washington, Seattle, WA 98195

Cervical motoneuron pools of specific forelimb muscles of Macaca mulatta were retrogradely labeled with horseradish peroxidase (HRP) to determine their relative location and size. In most cases HRP was applied in a cuff attached over the proximal end of the severed muscle nerve, for direct uptake by cut motoneuron axons. Alternately, HRP (Worthington, 30%) was injected into small hand muscles for uptake via motoneuron synaptic terminals. After 72-hour survival monkeys were perfused (1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer). Serial 80 um cord sections were reacted with tetramethyl benzidine (TMB). HRP-labeled motoneurons were counted only if their nuclei were visible. In two monkeys motoneurons of extensor digitorum communis (EDC) were labeled with an HRP cuff. In both animals, labeled motoneurons extended from the caudal portion of C7 segment to the rostral portion of T2. 495 EDC motoneurons were labeled in one animal, 374 in the other. In one of these animals, contralateral flexor digitorum profundus (FDP) motoneurons were also labeled by HRP cuff. The rostrocaudal extent of the 269 FDP motoneurons was virtually identical to that of the EDC pool, but FDP motoneurons lay more dorsally in the lateral ventral horn. In another monkey, a cuff applied HRP to the median nerve at the wrist, to identify its motoneurons of intrinsic hand and finger muscles; the 582 labeled neurons extended from the C7/C8 junction to the rostral portion of T2. Several labeled motoneuron pools appeared at slightly different positions in dorsolateral ventral horn. In one monkey, motoneurons of abductor pollicus brevis (APB), an intrinsic thumb muscle innervated by the median nerve, were labeled by HRP muscle injection on the left side and by HRP nerve cuff on the right. The muscle injection produced 271 densely labeled neurons, while the nerve cuff labeled 135 neurons less densely; this difference may have been due to compromised HRP transport in the six nerve twigs of APB. The rostrocaudal extent of both labeled APB pools was identical with that obtained by cuffing the entire median nerve at the wrist, but the APB motoneurons were more localized in the ventral horn.

For some muscles, motoneurons appeared to be preferentially grouped within the rostro-caudal extent of the pool. Motoneuron soma diameters ranged from 15-70 μm. The TMB reaction product was no denser in the smaller γ motoneurons than in the α motoneurons, making it impossible to assess the relative number of γ and α motoneurons, particularly in the intermediate range of soma diameters (30-40 μm). The TMB product labeled the axons of many motoneurons, and revealed surprisingly tortuous axon trajectories in the ventral horn, which have proven to be characteristic of particular motoneuron pools. Instead of exiting the lateral ventral horn by the shortest route, many axons travel ventro-medially for a considerable distance, and then exit the gray matter in the ventral direction.

12.2 ANATOMY OF THE FLEXOR DIGITORUM PROFUNDUS MOTOR NUCLEUS IN THE CAT SPINAL CORD. G.A. Iwamoto, J.A. Dixon* and W.J. Gonyea*. Depts. of Internal Medicine and Cell Biology, Univ. of Texas Health Science Center, Dallas, TX 75235.

As a necessary step in our ongoing examination of the structural and functional bases of prehensile motor activity in the cat forelimb, the motor nucleus of the flexor digitorum profundus (FDP), was mapped in detail. As described by Reighard and Jennings (Anatomy of the Cat, 1901) the muscle is composed of five distinct heads. The heads are commonly held (McClure, Dallman and Garrett, Cat Anatomy, 1973) to be innervated by the median nerve (heads 2,3,4,5) and the ulnar nerve (heads 1 & 2).

The FDP was injected with a 30% solution of horseradish peroxidase utilizing a microliter syringe. To isolate the contributions of individual heads of this muscle, injections of the entire muscle on one side of each animal were accompanied by injections of a selected head on the contralateral side. Following a 48-72 hr survival period, the animals were perfused with a 0.4% paraformaldehyde -2.5% glutaraldehyde solution. Frozen serial sections from the C_5-T_2 spinal segments were cut at 50 μm and reacted with tetramethyl benzidine after the method of de Olmos, Hardy and Heimer (J. Comp. Neurol. 181:213-244).

The overall distribution of the FDP motor nucleus extends from the mid C_6 to caudal T_1 levels. The cells are grouped on the dorsolateral border of the ventral spinal gray matter for the rostral half of this distribution. However, the caudal half of the nucleus, in addition to the large main group on dorsolateral border, shows a division of more scattered cells situated just ventromedial to the main group. The highest density of cells occurs in the mid C_8 to mid T_1 regions. Individual heads contribute thusly: Head 1 is concentrated between C_8 and T_1 while head 5 extends from C_6 to T_1. These results are similar to those reported previously for flexor carpi ulnaris (FCU) and flexor carpi radialis (FCR), respectively (Iwamoto et al, Anat. Rec. 196:85-86A). Head 5, however, does not show a high density of cells in the C_7 area (as in FCR), but reveals a higher concentration of cells in the C_8 and T_1 regions as is the case with all heads of the FDP. Heads 2, 3, and 4 show distributions which are intermediate (in varying degrees) between the patterns shown by 1 and 5. The data show that many similarities exist in motor cell groupings for muscles synergically involved with wrist flexion. The data also suggest that a possible basis for synergy between muscles involved in radial or ulnar deviation movements during prehensile motor activity may exist since the motor nuclei involved seem to be spatially grouped and selectively innervate medial or lateral antibrachial musculature. (Supported by NIH Grants AM-17615 and HL-07360.)

12.3 ELECTRICAL STRUCTURE OF MOTOR NEURONS: INFORMATION FROM THE RESPONSE TO A PULSE OF CURRENT. D.H. Edwards, Jr. and B. Mulloney. Dept. of Zoology, University of California, Davis, CA 95616.

The electrotonic structure of a neuron is the foundation on which the integration of all its synaptic currents and active responses rests. The electrotonic structure also determines what can be recorded from a given site in the neuron, and how effectively the potential of the neuron can be controlled.

We have developed a method that uses the voltage response of a neuron to a brief pulse of current to build a compartmental model of the electrotonic structure of the cell (Rall, 1962). We have used this method to study the electrotonic structure of neurons in the lobster's stomatogastric ganglion.

The method treats the recovery phase of the voltage transient as a series of exponentials, each of which has a coefficient and time constant. The terms of this series can be separated by "peeling." We have developed algebraic formulae that relate these coefficients and time constants to the resistances and capacitances of a compartmental model of the injected neuron that has one compartment for each term in the series. This model will accurately predict the linear voltage response of the cell to any waveform of current injected at the recording site (Perkel and Mulloney, 1978).

The input resistance of the neuron can be calculated from the pulse response. This method has the advantage that voltage is measured after current is injected, so bridge-balance is not a problem. The total capacitance, and therefore the surface area, of the injected region of the neuron can also be calculated from the pulse response.

Each of the identified stomatogastric neurons we have studied with these methods has a characteristic electrotonic structure. Their input resistances at the soma and their membrane time constants vary through a wide range, but these neurons share some common features. The soma of each neuron is its most resistive region; therefore, the soma is an excellent place to record voltage responses to synaptic currents. The axon of each neuron is its major current sink; most currents flow out of the integrative region down the axon. There is a significant coupling resistance between the soma and the neuron's integrative segment; therefore, it is not possible to space-clamp the neuron with a voltage clamp applied to the soma.

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12.4 MOTONEURONAL POOLS INNERVATING FIN MUSCLES IN THE ATLANTIC STINGRAY, DASYATIS SABINA. M.H. Droge and R.B. Leonard. Marine Biomed. Inst. and Dept. of Physiol. & Biophys., Univ. of Texas Med. Br., Galveston, TX 77550.

Pectoral fin muscles in the stingray consist of a series of antagonist sets of dorsal and ventral muscles separated by cartilaginous bars. The radially arranged muscles have distinct superficial and deep components in both the dorsal and ventral masses. The localization of motoneuron pools innervating the various portions are being investigated using retrograde transport of horseradish peroxidase (HRP). Dorsal or ventral muscle is split by gentle dissection to expose the interface of adjacent muscle bundles (myotomes?). The desired portion is injected along its radial length with 4-6μl of 30-40% HRP. Following 3-4 days of survival, animals are perfused with 1.25% gluteraldehyde and 0.75% paraformaldehyde. Serial frozen sections of the appropriate spinal cord levels are reacted with tetramethylbenzidine histochemistry.

Two groupings of relatively large neurons can be distinguished in the ventral horn of Nissl stained material in the region previously shown to contain motoneurons (Leonard et al., J. Neurophysiol., 41:108, 1978). One group of prominent cells extends in a loose row from the central canal ventrolaterally across the gray matter. The second consists of large cells scattered beneath the first group; particularly ventrolaterally. Labelled somata in both groups range in diameter from 20-45 μm with a unimodal distribution. This is expected from the absence of well organized muscle spindles with efferent innervation. Motoneurons innervating the ventral muscles are located dorsomedially in the first cell group. Dorsal muscles are supplied by motoneurons in the more scattered, ventrolateral second group. There appears to be some overlap among neurons to dorsal and ventral muscles. Preliminary evidence indicates more overlap among neurons to the superficial and deep components within the dorsal muscle mass. Following injection of a single muscle bundle, motoneurons are found over about two spinal cord segments (about 2.0 mm).

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12.5 MONOSYNAPTIC SUPRASPINAL INPUTS TO LONG DESCENDING PROPRIOSPINAL NEURONS IN CATS. R. D. Skinner and R. S. Remmel. Depts. of Anatomy and of Physiology, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

The cell bodies of origin and the peripheral inputs to long descending propriospinal (LDP) neurons were recently reported by this lab (J. Comp. Neurol. 188 (1979) 443; Brain Res. (in press); Soc. Neurosci. Abst. 4 (1978) 563). Adult cats were intercollicularly decerebrated under ether and then the ether was discontinued. Blood pressure, temperature and expired CO_2 were maintained within physiological limits. The midline cerebellum was removed. The cat was either paralyzed with gallamine or else unparalyzed for EMG recordings. The action potentials of LDP neurons were recorded in the cervical enlargement on the left side with extracellular micropipettes (1-3 MΩ, filled with 4M NaCl). LDP neurons were identified by means of antidromic excitation from the lumbar cord. In order to study inputs from the brain, monopolar stainless-steel electrodes were placed into the left medial longitudinal fasciculus (MLF) at 5 mm rostral of the obex and into the left lateral vestibular nucleus (LVN). Stimulating currents were ≤100 μA and 0.1 msec duration. Trains of 1-3 shocks at 300/sec were used.

Thirty-four of 50 (68%) LDP neurons were fired by ipsilateral supraspinal stimulation. Some responded to only one input (15 to MLF only, 4 to LVN only), but inputs from MLF and LVN converged upon 15 cells. Using the criterion that a response ≤1.0 msec following the arrival of the axonal volly (detected by a cord dorsum electrode) indicated a monosynaptic input, 24 neurons were monosynaptic. (The 10 longer-latency neurons might have had a monosynaptic input.) Seventeen (34%) were monosynaptic to MLF stimulation and 9 (18%) were monosynaptic to LVN. Only 2 were monosynaptic from both inputs. Some LDP neurons received both antidromic and orthodromic excitation from the lumbar cord.

The spontaneous "walking" rhythm of these neurons could in general be modulated by the lateral vestibulospinal tract, the MLF, somatosensory input from the forelimbs and/or lower parts of the body (see abstract by Remmel and Skinner, this volume). The LDP cells are hypothesized to synchronize the walking of the forelimbs with the hindlimbs.

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12.6 "WALKING" RHYTHMS IN LONG DESCENDING PROPRIOSPINAL NEURONS IN CATS. R.S. Remmel and R.D. Skinner. Depts. of Physiology and of Anatomy, Univ. of Ark. for Med. Sci., Little Rock, AR 72205.

The accompanying abstract (Skinner and Remmel, this volume) and papers referenced therein describe the cell bodies of origin and the peripheral and brainstem inputs to long descending propriospinal (LDP) neurons. This abstract describes "walking" rhythms in these neurons.

Methods were similar and most cats yielded data on both "walking" and on synaptic connections. The cat was either paralyzed with gallamine or else unparalyzed for EMG recording. The responses of LDP neurons antidromically excited from the lumbar cord were recorded extracellularly in the cervical enlargement. In order to study effects of brain stimulation upon the "walking" rhythm, both the medial longitudinal fasciculus (MLF) and the lateral vestibular nucleus (LVN) were stimulated, as described elsewhere. The spontaneous bursting ("walking") rhythm of spike trains and of the EMGs from arm extensor muscles were recorded on an FM tape recorder.

LDP and other cervical neurons often developed a "walking" rhythm for 6 or more hours. Forelimb stepping movements and EMGs confirmed that the single-unit rhythms were indeed "walking" rhythms. The average and standard deviation of at least 10 interburst periods were calculated. The average period was 0.61 ± 0.04 sec for one neuron. It was 1.19 ± 0.12 sec for a second neuron (23 ± 9 spikes/burst); this period, however, increased to 1.8 ± 1.0 sec and the number of spikes/burst decreased to 7.4 ± 4.6 when the ipsilateral digits were squeezed. It was 0.62 ± 0.04 sec for a third neuron; this period decreased to 0.49 ± 0.03 when the ipsilateral forepaw digits were squeezed and the period was increased when the contralateral paw was squeezed. The period was 1.6 ± 0.3 sec for a fourth neuron (3.3 ± 1.3 spikes/burst), but the firing rate increased when the skin over the triceps muscle of the contralateral forelimb was pinched. Thus for these and other neurons the rhythm could be modulated or in some cases stopped by stimulation of the LVN or the MLF or by natural stimulation (squeezing) of the pads, skin or muscles of the arm or of other body parts.

For one neuron the firing was correlated with the EMG recorded from the triceps muscle of the ipsilateral forelimb. Both the EMG and the LDP neuron fired at a period of 0.59 ± 0.04 sec, with the beginning of the neuronal burst leading the beginning of the EMG activity by 65 ± 18 degrees. This neuron fired 0 to 3 spikes/burst.

The LDP cells are hypothesized to synchronize the walking of the forelimbs with the hindlimbs. Supported by NIH grant NS-10304.

12.7 THE EFFECTS OF APOMORPHINE ON SPINAL CORD MONOSYNAPTIC TRANSMISSION. J.S. Carp* and R.J. Anderson (SPON: J.G. Kenimer). Dept. of Pharmacol., Geo. Washington Univ., Washington, D.C. 20037.

The effects of i.v. administration of the dopamine agonist apomorphine and the catecholamine antagonists haloperidol and phentolamine on spinal cord neurotransmission were evaluated in spinal cats. Compound action potentials of monosynaptic latency evoked by supramaximal stimulation of L_6, L_7 or S_1 dorsal roots were recorded from the appropriate ventral root during slow repetitive stimulation (2N response) and during posttetanic potentiation (PTP response).

The results of these experiments are summarized in the table below. Apomorphine (2 mg/kg) significantly depressed the amplitude of the 2N but not the PTP response. However, this depressant effect of apomorphine was not observed in animals pretreated with the dopamine receptor antagonist haloperidol (2.5 mg/kg). This dose of haloperidol alone depressed the amplitudes of the 2N and PTP responses to 90±4% and 82±5% of the predrug control, respectively. Pretreatment with phentolamine (1 mg/kg), an α-adrenergic antagonist, did not alter apomorphine's depressant effect on the 2N response. This dose of phentolamine alone had no effect on spinal monosynaptic transmission.

These data demonstrate a depressant effect of apomorphine on spinal cord transmission. This appears to be mediated by a dopaminergic mechanism, since the dopamine receptor antagonist haloperidol, but not the α-adrenergic receptor antagonist phentolamine, prevented the depressant effect of apomorphine on the 2N response.

Effect of Apomorphine
(mean % of predrug amplitude)

Pretreatment	none + apomorphine	haloperidol + apomorphine	phentolamine + apomorphine
2N	75 ± 5*	96 ± 5	76 ± 1*
PTP	96 ± 4	96 ± 3	90 ± 7
	n = 10	n = 5	n = 3

*$p < 0.01$, significantly different from predrug response

12.8 RECOVERY OF MOTOR FUNCTION FOLLOWING LESIONS OF THE DORSAL COLUMN NUCLEI, DORSAL COLUMNS, AND DORSAL ROOTS. A.J. Berman, D. E. Teodoru, and T.A. Tran.* Dept. of Neurosurgery, V.A. Hospital, Bronx, New York 10468.

This study was undertaken to further explore the role of the dorsal column medial lemniscal system (DCMLS) in recovery of motor function after dorsal rhizotomy (DR). A total of 14 monkeys were subjected to lesion of the dorsal column nuclei (DCN). Of these, five underwent DR six months later; six underwent dorsal columns section (DC) three months after DCN lesion, and of these, three were subjected to a third lesion, DR, after a further three months. Thus, all DR procedures were carried out six months after DCN lesion. All operative procedures were bilateral. Animals were trained on each of two tasks before the initial surgery, and all were retested starting two weeks after the final surgery except for two of the DCN plus DR animals, which were not retested until six months after the last surgery. One task involved reaching into a moving cylinder to remove a piece of food, and the other necessitated reaching for a small food pellet nested atop a narrow stationary platform.

DC section ameliorated the finger discoordination resulting from DCN lesion. The amelioration, however, is not as great as that seen when DR follows DCN lesion. On both tasks, reaching was more severely affected by DC than by DCN or by DCN plus DC lesion, confirming previous results of Melzack and Bridges. On the moving cylinder test, monkeys that had undergone lesion of DCN, followed by lesion of DC, then DR, exhibited a more rapid recovery than monkeys that had only undergone lesion of DCN then DR. On the stationary platform test, however, rate of recovery was similar for both groups of animals.

Melzack and Bridges have proposed that after DC lesion, intrinsic circuits in DCN are released from ascending control and are driven to fire aberrantly by descending cortical input, thus providing "misinformation" to motor centers. They proposed that lesion of DCN eliminates such misinformation. We suggest that our results, involving DR, can be interpreted similarly. After DCN lesion, spinocerebellar pathways within DC are still intact. DR may release intrinsic circuits of the spinal relay nucleus of this system from ascending control, thus sending "misinformation" to the cerebellum. Tracking performance, as on the moving cylinder test, is sensitive to spinocerebellar input, and would, therefore, be severely impaired by such misinformation.

12.9 EFFECTS OF DORSAL RHIZOTOMY AND DORSAL COLUMN MEDIAL LEMNISCAL SYSTEM INTERRUPTION ON PERFORMANCE OF A BIMANUAL TASK. T.A. Tran,* D.E. Teodoru, and A.J. Berman. (SPON: R. Hawkins). Dept. of Neurosurgery, V.A. Hospital, Bronx, New York 10468.

Dorsal rhizotomy (DR) is not only a "sensory" lesion depriving the CNS of peripheral information; it is also a motor lesion, for it interrupts the γ loop, debilitating those motor functions dependent on "α-γ coactivation"(Granit, 1976). Dorsal column medial lemniscal system(DCMLS) interruption is more of a sensory lesion for it deafferents the sensorimotor cortex (SMC) (Brinkman, et.al., 1978) without interrupting the γ loop. The present study seeks to differentiate the chronic effects of DR from those of DCMLS interruption. Three monkeys underwent unilateral DR and two, bilateral DCMLS lesions followed by unilateral DR. They were tested 18 months post-operatively on two bimanual tasks requiring proximal strength from one limb and digital coordination from the other. Each monkey had to pull a tray against a 6 lb. (heavy) or a 2 lb. (light) counterweight and to hold it while removing pellets from a flat surface (easy task) or from a dexterity board (hard task) which required use of a coordinated precision grip. All monkeys used the non-DR limb exclusively to pull the tray towards themselves. When the counterweight was light, the uDR monkeys switched hands: the intact limb pulled the tray and the DR limb held it while the intact hand grasped the pellets on both easy and hard tasks. When the weight was heavy, the DR limb was used to grasp the pellets on both hard and easy tasks, while the intact limb held the tray. The two monkeys with DCMLS interruption on one side and DR on the other used the limb on the DCMLS-lesioned side to hold the tray and the DR limb to remove the pellets on both hard and easy tests, when the weight was light as well as heavy. The strategy used by the unilateral DR monkeys indicates that the strength of the DR limb was inadequate to hold the tray when the weight was heavy but finger coordination was adequate to perform the hard tasks. This observation is consistent with the results of Hepp-Raymond, et.al. (1973), suggesting that coordinated movements can be executed via the "α route," but the development of strength requires α-γ coactivation. Since these uDR monkeys had undergone maximal recovery from post-DR ataxia, we conclude that chronic disuse of the DR limb is due to weakness and not discoordination. Exclusive use of the DR limb to grasp pellets by the monkeys subjected to DR on one side and DCMLS interruption on the other suggests that while post-DR digital coordination greatly improves, after DCMLS interruption it remains permanently impaired.

12.10 THE ROLE OF ATTEMPTED MOVEMENTS IN RECOVERY FROM UNILATERAL DORSAL RHIZOTOMY. D.E. Teodoru and A.J. Berman. Dept. of Neurosurgery, V.A. Hospital, Bronx, New York 10468.

After unilateral dorsal rhizotomy (uDR), the deafferented limb of monkeys was found not to be used purposefully in the free situation unless the intact limb was immobilized or amputated at the wrist. However, if the intact hand was covered, uDR monkeys could eventually be trained to use the DR limb to reach into a box and remove crackers. Furthermore, we observed spontaneous use of the DR limb in postural support, ambulation, and climbing by some uDR monkeys several years after surgery. The present study has sought to examine the pattern of spontaneous recovery of uDR monkeys (C2 to T3) after rendering the intact limb functionally useless. A hollow, transparent, lightweight polyethelene ball, 11.4 cm. in diameter, was placed over the intact hand permitting free movement of the hand within it. This was done to 3 uDR monkeys 3-1/2 months after surgery and to four at surgery. The ball was temporarily removed 1, 2, or 3 months after being put on in some cases and permanently 4 months later in all cases. Food could only be obtained by reaching blindly into a box through a hole 6.4 cm. in diameter. Animals were kept in a large "gym" cage and observed daily for over two years. Before the intact limb was covered, it performed all purposive movements; the DR limb only exhibited associated movements. Irrespective of when the DR limb was covered, all uDR monkeys exhibited almost the same pattern and rate of recovery. A sudden shift to exclusive use of the DR limb followed initial abortive attempts to use the covered intact limb. Early DR limb movements were very ataxic. During such movements, the intact limb exhibited associated movements inside the ball. Recovery followed a distal to proximal course (e.g., finger coordination preceeded accurate reaching). Each movement recovered in strength only several weeks after it recovered in form. When the ball was removed, bimanual cooperation was immediately evident in eating, climbing, ambulation, and other manipulative tasks. If removal occurred after more than 4 months, interlimb coordination persisted; if after less than 4 months, it deteriorated in days,and disuse of the DR limb resulted. We propose that higher centers substitute reefferent for reafferent feedback in order to regain control of lower centers disrupted by DR. Such internal feedback is available only when movements are attempted so that the post-operative period of disuse does not contribute to recovery. We feel that adjustment of the same motor circuits that are used to produce movements in intact monkeys would be responsible for post-DR recovery and not retraining to use alternate circuits as proposed by Chambers.

12.11 SUPRASPINAL FACILITATION OF SHORT LATENCY POLYSYNAPTIC EPSPs PRODUCED BY LOW THRESHOLD SURAL NERVE AFFERENTS IN MEDIAL GASTROCNEMIUS α MOTONEURONS. M. J. Pinter*, R. E. Burke, M. J. O'Donovan* and R. P. Dum. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Electrical stimulation of low threshold (<5xT) afferents in the sural nerve (SN) produces mixed polysynaptic PSPs in ankle extensor α motoneurons (MNs). The shortest-latency components are excitatory (EPSPs) and these are predominant in the SN PSPs recorded in fast twitch (type F) medial gastrocnemius (MG) MNs but are much smaller and often absent in slow twitch (type S) cells (J. Physiol. 207:709, 1970). The polysynaptic PSPs produced in the same MNs by stimulation of the red nucleus (RN) are qualitatively similar, suggesting that SN afferents and rubrospinal axons may converge upon the same spinal interneurons (op. cit.). This hypothesis was tested using intracellular recording from MG MNs in cats anesthetized with α-chloralose. Ventral roots were intact and MG MNs were identified by antidromic invasion from the cut MG nerve on the left side. The right RN and the pyramidal tract (PT) were separately stimulated (5 to 8 pulses, 0.2 msec duration at 500 Hz, 30 - 100 μA) with sharpened tungsten monopolar electrodes placed stereotaxically, guided by antidromic field potentials evoked from the left dorsolateral spinal cord quadrant at T13 (right half of the cord cut and dorsal columns removed at T13). Final RN and PT electrode positions were adjusted for point of minimum threshold for facilitation of the disynaptic group Ia IPSP (tibialis anterior nerve stimulation; Hongo et al., Exp. Brain Res. 7:365, 1969). Supraspinal conditioning trains to RN or PT preceeded the test SN PSP by 20 - 50 msec (train onset). Resulting PSPs were averaged by interleaving individual sweeps of test, conditioning alone, and conditioning plus test trials in different quadrants of the averager memory. RN and PT conditioning both produced net facilitation of the short-latency EPSP components in the SN response, which was particularly clear when SN stimulation was 1.1-1.5 x T (threshold for cord dorsum N wave and compound action potential in the intact sciatic nerve). At these low levels of SN stimulation, PT conditioning produced relatively clean increase in SN EPSP components while the effect of RN was complicated by considerable facilitation of SN IPSP components. Maximum SN EPSP facilitation was found in MG MNs with fast conduction velocity and short afterhyperpolarizations, presumed to be type F MNs. The existence of this excitatory convergence suggests that rubro- and corticospinal axons can modulate transmission in a polysynaptic cutaneous excitatory pathway that appears to be organized to produce differential control of fast and slow twitch motor units (see Exp. Brain Res. 29:57, 1977).

12.12 PATTERNS OF SPIKE GENERATION OF LUMBAR MOTONEURONS DURING SLEEP AND WAKEFULNESS. F. Morales*, S. O'Brien*, and M. Chase (SPON: J. Segundo). Departments of Physiology and Anatomy and the Brain Research Institute, Univ. of California, Los Angeles, CA 90024.

The present report describes the patterns of spike generation of lumbar alpha motoneurons during sleep and wakefulness. Intracellular experiments utilizing micropipettes filled with 3M K-citrate were performed on six chronic, undrugged, normally respiring adult cats according to procedures previously described (Morales and Chase, Exp. Neurol. 62:821-827, 1978). The patterns of motoneuron discharge in the chronic cat during wakefulness (W) and quiet sleep (QS) were similar to that reported during synergistic muscle stretch in the acute preparation: small depolarizing potentials summated, which resulted in sustained depolarization. When a critical level of depolarization was reached, rhythmic discharges appeared. The membrane potential time course between spikes, i.e., its "trajectory," was similar in W and QS to that reported for motoneurons discharging in the primary range in the acute preparation. Some lumbar motoneurons were continuously active during W or QS while others discharged sporadically.

When the animal entered AS, spontaneous activity ceased entirely. Concomitant with periods of intense rapid eye movements, large rhythmic depolarizing potentials often developed (4-6 waves in each episode with a wave duration of 100 to 200 msec and an amplitude of 6 to 8 mV). In spite of their large amplitude these potentials did not always spontaneously produce spike activity. Indeed, in most of the cases, discharge threshold was not reached. If a depolarizing bias was added (outward current injected through the microelectrode), spike doublets or triplets were observed riding on the summit of these rhythmic potentials. When these potentials were above threshold, two or three spikes appeared in conjunction with each wave.

Two types of action potentials were noted during AS. The first type was characterized by full sized spikes whose amplitudes were similar to those which appeared during QS and W. The main difference consisted of the almost complete abolition of afterhyperpolarization. The second type was characterized by partial spikes whose amplitudes were in the order of magnitude of an "A" spike. Typically "normal" motoneuron spikes could always be induced by orthodromic and/or antidromic excitation both before and after periods of rapid eye movements.

We postulate that the depolarizing potentials of AS may be due to phasic excitatory influences acting on spinal cord motoneurons during periods of rapid eye movements, and that the small spikes possibly originate in a pacemaker region other than the soma membrane. Supported by grants from the USPHS (NS 09999) and the NSF (BNS 79 12897).

13.1 SIMILARITIES AND DIFFERENCES IN THE STRUCTURE OF SEGMENTALLY HOMOLOGOUS NEURONS CONTROLLING HEARTBEAT IN LEECH. M. R. Shafer* and R. L. Calabrese. The Biological Laboratories, Harvard Univ., Cambridge, MA 02138.

The central pattern generator for heartbeat in the leech, Hirudo medicinalis, comprises an ensemble of synaptically interconnected heart motor neurons (HE cells) and heart interneurons (HN cells). We have investigated the structure of these neurons by iontophoretic injection of the dye Lucifer Yellow.

Bilateral pairs of HE cells have been identified in segmental ganglia 3-19 of the nerve cord. Their structure was found to be nearly identical from ganglion to ganglion and from animal to animal.

Bilateral pairs of HN cells have been identified in segmental ganglia 1-7 of the nerve cord. Their structure was found to vary from ganglion to ganglion. These segmental differences among HN cells were consistently observed from animal to animal. The HN cells also show segmental differences in the types of electrical activity recorded in their cell bodies and in their synaptic connections with the other neurons of the network. Some of these physiological differences were found to correlate with the observed structural differences.

No postsynaptic potentials can be recorded from the cell body of the heart interneuron in the second ganglion, cell HN(2). This observation correlates with the cell's lack of dendritic arbor in that ganglion. Cells HN(3) and HN(4) show similar electrical activity and have similar input and output connections. However, cell HN(3) connects monosynaptically with the ipsilateral heart motor neuron in its own ganglion while cell HN(4) does not. This difference is reflected by the presence of a well developed anterior branch in cell HN(3) that projects into the dendritic arbor of the ipsilateral cell HE(3). Such a branch is not present in cell HN(4).

Some physiological differences among the heart interneurons are not reflected in their dendritic structures. Cells HN(3) and HN(4) inhibit their contralateral homologues while cells HN(6) and HN(7) do not. However, the dendritic arbors of each of the neurons overlaps to the same extent with that of its contralateral homologue.

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13.2 SPIKE TRAIN ANALYSIS OF IDENTIFIED SIPHON MOTONEURONS IN INTACT APLYSIA AND SUBSEQUENTLY REDUCED PREPARATIONS. Lewis Eberly and Harold Pinsker. Marine Biomedical Institute, Depts. of Physiology and Psychiatry, UTMB, Galveston, Texas 77550.

To examine how a simple nervous system mediates different patterns of behavior, implanted cuff electrodes are used to record spontaneous and triggered activity from whole nerves in intact Aplysia californica. Firing patterns of individual units are characterized by analysis of 2-channel recordings based on spike amplitudes and conduction velocities. To record intracellularly from members of the same population, the intact animal is then anesthetized, restrained and surgically reduced to a semi-intact preparation in which the abdominal ganglion is lifted onto a stage. The spike train analysis must recognize the same units in the semi-intact preparation in order to trace the activity of these identified units back to the intact animal.

Electrical activity was recorded from the siphon nerve of intact animals during quiescence and reflex activation of siphon withdrawal. Computer analyzed siphon nerve samples typically contain 8 to 10 units with well isolated spike amplitudes and conduction velocities. After reduction to the semi-intact preparation, siphon nerve activity was again recorded and the same population of units could typically be recognized. Representative members of the population of siphon motoneurons were then identified by intracellular impalement and their extracellular spikes characterized in terms of amplitude and conduction velocity. Once characterized in the semi-intact samples, the same unit could be identified in samples from the intact animal using spike amplitude and conduction velocity as criteria. Additional criteria such as similar responses (excitation or inhibition) during spontaneous Interneuron II contractions and visual inspection of unitary waveforms aid in making the connection between the semi-intact preparation and the intact animal.

This approach will be used to characterize the firing patterns of identified neurons in intact animals and to specify the changes in firing at different stages of the experiment. For example, siphon motoneurons often fire more regularly in the semi-intact preparation and occasionally show prolonged spontaneous bursts, quite unlike their activity in the intact animal. We also hope to fill siphon motoneurons intrasomatically to label their axons in the nerve and to correlate axon diameter and infolding with the amplitudes and conduction velocities used to identify units by spike train analysis. (Supported by NSF grant BNS 76-17480 and NIH grants 16087 and 11255).

13.3 CENTRALIZED CONTROL OF DISTRIBUTED MOTOR NETWORKS IN PLEUROBRANCHAEA. C. S. Cohan* and G. J. Mpitsos (Spon: P. Crago). Dept. of Anatomy, Case Western Reserve Univ., Cleveland, OH 44106.

Coordination of cyclical appendage movements in the marine gastropod mollusc Pleurobranchaea californica has been explained by the coupling of multiple oscillatory centers distributed in the nervous system (Davis, W. J., Siegler, M. V. S., and Mpitsos, G. J., J. Neurophysiol. 36: 258, 1973). The neural responses, which were assumed to represent feeding behavior, were obtained from isolated nervous systems by electrical stimulation of the stomatogastric nerve (STGn), paracerebral command neurons (PCN), and the connective between the cerebral and buccal ganglia (CBC). In studying the mechanisms for coordination of activity between ganglia we first reexamined the evidence for distributed oscillators. The following results were obtained when activity in the CBCs was blocked by means of anodal electrical currents or sucrose gap perfusions: a) blockage during STGn stimulation immediately stopped cerebral ganglion rhythms but did not affect buccal ones; b) short duration blocks did not shift the phase relationship between cerebral and buccal activity; c) blockage during PCN stimulation stopped all cerebral and buccal rhythms. Tonic stimulation of the CBCs produced rhythmic activity in isolated cerebral ganglia as originally reported. In some instances, bursts in the CBC occurred simultaneously with the cerebral bursts, but, when individually isolated, the same CBC produced rhythmic activity to tonic electrical stimulation. Taking these observations together, we conclude that the patterned activity necessary for driving the distributed motor networks in isolated nervous systems arises only from loci in the buccal ganglion.

Intracellular recordings from PCN has shown that these cells are functionally heterogeneous in the types of motor patterns they produce. Moreover, corollary discharge neurons (CDN) in the buccal ganglion are activated during PCN or STGn stimulation, and, in turn, drive specific cerebral motoneurons. In addition, we have found that the body structures innervated by the buccal and cerebral ganglia participate in at least 5 different behaviors (feeding, regurgitation, rejection, defensive bite, and gill grooming), all of which appear to be produced by similar motor patterns, except for the active phase of regurgitation (McClellan, A. D., Soc. for Neurosci., 1978 and 1979). By our present hypothesis, these behaviors might be produced by different "command system requests" that turn on the buccal oscillator(s) and selectively orchestrate the movement of the anterior body structures through the CDN. (NSF BNS 76-8123)

13.4 Leech swimming:effects of interrupting the intersegmental path of swim-initiating vs. pattern generating neurons, and the ability of single ganglia to produce swim bursts. Janis C. Weeks. Dept. of Biology, Univ.Ca.San Diego, La Jolla, Ca. 92093

Two functionally distinct groups of segmentally iterated interneurons participate in leech swimming: 1) "oscillator" interneurons, whose intra- & interganglionic connections have been proposed to comprise the swim central pattern generator, & 2) swim-initiating interneurons. Both classes send interganglionic axons for considerable distances in anatomically separate connectives: oscillator interneuron axons run in the paired, lateral connectives, whereas swim-initiating interneuron axons run in the medial connective, Faivre's Nerve. Thus, section of the medial or lateral connectives interrupts functionally distinct neuronal pathways. The initiation and coordination of the swim motor pattern in isolated nerve cords was studied after producing such lesions in midbody segments. Faivre's Nerve section did not affect intersegmental coordination of the pattern, but interfered somewhat with the simultaneous initiation of the pattern along the cord in response to swim-initiating interneuron stimulation. After section of the lateral connectives, stimulation of a swim-initiating interneuron in either end of the nerve cord caused normally coordinated simultaneous swim activity in both ends, but individual swim cycles in the two ends were randomly phased with respect to each other. Thus, intersegmental coordination is normally mediated by axons running in the lateral connectives.

When cut away from the rest of the nerve cord, pairs of ganglia were able to produce the swim motor pattern, but individual ganglia were not. However, single ganglia left attached to the remainder of the nerve cord by one or both Faivre's Nerves produced swim bursts in response to swim-initiating interneuron stimulation anywhere in the nerve cord. Thus, intersegmental connections among oscillator interneurons are not required for rhythmic pattern generation by single ganglia.

13.5 FLIGHT ACTIVITY EVOKED BY INTRACELLULAR STIMULATION OF GIANT INTERNEURONS IN THE COCKROACH PERIPLANETA AMERICANA.
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Stimulation of giant interneurons (GIs) in the cockroach Periplaneta americana evokes a motor output which is believed to be associated with running in the wind-mediated escape response (Ritzmann and Camhi, J. comp. Physiol. 125: 305, 1978, Camhi and Tom, J. comp. Physiol. 128: 104, 1978). However, when none of the cockroach's legs are in contact with a surface, wind stimuli directed at the animal's cerci evoke flying movements rather than running movements. The initiation of both of these behaviors could be controlled by the GIs, if a switch were interposed between the GIs and the pattern generators for flying and running. The position of this switch would be determined by sensory information on leg contact. To test this hypothesis, we performed experiments to determine whether or not stimulation of GIs can in fact initiate flight in the absence of leg contact.

All legs were removed except the right metathoracic leg, which was left intact to allow for recording from leg nerves. In this leg,nerve 5, which contains the major sensory axons of the leg, was severed. Under these conditions an animal pinned to a cork will perform vigorous flight activity in response to gentle wind puffs. This is characterised as follows: 1. The wing stubs unfold and beat at 20-40Hz, 2. EMG recordings from flight muscles are indistinguishable from those recorded during tethered flight, 3. Leg levator motor neurons burst approximately in phase with wing elevator and depressor muscle potentials. This levator activity is probably involved in maintaining the proper leg posture during flight.

Under these conditions trains of current pulses (73 msec duration/2.0 - 3.5 msec interpulse interval) delivered intracellularly to any dorsal GI will evoke flight activity identical to that described above. This is a dramatic change from the response evoked by the same stimulus to the same GIs when leg contact is maintained. GI stimulation only evokes vigorous flying when nerve 5 of all 6 legs is severed. Moreover, even when they are all severed, stimulation of a single nerve 5 during GI stimulation prevents flight activity from occurring. Thus, the dorsal GIs are capable of initiating at least two completely different locomotor behaviors. The choice of which behavior is initiated at any given time is based, at least in part, on sensory activity in nerve 5 of each leg.

13.6 CENTRAL EFFECTS OF OCTOPAMINE AND SEROTONIN ON POSTURAL MOTOR SYSTEMS IN THE LOBSTER.
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Injection of octopamine into freely moving lobsters (Homarus americanus) causes the animals to assume a rigid, sustained posture characterized by hyperextension of all extremities. Serotonin injection induces flexion. These opposite postural effects are not due to excitatory effects of the amines directly on exoskeletal muscles: on muscles, amines have a synergistic action. Rather, the postures are generated by activation of diametrically opposite centrally co-ordinated motor programs. In root recordings from isolated abdominal ganglia, we have shown that bath application of octopamine selectively excites motor neurons to slow (postural) extensor muscles and the inhibitory neuron to slow flexors while simultaneously inhibiting activity of motor neurons to slow flexors and the inhibitory neuron to slow extensors. Serotonin activates an opposite pattern (Livingstone et al., Science 208:76 (1980).

To understand the mechanisms by which the amines generate these complex motor programs, we have recorded intracellularly from identified motor neurons in abdominal ganglia, primarily I1, the inhibitory neuron to slow flexors, and M15, an excitatory motor neuron to slow extensors. Recording from the somata, we can detect attenuated epsp's and ipsp's, and action potentials arising from the distal spike initiation zone. At least part of the amine effect appears to be on pre-synaptic inputs to the motor neurons: in both I1 and M15, octopamine increases the spontaneous epsp frequency (+255%) while serotonin decreases epsp frequency (-25%). These changes could be due to direct amine effects on the input resistance of the cells; this would raise or lower the signals into the noise level of the recording, causing only an apparent change in epsp frequency. We believe this is not correct for the following reasons: 1) spontaneous and evoked epsp's and ipsp's do not change in amplitude; 2) the ipsp frequency does not change; 3) the attenuated spike recorded in the soma does not change in amplitude; 4) the input resistance and resting potential recorded in the soma do not change. In addition to pre-synaptic effects, octopamine also lowers the apparent threshold for action potential generation in I1 when the spike is induced by current injection into the soma. At this time it is not known whether this effect is a direct action on I1 or results from increased excitatory input localized at the I1 spike initiation zone.

The motor patterns elicited by octopamine and serotonin are strikingly similar to those observed upon stimulation of command fibers for abdominal extension and flexion in crayfish (Evoy and Kennedy, J. Exp. Zool. 165:223 (1967)). Our results support a role for octopamine and serotonin in directing or modulating postural commands in Crustacea. Supported by NIH #NS-07848 and MDA.

13.7 SEROTONIN INCREASES THE PROBABILITY THAT THE LEECH NERVOUS SYSTEM WILL PRODUCE THE SWIM MOTOR PROGRAM.
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The isolated nerve cord of a leech can produce episodes of the swim motor program but usually does so only in response to stimulation of peripheral nerves or of swim-initiating interneurons (Kristan & Calabrese, J. exp. Biol., 65: 643, 1976; Weeks & Kristan, J. exp. Biol., 77:71, 1978). I have found that isolated nerve cords produce episodes of the swim motor program in the absence of such stimulation when they are exposed to $5x10^{-8}$-10^{-5}M serotonin. Such "spontaneous" episodes first occur 2-30 min after serotonin is added to the saline bathing an isolated nerve cord or one or a few ganglia within an isolated nerve cord. They continue to occur for as long as 2-3 hr after washing out the serotonin.

Each segmental ganglion has 7 serotonin-containing neurons. These cells are electrically coupled (Lent & Fraser, Nature, 266: 844, 1977) and include the Retzius cells, which are the largest neurons in the leech. Stimulation of the Retzius cells in a pair of ganglia of an isolated nerve cord is followed by "spontaneous" episodes of the swim motor program if the volume of saline around the ganglia containing the stimulated cells has been reduced to about 50 µl or less. This requirement for a reduced volume in order to see an effect of Retzius cell stimulation on the probability of occurrence of a swim episode suggests that serotonin may act as a circulating effector in the leech.

To test the physiological relevance of the effect of serotonin on isolated nerve cords, intact leeches were injected with 100 µg of serotonin. Swimming behavior of these and of control leeches (both uninjected and saline-injected animals) was recorded by an observer who did not know what treatment the animals being observed had received. In the absence of experimental stimulation, control animals swam relatively infrequently (about once per 10 min observation period per 2 animals), while leeches which had been injected with serotonin swam about 7 times per 10 min observation period. This effect of serotonin injections lasted 5-8 hr.

Thus, either administration of exogenous serotonin to isolated nerve cords or to intact leeches or stimulation of serotonin-containing neurons in isolated nerve cords can result in increased probability that the leech nervous system will produce episodes of the swim motor program. Experiments are in progress to find the neuronal basis for this phenomenon. (Supported by NIH fellowship 1 F32 NS 06182-01 to ALW and by USPHS grant 1 R01 NS14410-01A1 to WBK.)

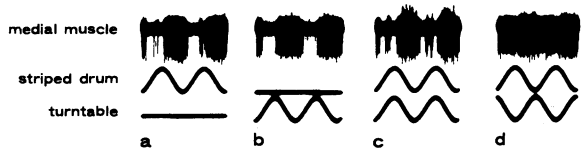
13.8 CEREBRAL CONTROL OF THE EXTRINSIC FEEDING MUSCLES IN APLYSIA.
Steven Fredman and Behrus Jahan-Parwar. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Feeding in Aplysia is the result of the coordinated cyclical activation of both the intrinsic and extrinsic muscles of the buccal mass. The former control the several steps involved in each bite and are innervated by buccal ganglia neurons, while the latter are largely responsible for the overall protraction and retraction of the buccal mass itself and are innervated, at least in part, by several identifiable cerebral ganglion neurons. We have examined the control of two of the extrinsic buccal mass protractors using an isolated brain-muscle preparation. Cerebral neurons innervating these muscles were identified by simultaneous intracellular, extracellular and tension transducer recordings. Cerebral innervation of the protractors is via the upper labial nerve (ULAB) and includes a single ipsilateral B cluster neuron and several neurons in the E clusters. Excitatory motor neurons had axons in the ULAB branches innervating the protractors; caused muscle contractions when depolarized by current injection and 1:1 extracellularly recorded potentials at the muscle. The B neuron is a strong exciter and could produce measureable muscle contractions at firing rates as low as 4 Hz. There are at least 2 excitatory E neurons. In addition 2 E cluster neurons appear to be inhibitory motor neurons for one of the muscles. Intracellular stimulation of these neurons produced no tension changes when the protractor was relaxed, but caused measureable decreases in tension when the muscle had contracted either spontaneously or following intracellular stimulation of an excitatory motor neuron. While the mechanism by which the inhibitory motor neurons produce their effect is not yet known, two possibilities are direct action at the muscle and/or presynaptic gating. E cluster neurons display cyclic firing, with the excitatory motor neurons firing during the rising phase of protractor contractions and the inhibitors firing during the falling phase. Both excitors and inhibitors exhibit synaptic input and firing which is coordinated with the firing of units recorded from the buccal roots and with individual intracellularly recorded buccal ganglia neurons. The mechanisms by which the neurons controlling the intrinsic and extrinsic buccal muscles are coordinated is under investigation. (Supported by Grant NS14388 to BJ-P).

13.9 OCULOMOTOR ACTIVITY DURING COMBINED OPTOKINETIC AND PROPRIOCEPTIVE STIMULATION OF THE CRAYFISH. Richard F. Olivo and DeForest Mellon Jr. Dept. Biol. Sci., Smith College, Northampton, MA 01063 and Dept. Biology, Univ. of Virginia, Charlottesville, VA 22901.

The crayfish's horizontal eye movements are driven by inputs from the visual system (the optokinetic response) and from proprioceptors in the legs; the two inputs add algebraically. We report here on experiments in which we recorded from the muscles that move the eye in the horizontal plane. Crayfish (Procambarus clarkii) were clamped above a turntable on which their legs rested and were surrounded by a striped drum. Fine wire electrodes were inserted in the medial and lateral muscle of the eye. The turntable and striped drum were oscillated sinusoidally through 38° at frequencies from 3 to 23 cycles/min (0.05 to 0.38 Hz), under four conditions: stripes moving with turntable clamped, turntable moving with stripes covered and clamped, stripes and turntable moving together (in phase), and stripes and turntable moving in opposite directions (out of phase).

Preliminary analysis of the data confirms the results of prior experiments on eye movements: optokinetic stimulation is most effective at low frequencies, proprioceptive stimulation is most effective at high frequencies, and the response to combined (in phase) stimulation is greater than the responses to either stimulus alone. Out-of-phase stimulation, which evokes very small movements of the eyes, evokes substantial motor activity. This surprising result is illustrated in the recordings below from a unit in the medial muscle of the right eye. The unit responds to counterclockwise (downward) movement of the stripes (a), the turntable (b), or both together (c). (Brief bursts during the clockwise phases of (a) and (c) correspond to flickbacks of the eye.) The unit's response to out-of-phase stimulation (d) is continuous, as if the unit were driven independently by the counterclockwise phases of each stimulus. This suggests that the reduced eye movements during out-of-phase stimulation are due to simultaneous activation of antagonist muscles, and it implies that at least some of the algebraic summation of visual and proprioceptive stimuli occurs at the level of the muscles themselves.



(Supported by a grant from the Whitehall Foundation).

13.10 Thoracic leg control of abdominal extension in the crayfish, Procambarus clarkii. Charles H. Page. Dept. of Physiology and Bureau of Bio. Research, Rutgers Univ., Piscataway, NJ, 08854.

Postural extensions of the abdomen of the crayfish, Procambarus clarkii can be evoked by mechanical stimulation of single thoracic legs. Movement of a single leg joint is sufficient to initiate an extension response. Vigorous abdominal extensions are initiated either by depression of the whole leg (WLD) or by flexion of the mero-carpal joint (MCF). Similar stimulation of the chelipeds does not elicit an abdominal extension response.

Single frame analysis of motion pictures of crayfish responding to WLD or MCF stimulation showed that the responses evoked by the two different stimulus situations are nearly identical. They differ principally in the responses of the leg located contralateral to the stimulated leg.

Movements of most of the cephalic, thoracic and abdominal appendages accompany the abdominal extension response. Only the eyes remained stationary throughout the response. The mean values of the latencies for the initiation of appendage movement ranged from 125 to 204 msec; abdominal movement had a mean latency of about 220 msec.

The abdominal extension reflex results from the activity of the tonic superficial extensor muscles. The deep phasic extensor muscles are silent during the response. The mean latencies for the initiation of superficial extensor muscle activity by WLD and MCF stimulation were 53.7 msec and 50.0 msec respectively. Supported by NIH grant NINDS #12262.

14.1 ALPHA-ADRENERGIC BINDING SITES IN BOVINE ANTERIOR PITUITARY GLAND AND CONTROL OF ACTH SECRETION IN RAT ANTERIOR PITUITARY CELLS IN CULTURE. L. Beaulac-Baillargeon*, T. Di Paolo*, V. Raymond*, V. Giguère* and F. Labrie* (SPON: C. Radouco-Thomas) School of Pharmacy & Dept. of Mol. Endocrinology, CHUL, Quebec G1V 4G2, Canada.

Three α-adrenergic ligands were used to identify and characterize α-adrenergic binding sites in bovine anterior pituitary membranes. The assay was performed as described (Caron et al., J. Biol. Chem. 253: 2244, 1978). [^{3}H]clonidine binds to a single class of high affinity binding sites at a K_D value of 0.45 ± 0.1 nM with a number of binding sites of 15 ± 4 fmol/mg protein. Specific binding measured in the presence or absence of 1 μM unlabeled clonidine (65% of total binding) is rapid, saturable and stereospecific. The specificity is characteristic of an alpha-adrenergic receptor: clonidine > epinephrine > norepinephrine > dopamine > isoproterenol. Yohimbine is much more potent than prazocin which has no effect below 10^{-6}M. The α-adrenergic antagonists phentolamine (K_D = 6 nM) and dihydroergocryptine (DHEC) (K_D = 15 nM) compete for binding while WB-4101 is less potent (K_D = 60 nM). The β-adrenergic antagonist propranolol and serotonergic agents do not interfere with [^{3}H]clonidine binding. No high affinity sites could be demonstrated for [^{3}H]WB-4101 at concentration up to 60 nM. As assessed in the presence or absence of 100 nM spiroperidol, 10-15% of total [^{3}H]DHEC binding is alpha-adrenergic and can be displaced by α-adrenergic agents. [^{3}H]clonidine binds with high affinity in rat anterior pituitary homogenate. A good correlation is observed between the potency of a series of adrenergic agents to displace [^{3}H]clonidine binding from bovine adenohypophyseal membranes and to modulate ACTH secretion, in rat anterior pituitary cells in primary culture. (-) norepinephrine, (-)epinephrine and clonidine induce a dose-dependent stimulation of ACTH secretion (6- to 8-fold) which can be reversed by phentolamine, yohimbine and DHEC. Isoproterenol and dopamine do not stimulate ACTH secretion up to 1 μM while phenylephrine has little effect. The present data demonstrate the presence of an α_1-adrenergic receptor in bovine anterior pituitary membranes which shows a close correlation with the control of ACTH secretion in rat adenohypophyseal cells in culture. This system should be a useful model to study the mechanisms of action of α-adrenergic receptors.

14.2 [^{3}H]SPIROPERIDOL LABELS DOPAMINE RECEPTORS IN THE BOVINE PARS INTERMEDIA PITUITARY. T. Di Paolo*, P. Poyet* and F. Labrie* (SPON: S. Radouco-Thomas) Department of Molecular Endocrinology, CHUL, Quebec, G1V 4G2, Canada.

Pars intermedia cells secreting ACTH- and β-LPH-related peptides are under stimulatory and inhibitory control by β-adrenergic and dopaminergic agents, respectively. Since the secreted peptides can be measured with precision by specific radioimmunoassays, this system could become a useful model to study the interactions between β-adrenergic and dopaminergic receptors. We have thus used [^{3}H]-spiroperidol, a potent dopaminergic antagonist, to study the dopamine receptor in membranes prepared from the pars intermedia obtained from adult bovine pituitaries. The binding assay was performed as described (Caron et al., J. Biol. Chem. 253: 2244, 1978) using 10 μM (+)butaclamol to determine non-specific binding. [^{3}H]spiroperidol binding shows high affinity with a single class of binding sites (K_D value = 0.7 ± 0.1 nM), saturability (number of binding sites = 300 ± 20 fmoles/mg protein) and reversibility. At 25°C, the second order association rate constant (k_1) is 1.1 ± 0.2 x 10^7 min^{-1} while the first order dissociation rate constant (k_2) is 5.1 ± 1.0 x 10^{-3} min^{-1}, thus giving a ratio (k_2/k_1) of 0.5 ± 0.2 nM, a value in good agreement with that obtained by equilibrium binding data. The rank order of potency of various agonists to compete with [^{3}H]spiroperidol binding sites is consistent with a dopamine receptor: apomorphine > dopamine >(-)epinephrine =(-)norepinephrine > isoproterenol = clonidine. Competition for binding of the tritiated ligand shows stereoselectivity,(+) butaclamol being 1,000 times more potent than (-)butaclamol. Dopaminergic antagonists compete for [^{3}H] spiroperidol binding at nanomolar concentrations whereas adrenergic (phentolamine, propranolol, clonidine, (-)epinephrine, (-)norepinephrine and isoproterenol) and serotonergic (serotonin, cyproheptadine and methysergide) drugs do not compete or are only weak competitors at micromolar concentrations. GTP decreases the affinity of the agonists apomorphine and dopamine for [^{3}H]spiroperidol binding but does not change the potency of unlabeled dihydroergocryptine or spiroperidol. The influence of GTP suggests that the dopaminergic receptor of the pars intermedia pituitary is associated with a dopamine-sensitive adenylate cyclase. The present data show the presence of a typical dopaminergic receptor in pars intermedia and suggest that this system, in analogy with the adenohypophyseal mammotrophs where binding to the dopamine receptor can be correlated with prolactin secretion, could be a useful model where changes of receptor binding could be correlated with changes of secretion of ACTH- and β-LPH-related peptides.

14.3 EFFECTS OF LONG TERM DOPAMINERGIC TREATMENT ON PROLACTIN REGULATION IN THE RAT.
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Evidence suggests that tonic hypothalamic inhibition of pituitary prolactin (PRL) secretion is mediated by pituitary dopamine receptors responding to dopamine (DA) released from tuberoinfundibular neurons into portal blood. Prompted by an interest in observing the effects of a long term increase in DA stimulation on the regulation of PRL, we performed the following experiment. Male Wistar rats were given 16mg/kg of the DA agonist apomorphine in the form of a diester pro-drug apomorphine dipivaloyl ester (ADPE), for 12 days. Following 3 drug free days animals were decapitated and anterior pituitaries and trunk blood collected. Hemipituitaries were pre-incubated for 1 hour in medium 199 at 37°C in a shaking incubator under 95% O_2, 5% CO_2, then transferred to fresh medium. One of each pair was incubated for 3 hours in medium containing 250 nM DA, the other in drug free medium. Media were sampled at 90 mins. and 3 hrs., following which pituitaries were sonicated in saline.

Results of PRL radioimmunoassay revealed (1) lowered serum PRL levels, (2) reduced PRL secretion into drug free medium and (3) reduced pituitary PRL content, in animals pre-treated with ADPE. ADPE treated animals also showed a smaller percentage inhibition of PRL secretion in vitro in response to 250nM DA, but this was not a consistent observation and may simply be a reflection of the greatly lowered basal levels.

In earlier studies on the effect of long term DA agonist treatment, DA agonists have been shown to inhibit growth of cultured mammotrophs and cause tumor shrinkage in vivo. Our results indicate that either mammotroph PRL content or mammotroph numbers are lowered following long term dopaminergic stimulation in vivo, and suggest that downregulation of mammotroph proliferation and prolactin content may be a physiological role of DA.

(Supported by MRC grant #MT4749. G.M.B. is an OMHF Research Associate.)

14.4 CEREBELLAR INFLUENCES ON BASAL AND STRESS-INDUCED CORTICOSTERONE AND ACTH SECRETION IN THE RAT. J. P. Allen and T. Kepic*. Dept. of Neurosciences, Peoria Sch. of Med., Peoria, IL 61656.

The cerebellum is known to have an inhibitory influence on several CNS systems. Its possible role in neuroendocrine regulation is incompletely understood. Therefore, we studied the effects of midline and lateral cerebellar lesions on basal and stress-induced corticosterone (B) and ACTH secretion in 200 gm male Sprague Dawley rats. The study was accomplished by completely removing midline cerebellar (ML) structures or a portion of the left lateral cerebellar (LL) hemisphere using a vacuum operated suction apparatus. Four days following surgery the rats were decapitated immediately at 0700 h (AM) or 1600 h (PM) or following the exposure to a diethyl ether atmosphere for 2.5 min. Trunk blood was collected and plasma B and ACTH concentrations were measured by sensitive and specific radioimmunoassays. There was a reduction ($P < 0.05$) in the mean AM but not PM basal plasma B concentration in those with ML lesions compared to those with LL lesions. In each group there was preservation of the normal diurnal difference in plasma B concentration; however, there was no diurnal difference in plasma ACTH levels. Following the ether stress, mean plasma B levels were significantly higher ($P < 0.01$) in the ML group compared to the LL group. Similarly, the mean plasma ACTH levels were slightly but not significantly higher in the ML compared to LL group. We conclude from our data that an inhibitory outflow from midline cerebellar structures may modify both basal and stress-induced corticosterone secretion in the adult male rat. Moreover, the cerebellum does not appear to significantly alter the pattern of diurnal secretion of corticosterone or ACTH.

14.5 THE OLFACTORY BULB IS RICH IN TRH IMMUNOREACTIVITY. M.S. Kreider*, A. Winokur, N.R. Krieger, Departments of Pharmacology and Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

The tripeptide thyrotropin releasing hormone (TRH) was originally isolated from hypothalamic tissue. Its presence was subsequently demonstrated throughout the central nervous system, including retina, cortex, brain stem and spinal cord. We report that the rat olfactory bulb is rich in TRH immunoreactivity. Tissue was homogenized in 0.15 M NaCl, 0.01 M NaH_2PO_4, pH 7.5 at 0°C, diluted 5 fold with methanol and centrifuged for 10 minutes at 2000 rpm. The supernatant was dried and redissolved in 0.25% bovine serum albumin/PBS at 25°C. TRH content was determined according to the radioimmunoassay method of Bassiri and Utiger (Endocrinol, 90:722 - 727, 1972). The concentration (mean ± S.E.M., n=10) of TRH in olfactory bulb (60 ± 10 pg/mg wet weight) was 23% of the concentration in the hypothalamus, and was at least twice that of other brain regions examined. The 2 olfactory bulbs (mean wet weight 65 mg/2 bulbs) contained 3.9 ± 0.3 ng TRH. The TRH immunoreactivity could be separated into high and low molecular weight forms. The high molecular weight form sedimented at 15,000 xg and eluted in the void volume after gel filtration on Sepharose 4B. The low molecular weight form co-chromatographed with authentic TRH (MW 362) on gel filtration and thin layer adsorption chromatography and caused the release of thyrotropin from pituitary tissue incubated in vitro. The neuronal organization and functions of the olfactory bulb are well described. Studies of the localization and metabolism of TRH in this region may help clarify the role of this tripeptide in the central nervous system.

14.6 IMMUNOCYTOCHEMICAL ANALYSIS OF MAGNOCELLULAR ELEMENTS IN RAT HYPOTHALAMUS: DISTRIBUTION AND NUMBERS OF NEUROPHYSIN, OXYTOCIN, AND VASOPRESSIN CELLS. C. H. Rhodes*, J. I. Morrell and D. W. Pfaff. The Rockefeller University, New York, NY 10021.

A cell by cell analysis of the magnocellular elements in hypothalami of forty Long-Evans (normal) and Brattleboro (diabetes insipidis) rats was done using the unlabeled antibody enzyme technique (PAP) with primary antisera directed against oxytocin (OXY), vasopressin (ADH), and the neurophysins.

In the Long-Evans rat the supraoptic nucleus (SON) has, on the average (uncorrected for section thickness), 3849 OXY and 7729 ADH cells; the paraventricular nucleus (PVN) has 3130 OXY and 2602 ADH cells; and the accessory magnocellular groups in the hypothalamus have 3393 OXY and 1473 ADH cells. The Brattleboro strain animal has similar numbers of cells in these nuclei. (The cells which contain ADH in normal animals were identified in the Brattleboro rat as large, neurophysin negative cells.) Thus, a large fraction of the magnocellular oxytocin and vasopressin producing cells in the rat are located outside of the PVN and SON. One accessory cell group in particular, the anterior commissural nucleus, has 1643 OXY cells, or about 50% as many as PVN. In each nucleus the sum of the numbers of OXY and ADH cells was approximately the number of neurophysin cells.

SON is divided by the optic tract into the principal part and retrochiasmatic SON. In retrochiasmatic SON a majority of the cells contain vasopressin. Within the principal part oxytocin producing cells tend to be found rostrally and dorsally while the vasopressin cells are more ventral. PVN can be divided into three subnuclei, the medial, lateral (as described by Hatton et al., Br. Res. 108:187, 1978), and posterior subnuclei, on the basis of cellular morphology and peptide content. The magnocellular cells of the medial and lateral PVN are closely packed together and nearly round, while those of posterior PVN are more separated and are fusiform in shape with their long axis running in a medio-lateral direction. Medial PVN consists primarily of oxytocin producing cells while lateral PVN is formed of a core of vasopressin producing cells with a rim of oxytocin cells. Posterior PVN contains largely oxytocin producing cells. Both ADH and OXY cells are found in the accessory nuclei.

This work was in part supported by HD-10655.

14.7 Selective Depletions of Somatostatin in Discrete Brain Nuclei by Hypophysectomy, Periventricular Hypothalamic and Medial Basal Amygdaloid Lesions. L.C. Terry and W.R. Crowley. Depts Neurol. and Pharmacol., Univ. Tenn., Memphis, TN 38163.

Several hypothalamic and extrahypothalamic sites that have high concentrations of somatostatin (SRIF)-positive nerve terminals and/or cell bodies are important in the regulation of growth hormone (GH) secretion. GH is capable of inhibiting its own secretion under certain prescribed conditions and a short-loop regulatory feedback mechanism may involve SRIF systems. Immunohistochemical studies have demonstrated the presence of SRIF-positive cell bodies in the periventricular nucleus of the hypothalamus (PVN) and in the medial basal amygdala (MBA). It has been suggested that SRIF in the median eminence (ME) derives from cells located more rostrally because large lesions of the preoptic area (POA) and anterior hypothalamus, or deafferentation of the medial basal hypothalamus reduces SRIF within the ME. It is unclear whether these effects are due to destruction of the PVN SRIF system or to interruption of SRIF-containing fibers from other forebrain structures. The objectives of the present investigations were: 1) to determine the effect of GH removal by hypophysectomy on SRIF in discrete brain nuclei, and 2) to define more precisely the specific projection patterns of the PVN and MBA SRIF-positive cell groups by employing the biochemical mapping approach. To these ends, SRIF was measured in individual nuclei microdissected from frozen sections of the hypothalamus and other forebrain structures in: 1) hypophysectomized (hypox) and sham-operated rats, and 2) animals with discrete lesions placed in the anterior PVN and MBA. The tissue content of SRIF was determined by a specific radioimmunoassay.

The content of SRIF in the ME of hypox rats was reduced significantly by 38%, compared to sham-operated controls (278 ± 53 vs 447 ± 57 pg/ug protein, respectively). Significant reductions of SRIF in the MPOA (50%), arcuate (33%) and periventricular (30%) nuclei were also observed in hypophysectomized animals. No significant changes were detected in the ventromedial, suprachiasmatic, medial, central or cortical amygdaloid nuclei nor in the nucleus interstitialis stria terminalis. Bilateral destruction of the PVN significantly reduced SRIF in the ME (72%)and the rostral PVN(44%) MPOA (33%) and arcuate nuclei (50%). Bilateral MBA lesions significantly decreased SRIF in the ME (36%) and suprachiasmatic nucleus (28%). Similar depletions were also observed following lesions of the stria terminalis.

These data suggest: 1) GH may exert "feedback" effects on specific hypothalamic nuclei that involves SRIF-containing systems, and 2) both the periventricular and amygdaloid SRIF systems may participate in such regulation of GH secretion via their projections to the ME and other hypothalamic nuclei.

Supported by grants from NIH and Univ. of Tenn.

14.8 SYNTHESIS OF SOMATOSTATIN PROHORMONES IN CEREBRAL CORTICAL CELL CULTURES. Richard J. Robbins* and Seymour Reichlin. Endocrine Div., Tufts-New England Med. Center Hospital, Boston, MA 02111.

We have previously reported that dissociated monolayer cultures derived from fetal rat telencephalon produce progressively increasing amounts of immunoreactive somatostatin (IRS) (Delfs et al, Nature 283:676). To demonstrate that this represents de novo synthesis, we exposed the cultures to labeled amino acid precursors of the peptide. During the period of rapid IRS accumulation (Day 13), the cultures were deprived of phenylalanine for 18 hours, and then incubated with ^{3}H-Phe for 15-120 min. This was followed by a "chase" incubation in normal medium (MEM-10% Heat Inactivated Horse Serum) for 0-180 min. Cells were then harvested in 1N acetic acid (HAc), heated to 100°C for 7 min., sonicated, and centrifuged. The supernatant was purified on an anti-somatostatin affinity column and subjected to gel filtration and high performance liquid chromatography (HPLC).

HPLC analysis revealed an IRS which comigrated with cyclic somatostatin-14, and 2 more polar species, eluting earlier on a reverse phase C_{18} column. After a 15 min. pulse, only the most polar peak of IRS was labeled; following 30-60 min. chase the predominant form was an IRS of intermediate polarity. After a 2 hour pulse and a 60 min. chase, the least polar peak of IRS, which comigrated with cyclic somatostatin-14, was labeled.

On a calibrated Biogel P-10 sizing column, eluted with 2N HAc, four types of IRS were noted. The largest form had an apparent molecular weight of 11.5-12.5 K, the intermediate form migrated in the 7.5-8.5 K range, the third species comigrated with cyclic somatostatin-14, and the final IRS was apparently smaller, in the 1200-1400 dalton range.

We conclude that cerebral cortical cells can synthesize somatostatin de novo and that there are two prosomatostatins in these cultures which are precursors in the biosynthetic pathway, as well as a smaller form of IRS which may represent a somatostatin metabolite or a separate type of somatostatin, as has recently been reported in catfish islets.

14.9 EFFECTS OF THE ABLATION OF THE LEFT CEREBRAL CORTEX ON T-CELL NUMBER AND CELL-MEDIATED RESPONSES IN THE MOUSE. K. Bizière, G. Renoux*, M. Renoux*, L. Gyenes*, D. Degenne*, J.M. Guillaumin*, P. Bardos*, and Y. Lebranchu*. Laboratoire d'Immunologie, Faculté de Médecine, B.P. 3223, 37032 Tours Cedex, FRANCE. Laboratoire de Pharmacologie, Faculté de Médecine. Tours.

The nervous system and the immune system are both involved in the maintenance of homeostasis and body integrity in relation to the external environment. They both possess recognition, memory and cell to cell learning through the production of mediators. Several investigators have observed relationships and mutual influences between the thymus and the pituitary gland or the hypothalamus. In the present report we describe the effects of a partial cortical ablation on T-cell differentiation and immune function of female C3H/He mice.

The amount of circulating T-cell selective inducer, relative and absolute numbers of splenic Thy-1-2$^+$ and sIg$^+$(B) cells, direct (IgM) and indirect (IgG) primary α-SRBC responses, cytotoxic (NK, CML, ADCC) responses, lymphoproliferative responses to mitogens (PHA, Con A, PWM) were evaluated and compared to those of sham operated controls, 8 weeks after a partial left decortication.

Cortical ablation : a) suppressed the production of the hormone active on T-cells and, concomitantly a sharp reduction in the number of spleen T-cells, b) depressed the IgG α-SRBC response c) depressed the mitogen-induced lymphoproliferations d) inhibited the NK activity, e) suppressed the CML response to allogeneic cells, and f) increased the ADCC, probably associated with an intact macrophage population.

Our results can hardly be attributed to an increased secretion of glycocorticosteroid hormones resulting from surgical stress and anesthesia. In mice, an excess of steroid hormones induces a reduction in spleen weight, and a depletion of the number of spleen lymphocytes, without altering the functional capacity of a constant number of live splenocytes and preferentially depresses the B cell responses and macrophage activity. In the present study, the spleen and organ weights, the number and viability of spleen lymphocytes, and the number and the activity of B cells were identical in decorticated and sham operated animals, and the macrophage-mediated cytotoxicity (ADCC to CRBC) of spleen cells from decorticates was found above that of controls.

The present findings strongly suggest that, in the mouse, the neocortex plays an important role in maintaining body integrity in response to environment through the control of T cell recruitment and function.

14.10 EFFECT OF CHOLINERGIC AND ADRENERGIC INHIBITION ON THE ADRENAL CORTEX REGENERATION, M. O. Udoye* and K. F. A. Soliman. School of Pharmacy, Florida A&M University, Tallahassee, Florida 32307.

In these experiments, adult Sprague-Dawley male rats weighing 150-250 grams were used. In one experiment animals were sympathectomized by chronic treatment with guanethidine (Ismelin Sulfate). Two groups of rats received daily injection of 30 mg/kg of guanethidine (i.p.) for a period of 7 weeks, while another two groups were placed on the drug vehicle. Adrenal enucleation and sham surgery were performed following the end of the 5th week of guanethidine without interrupting the treatment. Animals were sacrificed by decapitation at 2, 7, and 11 days post-surgery. Plasma and adrenal tissue were assayed for corticosterone. Significantly high levels of plasma and adrenal tissue corticosterone were observed in the 2 day group post-surgery in the guanethidine treated animals in contrast to the low levels of plasma and adrenal tissue corticosterone seen in the 7 day groups of rats. The result from 11 day post-surgery guanethidine treated animals, indicates that guanthidine treatment had no significant effect on corticosterone levels in the plasma or in the adrenal tissue. In another experiment the peripherally acting antimuscarine drug propantheline bromide was used. Adrenal enculeation and sham surgery were performed prior to treatment. Four groups of rats received daily injection of 5 mg/kg of propantheline bromide (i.p.) while the other four groups were placed on the drug vehicle. All treatments started 1 day post-surgery and continued till the animals were sacrificed by decapitation. Plasma and adrenal tissue corticosterone were determined at the high (1800 h) and low (0600 h) points of the circadian rhythm in control rats and in propantheline bromide treated animals 7 and 11 days post-surgery. The obtained results indicate the adrenal tissue corticosterone was significantly lower in propantheline bromide treated groups of rats than in the control animals at 7 and 11 days after surgery. The regenerating adrenal mass was also found to be reduced significantly by propantheline bromide treatment. From these studies, therefore, it seems that cholinergic, and not adrenergic, neural function is probably involved in the regeneration of adrenal cortex. (Supported by a grant from NASA NSG #2183)

14.11 CHANGES IN NORADRENERGIC TRANSMISSION ALTER THE CONCENTRATION OF CYTOPLASMIC PROGESTIN RECEPTORS IN HYPOTHALAMUS. B. Nock*, J.D. Blaustein and H.H. Feder*. Inst. of Animal Behavior, Rutgers University, Newark, NJ 07102

Experiments were designed to determine whether changes in noradrenergic (NA) transmission alter steroid action within hypothalamic target cells in female guinea pigs. We first examined the effects of the dopamine-β-hydroxylase inhibitor U-14,624 on cytoplasmic progestin receptors (PR, measured using a [^{3}H]R 5020 binding assay) in hypothalamus (HYPO) of estradiol benzoate (EB)-primed females. At 12 h after U-14,624 injection, the concentration of PR was 35 % less in HYPO (PR in other brain areas were not affected by U-14,624) in drug treated than in non-drug treated controls. Activation of α-adrenergic receptors with clonidine reversed the effects of U-14,624 on PR in HYPO of EB-primed females, although clonidine had no effect on PR when administered alone. Blockade of α-receptors by either intraperitoneal or intraventricular injection of phenoxybenzamine (Pb) resulted in a reduction of the concentration of PR in HYPO of EB-primed females. There was a 3 - 4 h delay between the blockade of α-receptors by Pb (determined using a [^{3}H]WB 4101 binding assay) and a significant effect on the concentration of PR in HYPO. The lower concentration of PR in HYPO after drug treatment did not appear to be due to the release of adrenal progesterone (P). The concentrations of P in plasma at .5, 2, 4 and 12 h after Pb and at 12 h after U-14,624 injection were not different from control values. Furthermore, the concentration of PR was lower only in HYPO after Pb and U-14,624 treatment, whereas injection of 100 µg P caused a reduction in the concentration of PR in all brain areas examined. The lower concentration of PR in HYPO after drug treatment might be due to interference with the EB-induced increase in the concentration of PR. This hypothesis is supported by the finding that Pb had no effect on HYPO PR in the absence of EB priming. Changes in NA transmission, therefore, might alter EB action in HYPO, in addition to altering target cell sensitivity (through effects on PR concentration) to progestins.

These results indicate that neurotransmitters affect target tissue sensitivity to steroids. The existence of such a relationship may provide a means by which environmental, behavioral, and emotional events could rapidly influence steroid-dependent behaviors and anterior pituitary function.

14.12 DIFFERENTIAL EFFECTS ON WARM AND COLD AMBIENT TEMPERATURE ON BLOOD LEVELS OF BETA-ENDORPHIN AND PROLACTIN IN RATS. W.T.Deeter, III* and G.P. Mueller. Dept. of Physiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20014.

Recent findings indicate that beta endorphin (B-END) may be involved in the stimulatory control of pituitary prolactin (PRL) secretion. To further investigate the neuroendocrine mechanisms controlling pituitary B-END and PRL release, the effects of warm and cold ambient temperature on blood levels of immunoreactive B-END and PRL were studied. Mature male rats were accustomed to daily handling for five days and then placed in lighted and ventilated chambers maintained at either 36°C or 4°C. All blood samples were collected by rapid decapitation and plasma concentrations of B-END and PRL were estimated by RIA. The B-END RIA used here can detect 10 pg of B-END and does not cross-react with related peptides including B-LPH (<3%) (Mueller, Endo,A518,1979). The results of a representative experiment are shown in the table below.

Treatment	B-END (ng/ml)	PRL (ng/ml)
Controls (22°)	0.35±.06	12±1
36° for 30 min	1.08±.10	54±7
4° for 60 min	0.89±.18	6±1

It was observed that exposure to warm ambient temperature evoked significant ($p<0.05$) increases in blood levels of both B-END and PRL. By contrast, cold exposure increased circulating B-END ($p<0.05$) and decreased PRL ($p<0.05$). To better characterize the effects of cold ambient temperature on blood levels of B-END and PRL, a time-course experiment was carried out. In this study blood samples were collected after 7½,15,30,60,180, and 360 min exposure to 4°C. Circulating levels of B-END were maximally elevated (3 fold,$p<0.05$) by 30 and 60 min and then returned to control values (.38±.02 ng/ml) by 180 and 360 min cold exposure. Plasma levels of PRL were significantly decreased from a control mean of 38±10 ng/ml to 23±4 ng/ml by 15 min cold exposure. At the later time intervals circulating PRL was further decreased to a mean of 10±1 ng/ml by 360 min.

Together these results show that warm ambient temperature stimulates the release of both B-END and PRL whereas acute cold exposure stimulates the release of B-END but inhibits the release of PRL. Thus, cold ambient temperature constitutes an enviromental stimulus which has opposite effects on the secretion of B-END and PRL. Although this finding does not preclude a role for opiate peptides in the stimulatory control of PRL, under the condition of cold exposure the acute release of pituitary B-END does not enhance the release of PRL.

15.1 GABA INJECTED INTO THE GLOBUS PALLIDUS ATTENUATES WATER INTAKE ELICITED BY CENTRAL ADMINISTRATION OF ANGIOTENSIN II BUT NOT CARBACHOL. D. L. Jones, D. Sztorc* and G. J. Mogenson. Depts of Physiolgy and Faculty of Medicine, University of Western Ontario, London, Canada N6A 5C1.

The globus pallidus has been shown to receive GABAergic projections from the nucleus accumbens, implicated in the initiation of locomotor activity (Jones and Mogenson, 1980, Am. J. Physiol., 238: R63-R69). The nucleus accumbens has been shown to contribute to oral-motor performance and ingestive behaviors as well as to locomotor activity. Whether the nucleus accumbens to globus pallidus projection also contributes to ingestive behaviors is unknown. The present study investigated the possibility that the nucleus accumbens to globus pallidus GABAergic projection contributes to the initiation of drinking, elicited by the dipsogens angiotensin II and carbachol, injected into the ventricles of chronically cannulated rats.

Rats were prepared with guide tubes implanted above the lateral cerebral ventricle and globus pallidus. Injections of angiotensin II or carbachol made into a lateral ventricle of the rat routinely elicited copious drinking, averaging 10 ml in 15 min, with latency of onset usually less than 3 min. Pretreatment of the globus pallidus with GABA produced a dose-dependent decrease in the volume of water consumed, the number of laps taken during the test interval and an increase in the latency to drink. These responses did not appear to be nonspecific motor effects. There were no effects on lap volume at any dose. Following injections of GABA, the animals continued to explore the home cage and drinking spout and often were observed sniffing at the drinking spout without drinking. In contrast, the administration of GABA into the globus pallidus was without effect at any dose on water intake stimulated by the ventricular administration of the dipsogen, carbachol. There were no alterations in the latency to drink, the amount consumed or the number of laps taken during the test interval.

These results suggest that GABAergic projection from the nucleus accumbens to the globus pallidus is involved in the control of water intake stimulated by central angiotensin II administration but not by carbachol administration. Further, they are consistent with the suggestion that these projections may be part of the interface between the limbic and motor systems necessary for the initiation of the behavior.

(Supported by the Medical Research Council of Canada. D. L. Jones is a Medical Research Council of Canada Fellow)

15.2 BRAIN STEM MECHANISMS IN CONTROL OF SALT INTAKE AND BLOOD PRESSURE. Robert J. Contreras & Paul W. Stetson.* Dept. of Psychol., Yale University, Box 11A Yale Station, New Haven, CT 06520.

Area Postrema (AP) and the nucleus of the solitary tract (NST) are two adjacent structures in the dorsal medulla. In rats, the AP is a midline structure at the caudal end of the 4th ventricle; it has been implicated as a chemoreceptor trigger zone for integration of emetic responses. The AP can be influenced chemically via the blood stream due to the absence of a blood brain barrier in this region, and possibly via the cerebrospinal fluid. Ventrolateral to the AP is the NST, which receives incoming afferents from gustatory and visceral receptors. The AP and the NST, together, due to their location, composition and proximity to each other, may integrate information from a variety of sources. More recent reports suggest a role for the AP and NST in cardiovascular function. Damage to these structures produced a chronic labile hypertension, although the relative contribution of each structure to the syndrome is a subject of controversy. We have obtained the following data that may speak to this conversy.

18 experimental rats received lesions to the AP while 6 control rats received sham lesions. After the lesions, we gave the animals a two-bottle preference test between water and various molar concentrations of NaCl (.03, .1, .3, .5), glucose (.1, .3) or KCl (.1, .3) solutions. We found that AP lesioned rats exhibited a significant increase in intake of the NaCl solutions, but their intakes of glucose and KCl solutions were unchanged. These changes in intake were apparently not secondary to changes in output. Urinary sodium and potassium levels were the same for both groups of rats while on a control, sodium replete diet or on a sodium free diet. Histological observations of the brains revealed a significant correlation between the lesion and the salt intake behavior of the rat. Rats, with lesions restricted to the AP, showed an enhanced salt intake. When the AP was partially spared or when the lesion extended well into the NST, the rats did not, however, exhibit an increased salt intake.

We believe that these changes in intake are secondary to the hemodynamic effects of the lesions. From our data as well as data from other labs, we propose that hypertension results from damage to the NST, not to the AP. AP ablations result in hypotension and hypovolemia and the animals may try to compensate by drinking more saline. It would be counterproductive for a hypertensive, NST lesioned rat to increase its saline intake, inasmuch as excessive salt intake leads directly to hypertension in rats. Our data suggest that the AP and the NST may operate synergistically to maintain sodium balance and normal blood pressure.

This research was supported by NIH, NHLBI Grant HL-24732.

15.3 EXAGGERATED SODIUM APPETITE AND ANGIOTENSIN-ELICITED DRINKING BY AREA POSTREMA-LESIONED RATS. Gaylen L. Edwards and Robert C. Ritter, Department of Veterinary Medicine, University of Idaho, Moscow, ID 83843 and Department of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Recently we reported that lesions of the area postrema (AP) enhanced ingestion of glucose solutions and preferred foods (Edwards and Ritter, Neurosci. Abstr., Vol. 5, p. 215, 1979; Ritter and Edwards, Neurosci. Abstr., Vol. 6, 1980). These findings implicate the AP in the control of food intake. Several reports also suggest AP involvement in control of fluid and electrolyte balance. In order to investigate potential AP participation in the behavioral control of fluid and electrolyte regulation, we have tested AP-lesioned rats for drinking in response to AII, isoproterenol, cell dehydration and water deprivation. Our results show that AP-lesioned rats drink more than two times as much distilled water as intact rats when treated with subcutaneous AII. For example, AP-lesioned rats drank 8.6 ± 2.6 ml while intact rats drank 3.2 ± 0.9 ml in response to subcutaneous AII (2 mg/kg). Lesioned rats also drank more than intact rats in response to isoproterenol (50, 100 and 150 ug/kg). This difference was most evident during the first 60 min of a 120 min test. Drinking by AP-lesioned rats in response to water deprivation or cell dehydration did not differ from that of intact rats. These results suggest a role for the AP in control of extracellular thirst.

Because sodium intake, as well as water intake, is essential for maintenance of extracellular fluid volume, we examined sodium balance and sodium appetite in AP-lesioned rats. Plasma sodium concentrations, plasma AII concentrations, sodium intake and sodium excretion are indistinguishable for AP-lesioned and intact rats maintained on lab chow and distilled water. Furthermore, 24 hr sodium excretion and urine volume does not differ between intact and AP-lesioned rats deprived of food. Nevertheless, the NaCl preference threshold of AP-lesioned rats is one to two orders of magnitude below that of intact rats. Lesioned rats also consumed significant amounts of 2.5% and 3.0% NaCl. These salt concentrations were virtually rejected by intact rats.

Our data suggest that the AP is involved in inhibition or modulation of extracellular thirst and sodium appetite. AP-lesions may result in release from inhibition and enhancement of extracellular thirst and sodium appetite. Further studies concerning the neuroendocrine and chemosensory role of the AP in mediating these behavioral responses are in progress.

15.4 NEW EFFERENTS AND AFFERENTS OF THE SUBFORNICAL ORGAN: A STRATEGIC NEURAL CIRCUITRY FOR THE CONTROL OF WATER BALANCE BEHAVIORALLY AND PHYSIOLOGICALLY. Richard R. Miselis. Animal Biol., Sch. Vet. Med., Inst. Neurol. Sci., Univ. of Penn., Phila. PA 19104.

The subfornical organ (SFO), one of the circumventricular organs of the brain, has attracted attention because of its role in drinking behavior, water balance and cardiovascular function. The SFO mediates drinking to angiotensin II (AII) and perhaps contributes to drinking to hypovolemia. Lesions of the anteroventral third ventricular area (AV3V) which includes the nucleus medianus (NM) and the organum vasculosum of the lamina terminalis (OVLT), cause drinking deficits, plasma Na and osmolality imbalances and transient hypertension. In previous work we described efferent projections from the SFO which were appropriate for these functions (Sci. 205:1022, 1979). It projects to the NM, OVLT and supraoptic nucleus (SON). Further work where ^{3}H leucine and ^{3}H proline were slowly infused into the SFO and adjacent structures shows additional projections from the SFO and some afferents to it. Efferent projections were observed passing to the NM, OVLT, SON (anterior and tuberal) the entire paraventricular nucleus (PVN), nucleus circularis, dorsal perifornical area and periventricular area of the hypothalamus. Precommissural fibers of passage emerging from the SFO remain on the midline and either descend along the anterior border of the NM or enter the periventricular area beneath the anterior commissure. Postcommissural fibers enter the columns of the fornix and then separate with the ventral stria medullaris (SM) as it descends into the hypothalamus. It separates from the SM to pass medially and laterally to the column of the fornix at the anterior dorsal level of its course through the hypothalamus. Small injections into the dorsal area of the medial septal nucleus and the anterior ventral triangularis nucleus of the septum (TS) resulted in labelling of the SFO, implicating these nuclei as afferent sources to the SFO. However, small injections into the dorsal TS did not label the SFO. The connectivity described above reveals the strategic relationship between the SFO and the magnocellular neurosecretory system of the hypothalamus. With its lack of a blood brain barrier giving it a view of the plasma and its connectivity, the SFO can modify water balance via an influence on ADH secretion. Its projection to the AV3V area and hypothalamic areas such as the PVN known to have a considerable projection field other than its projection to the posterior pituitary may provide the neural substrate for the behavioral response.

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15.5 IMPAIRED DRINKING RESPONSES OF RATS WITH LESIONS OF THE SUBFORNICAL ORGAN: NONSPECIFIC FEATURES. Jean A. Hosutt*, Neil Rowland*, and Edward M. Stricker. Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

Electrolytic lesions of the subfornical organ (SFO) in rats are known to abolish their drinking response to intravenous (iv) infusion of angiotensin II(AII). Such lesions also attentuate drinking to 20% polyethylene glycol solution (PEG; 5 ml sc), suggesting that AII may play an important role in mediating thirst during hypovolemia. However, we now report that rats with SFO lesions, which showed impaired drinking in response to 20% PEG, usually drank normal amounts when larger plasma volume deficits were caused by 30% PEG treatment. They also drank normally in response to 20% PEG when pretreated either with caffeine (12.5 mg/kg, ip) or with a small dose of hypertonic saline (0.5 ml of 1 M NaCl, ip). These results indicate that rats with SFO lesions are not incapable of drinking when hypovolemic, but may simply require a greater amount of stimulation before they do so.

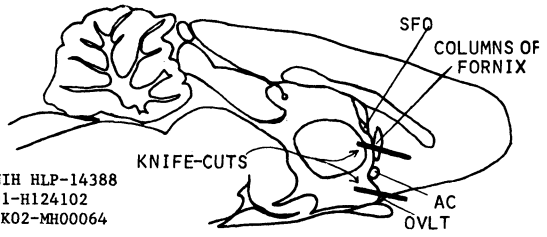
A second series of experiments reexamined the drinking response of lesioned rats to AII. Although rats with SFO lesions usually did not drink after AII administration (up to 128 ng/min for 60 min, iv), they did drink when pretreated with caffeine (12.5 mg/kg, ip). Furthermore, the lesioned rats usually failed to drink in response to a low dose of hypertonic saline (0.5 ml of 2 M NaCl, iv) which provoked drinking in control rats, but drank normally when given a larger dose (2 ml of 2 M NaCl, iv). In general, the same rats that showed drinking deficits in response to AII also had impaired responses to hypertonic saline. These results indicate that the drinking impairment in rats with SFO lesions may not be specific to AII receptor loss. Instead, the lesioned animals seem to be insensitive to relatively low levels of all thirst-provoked stimuli, but can be induced to drink water either by raising the level of such stimulation or by increasing the general activation of the animals.

15.6 KNIFE CUTS BETWEEN THE SUBFORNICAL ORGAN (SFO) AND ANTERO-VENTRAL THIRD VENTRICLE (AV3V) BLOCK DRINKING TO PERIPHERAL ANGIOTENSIN II. R. Wallace Lind and Alan Kim Johnson, Department of Psychology & the Cardiovascular Center, University of Iowa, Iowa City, IA 52242

Ablation and injection studies (Johnson and Buggy, AJP,234(3): R122-R129, 1978; Simpson, et al., JCPP,92(4):581-601,1978; Phillips, et al., Fed.Proc.,38:438,#1204, 1978) have implicated the SFO, the organum vasculosum of the lamina terminalis (OVLT), and the periventricular tissue of the AV3V in the dipsogenic action of angiotensin II. Anatomical studies by Miselis et al. (Science, 205: 1022-1024, 1979) have identified efferents from the SFO to the AV3V and electrophysiological data from Buranarugsa and Hubbard (J. Physiol. (Lond), 291:101-116, 1979) suggest that these fibers may reside in the columns of the fornix. The present experiment examined angiotensin-induced drinking in rats with knife-cuts separating these circumventricular organs from the dorsal AV3V.

Eighteen rats were tested before and after surgery for drinking responses to subcutaneous injections of hypertonic saline (12%, 1 ml/100 g) and angiotensin II (2 and 4 mg/kg). A Knigge knife was used to make a circular cut of approximately 3 mm diameter between the SFO and anterior commissure (AC) in six animals and between the OVLT and dorsal AV3V in six others. The approximate size and position of these cuts are represented in the figure below. The remaining six rats served as controls.

The controls and the rats with the ventrally placed knife-cuts evidenced no long-term deficits in drinking to either dipsogen following surgery. However, the rats that received cuts between the SFO and AC manifested a specific and persistent abolition of drinking responses to angiotensin II. Drinking to hypertonic saline was unaffected. These animals were found to have bilateral interruptions of the columns of the fornix ventral to the SFO with inconsistent damage to the septum, dorsal thalamus, and fibers of the stria medullaris. These findings converge with the work cited above to suggest that the subfornical organ is a receptor site for the dipsogenic action of peripheral angiotensin with efferents passing via the columns of the fornix to the dorsal AV3V.



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15.7 LESIONS OF THE ORGANUM VASCULOSUM OF THE LAMINA TERMINALIS INHIBIT HYPERTONIC NaCl AND ANGIOTENSIN INDUCED DRINKING IN DOGS. T. N. Thrasher*, J. B. Simpson, L. C. Keil* and D. J. Ramsay. Department of Physiology, University of California, School of Medicine, San Francisco, CA 94143 and Ames Research Center, NASA, Moffett Field, CA 94035

The tissue surrounding the anteroventral region of the 3rd ventricle (AV3V) has been suggested to play a role in body fluid regulation. The importance of this region in drinking and vasopressin (AVP) secretion in response to angiotensin II (20 ng/kg/min, iv) and 5% NaCl (3.8 ml/min, iv) was tested before and after electrolytic destruction of the AV3V in dogs. In addition, maintenance of salt and water balance was examined in these dogs. Accurate placement of the lesion was accomplished by injecting contrast media (Conray) into the third ventricle and taking an X-ray photograph which allowed visual verification of the electrode position. In 5 dogs histological examination indicated damage confined to the midline, bounded superiorly by the anterior commissure and inferiorly by the floor of the 3rd ventricle and included destruction of the organum vasculosum of the lamina terminalis, anteriorly. Mean intake of water in response to hypertonic NaCl was 210 $\pm$ 37 ml and fell to 54 $\pm$ 37 ml after lesioning. Angiotensin-induced drinking fell from 188 $\pm$ 26 ml to 83 $\pm$ 42 ml after lesioning the AV3V. The effect of the lesion on daily salt and water balance in these dogs was variable. One dog was completely adipsic but would readily lap a commercial liquid beverage mix. Other dogs showed a reduction in water intake and a mild elevation in plasma osmolality or no change at all. The increase in plasma AVP induced by hypertonic NaCl appeared blocked by AV3V destruction but the lesion did not alter the AVP secretion in response to angiotensin.

Subfornical organ (SFO) lesions in the dogs block angiotensin-induced drinking but not drinking in response to hypertonic NaCl. Lesioning the AV3V blocked NaCl induced drinking and also reduced drinking to angiotensin. In order to account for these two sets of results, we suggest that the SFO is essential for angiotensin-induced drinking and that the AV3V region contains osmoreceptive elements which are important in NaCl induced drinking. We have previously shown that intracranial osmoreceptors must lie outside of the blood-brain barrier (Thrasher et al., 1980, Am. J. Physiol.). Large lesions of the AV3V destroy these elements and also cut fibers from the SFO, thus affecting angiotensin induced drinking. Small lesions of the AV3V, specifically affect NaCl drinking leaving angiotensin drinking intact.

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15.8 KNIFE CUTS OF THE ANTERIOR STALK OF THE SUBFORNICAL ORGAN PRODUCE DRINKING DEFICITS TO ANGIOTENSIN II BUT NOT TO OTHER DIPSOGENIC CHALLENGES. Ricardo Eng, Richard R. Miselis, and Gitta Salanga*. Inst. Neurol. Sci., Anim. Biol., Sch. Vet. Med., Univ. of Penn., Philadelphia, PA 19104.

The subfornical organ (SFO) and the anteroventral third ventricular (AV3V) area have been shown to play a role in the dipsogenic action of angiotensin (AII). Lesions of either of these structures abolish AII-induced drinking. Anatomical studies from this laboratory have demonstrated SFO efferents which pass via its anterior stalk to the nucleus medianus and the organum vasculosum of the lamina terminalis (OVLT), which are within the AV3V area, the supraoptic nuclei (SON), the paraventricular nuclei, the nucleus circularis, the periventricular area, and the perifornical nucleus. In the present study we made wire knife cuts of the anterior stalk to test the role of SFO efferents in mediating the drinking response to AII. The anterior stalk of the SFO in adult male albino rats was cut using a retracting 2 mm. wire knife made from a modified Hamilton one microliter syringe. An intrajugular catheter was implanted and externalized through a head cap. After a brief recovery period, the rats were remotely infused with AII (192 ng/kg/min. for 20 min. i.v.). In other drinking tests, the rats were challenged with hypertonic saline (10% NaCl, 1cc/rat, s.c.), 20-hr. water deprivation or 24-hr. food deprivation. At the conclusion of these experiments the rats were perfused, the position of the catheter tip was verified, and the brains were processed in celloidin (40 µm sections, cresyl violet) for histological analysis. Rats that did not drink to AII (1/2 hr. test, 3 tests each on different days) had transections of the anterior stalk (6 rats). Rats with cuts sparing the stalk drank 1.6 $\pm$ .3 ml. and intact brain rats drank 1.7 $\pm$ .2 ml. In the two hours after hypertonic saline, rats with cuts of the anterior stalk drank 6.3 $\pm$ 1.3 ml., rats with control cuts drank 7.4 $\pm$ 1.1 ml. and intact brain rats drank 7.2 $\pm$ 1.7 ml. In the first two hours of water access after a 20-hr. water deprivation rats with the anterior stalk cut drank 21.6 $\pm$ 3.0 ml., rats with control cuts drank 18.3 $\pm$ 6.7 ml. and intact rats drank 17.0 $\pm$ 2.3 ml. During 24 hours of food deprivation rats with anterior stalk cuts drank 54.8 $\pm$ 4.8% control cut rats drank 56.5 $\pm$ 10.2%, and intact brain rats drank 33.8 $\pm$ 5.5% of their respective mean intakes when food was available. These results support a role for the anterior stalk SFO efferents in i.v. AII-induced drinking and exclude a primary role for SFO neurosecretion in mediating the behavioral response. (Supported by the Sloan Foundation, RR-07083 and ITG MH 15092-03)

15.9 ANGIOTENSIN-INDUCED SODIUM APPETITE: EFFECTS OF SODIUM BALANCE AND POSSIBLE SYNERGY WITH MINERALOCORTICOIDS.

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Continuous intracerebroventricular infusion (cICV) of angiotensin II (AII) elicits ingestion of both water and Na^+ solutions (Bryant et al., Fed. Proc. 37:1978; Avrith and Fitzsimons, J. Physiol. 282:1978). The salt intake is dose-related and specific for Na^+. However, cICV AII also results in excessive Na^+ loss (Severs et al., Pharmacol. 6:1971) and thus the Na^+ ingestion may be a behavioral compensation for this loss. We therefore examined the temporal relationship between Na^+ ingestion and urinary Na^+ loss during cICV AII. Animals were housed in metabolism cages with water and 3% NaCl available ad lib but without food. Intakes and urinary Na^+ loss were monitored every 1/2 hr during an 8hr infusion of either AII (60ng/5µl/hr; n=10) or isotonic saline (5µl/hr; n=7).

Na^+ ingestion is increased within the first 1/2 hr of the AII infusion and by 2 hrs is approximately 540µEq Na^+. During this time intake exceeds urinary Na^+ loss and consequently the AII-infused animals are in positive Na^+ balance. However, during the next 6hrs, the rate of Na^+ ingestion slows while urinary loss continues and the AII-infused animals descend rapidly into negative Na^+ balance (-2.1mEq Na^+ at 8hrs). When the AII infusion is extended to 24hrs Na^+ ingestion is markedly increased.

When 8hr of cICV AII is combined with a mineralocorticoid (DOCA; 2mg sc), the negative Na^+ balance is reduced although not completely abolished (-1.1mEq Na^+ at 8hrs). Nevertheless, 24hr Na^+ intake during this combined treatment remains unchanged. Moreover, when the AII infusions and DOCA treatments are continued for three successive days an excess Na^+ intake is elicited by as little as 60pg/hr of AII which is two orders of magnitude less than the minimally effective dose when exogenous mineralocorticoid is not given. Intake increases with increasing dose of AII. The magnitude of the intake exceeds the sum of the intakes that are produced by the DOCA and the AII acting alone, and is therefore potentiated.

Thus, 1) cICV AII alone elicits an early phase of Na^+ ingestion that is not the result of prior Na^+ loss but the magnitude and maintenance of the sodium appetite is probably affected by Na^+ loss. And 2) when DOCA is combined with AII, at a dose which attenuates the AII-induced Na^+ loss, the appetite is potentiated. Therefore, since both endogenous AII and mineralocorticoids are concurrently elevated during sodium deficiency, the sodium appetite may be aroused by a synergy of the peptide and steroid. Supported by NS-03469 & MH07753-02.

15.10 ATTENUATION OF SPONTANEOUS AND ANGIOTENSIN STIMULATED DRINKING DURING OESTROUS CYCLE OR CENTRAL OESTROGEN TREATMENT.

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In adult female rats with continuous access to water and 1.8% NaCl, 24 hr. ad lib fluid intakes were reduced on the day of oestrus compared to other days of the cycle. When thirst and sodium appetite were acutely stimulated by intracerebroventricular [ICVT] injections of angiotensin II (AII, 100 ng/ul), both water and saline intakes were again depressed on the day of oestrus, mirroring the fluctuating pattern of fluid intakes seen with spontaneous drinking. The ratio of saline to water intake remained constant, however, for both AII-stimulated and ad lib conditions. In contrast, after ICVT injections of carbachol (CARB, 200 ng/ul) which acutely stimulated water but not saline intake, the volume drunk was similar regardless of the day of oestrous cycle. These data provide two functional contrasts between CARB-stimulated versus AII-stimulated and ad lib drinking, namely, sodium preference and variation of amount drunk across the oestrous cycle. Since oestrogen levels peak the day before oestrus and since drinking evoked by central AII injection as well as ad lib drinking is depressed at oestrus, we decided to determine whether an action of oestrogen directly on the brain could modulate certain thirst states. Adult male and adult, ovariectomized female rats with chronic cannula terminating in the anteroventral third ventricle at the preoptic-hypothalamic level (AV3V) were used. Thirst states were each studied for four consecutive days. On the second day of each test series, 1 ug of oestradiol benzoate or vehicle was injected into AV3V 2 hrs before the test period. CARB (125 ng/ul, AV3V) or hypertonic saline (5 ml of 12% NaCl/kg, SC) induced drinking was unaffected by central oestrogen treatment. In contrast, 24 hr. ad lib and AII-induced (100 ng/ul, AV3V) drinking were depressed one day but not two days after central oestrogen treatment in overiectomized females (but not males!). The time course of central oestrogen effect is consistent with a delayed time course for expression of steroid action. The full recovery 2 days after oestrogen treatment implies a rapidly reversible central effect; subcutaneous injection of this dose of oestradiol had no effect. Preliminary data suggest that the effect of oestrogen on central AII mechanisms is not limited to the thirst response since pressor responses to AV3V injections of AII are also attenuated the day after central oestrogen treatment. From these studies, we conclude that oestrogen reversibly affects female brain sites to modulate certain forms of thirst, perhaps through an action on central AII receptors. Supported by American Heart Association.

16.1 DUPLICATION BY LANTHANUM OF SOMATOTOPIC AND OTHER OPIATE ANALGESIA EFFECTS WITH TOTAL NALOXONE REVERSIBILITY. P. Keresztes-Nagy* and J.P. Rosenfeld, Cresap Neuroscience Lab., Dept. of Psychology, Northwestern University, Evanston, Illinois 60201.

1μ mole of Lanthanum (La^{3+}) was microinjected into midbrain Periaqueductal Gray (PAG) of rats. Latency to paw lick (hot plate test), tail flick, and face-rub (to noxious heat; Rosenfeld, J.P., et al., Physiol. Behav., 21:287, 1978), and aversive reaction to midbrain Dorsal Central Gray stimulation were tested. Compared to saline controls, La^{3+} increased the response latency to hot-plate and tail-flick by 180% ($p<.05$), and the latency to left and right face-rub by at least 250%. Many rats' responses were totally blocked. The difference between the face-rub and tail flick effects was significant at beyond the $p<.01$ level. However La^{3+} had no effect on aversive midbrain stimulation. The analgesic effect of La^{3+} on hot-plate, tail-flick and face-rub was completely blocked by coinjection of 1μ mol Ca^{2+}, or by 15 min. pre-treatment with 20 mg/kg naloxone. These findings support the existence of a descending inhibitory analgesia system since La^{3+}, like morphine (Rosenfeld and Holzman, Brain Res. (1977) 124:367-372) and enkephalin (Rosenfeld and Keresztes-Nagy, Pain, 1980, in press) does not alter central nociceptive thresholds even while producing profound peripheral analgesia. The striking duplication by La^{3+} of both somatotopic (face vs tail effects) and differential central-peripheral effects of opiates, and their complete naloxone and Ca^{2+} reversibility suggest that both types of substance produce analgesia by interfering with Ca^{2+} metabolism specifically at opiate receptor neurons.

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16.2 ALTERATIONS IN RAT CENTRAL NERVOUS SYSTEM ENDORPHINS FOLLOWING TRANSAURICULAR ELECTROACUPUNCTURE. L. Ng, R. Dionne, E. Bragin,* C. B. Pert and A. Pert. Biological Psychiatry Branch, NIMH and Neurobiology and Anesthesiology Branch, NIDR, Bethesda, MD 20205

Several lines of evidence suggest that acupuncture may induce some of its analgesic actions by activating, at least in part, the endogenous pain suppression mechanism encoded by opioid peptides. We have evaluated the effects of auricular electroacupuncture in rats on levels of endorphins in the cerebrospinal fluid (CSF), plasma and various brain regions. Electroacupuncture, sufficient to induce analgesia, produced a significant increase in cerebrospinal fluid levels of endorphins as assessed by the radioreceptor assay. Gel filtration chromatography revealed that rat CSF contains only endogenous opiate ligand material with enkephalin-like molecular weight. This ligand was not only increased by acupuncture treatment, but was also shifted slightly toward higher molecular weight fragments. Concomitant with an increase of endorphins in CSF, there was also a decrease of endorphins in the hypothalamus, medial thalamus and periaqueductal gray matter as assessed by the radioreceptor assay. The radioimmunoassay for β-endorphin revealed only a decrease in β-endorphin-like immunoreactivity in the hypothalamus and medial thalamus. These data, taken together, suggest that acupuncture may activate the central endorphin systems coded by both β-endorphin and enkephalins. This activation results in a depletion of both opioids in strategic brain regions as well as a concomitant increase in the CSF of enkephalin-like material. While auricular acupuncture had a significant effect on brain endorphins, no change was seen in plasma endorphins as assessed by the radioreceptor assay. This seems to rule out the participation of the hypophyseal endorphin system in acupuncture analgesia.

16.3 ADRENALECTOMY ENHANCES ENDOTOXIC SHOCK SUSCEPTIBILITY AND ALTERS THE THERAPEUTIC EFFECTS OF CENTRALLY OR PARENTERALLY INJECTED NALOXONE. John W. Holaday and Alan I. Faden. Dept. of Med. Neurosci., Div of N.P., Walter Reed Army Inst. of Res., Wash. DC 20012

In prior work, we have shown that naloxone reverses the hypotension and improves recovery from shock produced by endotoxemia, hemorrhage, spinal transection, and spinal injury in a variety of species. Hypophysectomy was shown to block the therapeutic effects of naloxone in rat hemorrhagic shock (Neurosci. Abs. 5:528, 1979), suggesting that pituitary endorphins were involved in shock hypotension. Adrenalectomy (adrex) is known to elevate pituitary endorphins (Guillemin et al, Science 197:1367, 1977). The purposes of the present studies were to evaluate the effects of adrex upon endotoxic shock susceptibility and to determine the therapeutic efficacy of centrally and parenterally administered naloxone in sham-adrex and adrex rats.

One to two weeks following adrex or sham-adrex in male Sprague Dawley rats (250-350 g), cannulae were surgically placed in the tail artery and external jugular vein; a guide-tube was affixed to the cranium for drug injections into the lateral ventricle (ivt). Conscious rats were studied. Adrex itself resulted in a significant decrease in mean arterial pressure (MAP) of 20 mm Hg and increased heart rate (HR) 60 beats/min. Sham-adrex rats were injected intravenously (iv) with 15.0 mg/Kg lipopolysaccharide endotoxin which was 50% lethal in 24 h. All adrex rats injected with as little as 0.25 or 1.0 mg/Kg endotoxin died within 2 h. When MAP fell 20-30 mm Hg, 30 μg naloxone was injected ivt. This central injection produced a robust increase in MAP only in rats with intact adrenal glands. Fifteen min later, all rats received a second dose of naloxone (3.0 mg/Kg naloxone iv). This parenteral naloxone dose had therapeutic effects in both adrex and sham-adrex rats.

These results show that endotoxic shock is reversed by centrally injected naloxone only in rats with intact adrenal glands. From these studies we conclude that the enhancement of endotoxic shock suceptibility produced by adrex in rats is not solely a consequence of the feedback-mediated elevations in pituitary endorphin levels, but may also involve the absence of adrenal corticosteroids and/or catecholamines.

16.4 THE EFFECTS OF CHRONIC HEAT EXPOSURE ON OPIATE CHALLENGE AND THE OPIATE-LIKE EFFECTS OF ELECTROCONVULSIVE SHOCK (ECS) IN RATS. G.L. Belenky, V.E. Bates and J.W. Holaday. Dept. of Med. Neurosci., Walter Reed Army Inst. of Res., Washington, DC 20012.

We have previously shown that electroconvlusive shock (ECS) produces naloxone sensitive, opiate-like effects in rats. Prior work has also indicated that naloxone challenge in acutely or chronically heat-exposed rats produces opiate withdrawal-like signs (Holaday et al, PNAS 75, 2923, 1978). Because of the putative activation of endorphin systems by these two stressors, we were interested in evaluating the possible alteration of the opiate-like effects of ECS following chronic heat exposure. Additionally, we have shown that repeated ECS sensitizes rats to opiate challenge and, in a complimentary study, chronic morphine exposure sensitizes rats to the opiate-like effects of ECS. A further purpose of these present studies was to determine if chronic heat exposure, like chronic ECS, also potentiates the effects of challenging doses of opiates.

Our subjects were male, Sprague Dawley rats (250-300g). Transauricular ECS was delivered for 2 sec. at 160 v, 60Hz in rats maintained at 23°C or 35°C for 5 days. The duration of tonic-clonic seizures following ECS was significantly reduced in the heat-exposed rats (23.2 + 2.5 sec at 23°C; 15.2 + 1.0 sec at 35°C, $p<.01$). Heat alone significantly diminished both hot-plate escape latencies and tail-flick latencies and increased both colonic temperature and respiratory rates. The increased hot-plate escape latencies, increased colonic temperatures, and decreased respiratory rates produced by ECS were not significantly modified by chronic heat exposure when adjusted for the altered baseline values characteristic of heat-exposed rats. However, the transitory increase in tail-flick latencies produced by ECS was completely blocked by chronic heat exposure. Additionally, when these same rats were tested for catalepsy following ECS, there was evidence for a diminished cataleptic response in the heat-exposed animals. The decrease in some of the opiate-like effects of ECS produced by heat exposure suggests the possibility of cross-tolerance to some of the effects produced by these two stressors. In additional studies, we found that chronically heat-exposed rats were sensitized to the analgesic and cataleptic effects produced by central or parenteral opiate injections, a finding consistent with the cross sensitization between repeated ECS and responses to subsequent opiate challenge (vide supra).

Collectively, these findings suggest that the physiological activation of endorphin systems may result in cross-tolerance to some measures across stress systems. However, these same stressors (heat or ECS) potentiate the pharmacological effects of challenging doses of opiates.

16.5 CHRONIC NALOXONE INDUCES ABSTINENCE-LIKE SYNDROME. David H. Malin, Michael P. Layng* and Paul R. Swank*. Univ. of Houston at Clear Lake City, Houston, Texas 77058.

Prolonged blockade of endorphin receptors eventually produced a state which resembled an opiate abstinence syndrome, without any exposure to opiates. Rats were injected with 0.6 mg/kg naloxone sub. cut. twice a day for six days or with injection vehicle alone. They were observed under blind conditions twice a day for four subsequent days. The naloxone-pretreated rats developed symptoms resembling morphine abstainence syndrome. Their "wet dog" body shakes, head shakes, scratches and total symptoms were significantly elevated over controls.

Analysis of variance (one repeated measure) of total symptoms revealed a significant effect of pretreatment (naloxone versus water), and of days, but not of interaction (pretreatment x days). Symptoms were dramatically reversed by a small dose of morphine but not by naloxone.

In a second experiment, rats were injected for 10 days with the same dose of naloxone. The abstinence-like syndrome began after 6 days of naloxone and continued for several days after cessation of injections. Total symptoms, body shakes, scratches and aggression were significantly elevated over controls.

Analysis of variance of total symptoms (one repeated measure) revealed a significant effect of pretreatment (naloxone versus water) as well as days and interaction (pretreatment x days). In view of the significant interaction, an analysis of Simple Main Effects was performed, indicating significant effects on days 8 and 10 of the treatment period and day 1 of the post-treatment period.

These results suggest that organisms may depend on endogenous endorphin receptor stimulation to prevent abstinence-like discomfort. However, the fact that at least six days of receptor blockade were required to produce the syndrome suggests that continuous receptor stimulation is not necessary to prevent this syndrome, but that occasional cyclical or episodic stimulation will suffice.

16.6 DIFFERENTIAL BEHAVIORAL EFFECTS OF TYPE I AND TYPE II OPIATE RECEPTOR ACTIVATION IN RAT BRAIN. K. Trujillo* and A. Pert (SPON: L. Ng). Biological Psychiatry Branch, NIMH, Bethesda, MD 20205

There are at least two different subclasses of opiate receptors through which opiates and opioid peptides exert their pharmacological and physiological effects. One class of receptors appears to be coupled to adenylate cyclase (Type I) and the other appears to be uncoupled (Type II). There is a distinct regional distribution for each receptor subclass in brain. Regions of the brain that appear to be involved in mediating the analgesic and depressant actions of opiates (e.g., periaqueductal gray matter) have a higher concentration of Type I than Type II receptors. Type II receptors appear to be associated predominantly with limbic forebrain structures such as the nucleus accumbens, amygdala and hypothalamus through which the excitatory and euphorogenic actions of opiates are mediated. Morphine has a preferential affinity for the Type I receptor, while D-Ala^2-D-Leu^5-enkephalin (DADL) has a preferential affinity for Type II receptors. Intraventricular injections of 1, 5 or 25 µg of DADL produced a dose-dependent increase in locomotor activity, which was only partially antagonized by naloxone. Intraventricular injections of morphine produced a dose-dependent depression of locomotor activity which was antagonized by naloxone. Injections of morphine into the periaqueductal gray matter also produced a dose-dependent depression of locomotor activity, while injections of DADL into the same structure produced no effect or slight hypermotility. Injections of DADL as well as morphine into the nucleus accumbens increased locomotor activity without depression. DADL, however, was considerably more potent. Injections of DADL into the caudate nucleus produced contralateral rotation behavior which increased in intensity with repeated injections and was relatively resistant to antagonism by naloxone. Morphine had no apparent effect in this structure. The data suggest that Type I and Type II opiate receptors located in various brain regions mediate different behavioral actions of opiates.

16.7 β-ENDORPHIN: EFFECTIVENESS IN SUPPRESSING ACUTE WITHDRAWAL IN MORPHINE-DEPENDENT MONKEYS BY INTRAVENTRICULAR INJECTION. E.F. Domino, Dept. of Pharmacology, Univ. of Mich., Ann Arbor, MI 48109 and C.H. Li*, Hormone Res. Lab., Univ. of Calif., San Francisco, CA 94143.

The evidence that β-endorphin is a narcotic agonist when given intraventricularly to rodents is impressive. The fact that many drugs are reported to be effective in suppressing morphine abstinence signs in rodents and even in humans but not in morphine dependent monkeys makes additional studies in primates imperative. Six female monkeys, weighing from 3 to 5 kg, were made dependent upon morphine with daily injections of 3.0 mg/kg of morphine sulfate s.c. every 6 hr. at 7:00 a.m., 1:00 p.m., 7:00 p.m., and 1:00 a.m. for periods up to 2 yr. Each animal was prepared surgically under ketamine anesthesia with a chronic indwelling stainless steel needle which contained a rubber septum covered with silastic for sterility. Evidence that the needle was in the lateral ventricle was obtained by withdrawal of ventricular fluid and via skull x-rays obtained after injection of 0.2 and 1.0 ml of the radiocontrast medium metrizamide (250 mg of iodine/ml). At approximately 1 to 4 week intervals the animals were subjected to 14 hr. abstinence in which the 1:00 and 7:00 a.m. doses of morphine were omitted. Each experiment began at about 9:00 a.m. or 14 hr. after the previous 7:00 p.m. dose of morphine. Abstinence signs were scored before drug injection and at various times thereafter. Morphine and synthetic human β-endorphin were dissolved in artificial cerebrospinal fluid. To maintain sterility, all solutions were passed through separate disposable millipore filters prior to injection. Each substance was given intraventricularly in doses of 10, 32, 100, 320, 570 and 1000 µg total or /kg i.v. in a random fashion. The drug dosages were compared with equal volumes of artificial cerebrospinal fluid. Abstinence scores were obtained using an 8 point scale. Signs were grouped in the following categories: general behavior in the home cage, facial, trunk and extremity characteristics, autonomic signs, and those signs elicited by a handling routine. The handling routine was by far the most sensitive means of measuring abstinence. Both β-endorphin and morphine, in a dose dependent manner given intraventricularly, suppressed the signs of 14 hr. acute morphine abstinence. On a molar basis, β-endorphin was more active than morphine. β-endorphin, in comparable abstinence suppressing amounts, also produced more central nervous system depression and no gross convulsions, in contrast to morphine. Morphine, given i.v., suppressed morphine abstinence but β-endorphin in equivalent doses did not, indicating that the latter does not readily cross the blood-brain barrier in this species.

16.8 CHRONIC LITHIUM INDUCES APPARENT ANALGESIA AND HYPERSENSITIVITY TO OPIATE ANTAGONISTS. SHIMON AMIR and RABI SIMANTOV*. Isotope Department, The Weizmann Institute of Science, Rehovot, Israel.

Lithium is commonly used in treatment of manic depressive patients. However, its mechanism of action is not entirely clear. Increased levels of methionine enkephalin in specific brain regions has been observed in rats treated chronically with lithium (Gillin et al., PNAS, 75, 2991-2993, 1978). Lithium also affects the binding of opioid peptides and alkaloids to opiate receptors. In the present study we investigated the effect of chronic lithium on pain sensitivity and responsiveness to the opiate antagonists, naloxone and naltrexone.

Male C57Bl/6 mice were chronically fed with lithium containing diet, and at the day of the test they were injected intraperitoneally with saline which contained different concentrations of naloxone or naltrexone. Pain sensitivity to thermal stimulus was assessed in a modified hot plate test at 46°. Ten days feeding with lithium diet increased the latency and reduced jumping (by 56%) but had no such effect on grooming and rearing. Injection of 0.156-160 mg/Kg naloxone caused only 0-20% increase in jumping response in control mice, whereas 0.156 mg/Kg naloxone caused > 100% increase in jumping of lithium treated mice. The effect of naloxone observed in lithium treated mice was increased in a dose dependent fashion. A similar dose dependent relationship was obtained also with naltrexone, except that the highest dose of this alkaloid (160 mg/Kg) caused marked depression in all behavioral measures in both groups. The increased jumping in the naloxone treated mice was accompanied by a decrease grooming and rearing. It is concievable that the lithium induced reduction in pain responsiveness is a behavioral correlate of the increased levels of endogenous methionine enkephalin. However, since this increase was observed only in the nucleus caudatus and the globus pallidus, other mechanisms such as alteration in the opiate receptor cannot be excluded. The hypersensitivity to naloxone and naltrexone in the lithium treated mice may reflect an increase in endogenous enkephalins. The possibility that chronic lithium causes an apparent dependency to the increased levels of endogenous enkephalins will be discussed.

16.9 BEHAVIORAL EFFECTS OF INTRAVENTRICULARLY ADMINISTERED DYNORPHIN-(1-13) AND D-ALA2-DYNORPHIN-(1-11) IN RATS. B.H. Herman* and A. Goldstein, Addiction Res. Fdn., 701 Welch Rd., Palo Alto, CA 94304.

Lateral intraventricular (LV) or cerebral aqueduct (CA) administration of the opioid peptide, dynorphin-(1-13), induced catalepsy and analgesia in rats. Onset was earlier and duration shorter than with morphine or B_C-endorphin. The dose required to induce analgesia was reduced at least tenfold when dynorphin-(1-13) was administered into CA rather than LV. An analogue, D-Ala2-dynorphin-(1-11), produced a comparable degree of catalepsy and even more profound analgesia at one-tenth the dose. These effects of dynorphin-(1-13) and D-Ala2-dynorphin-(1-11) were significantly antagonized by naloxone pretreatment. Rats treated with dynorphin-(1-13) or a high dose of D-Ala2-dynorphin-(1-11) exhibited bizarre postures immediately following LV admistration with limb rigidity and "barrel rolling". These effects were not blocked by naloxone.

16.10 STUDIES ON THE BEHAVIORAL AND NEUROCHEMICAL INTERACTIONS BETWEEN NALOXONE, AMPHETAMINE AND APOMORPHINE. J. Curell*, B. Hitzemann*, H. Loh* and R. Hitzemann (SPON: D. Garver). Lab. of Psychobiology, Dept. of Psychiatry, Univ. of Cincinnati Coll. of Med., Cincinnati, OH 45267.

The effects of naloxone, in the absence of analgesics, on behaviors generated by activation of the mesolimbic and/or nigrostriatal dopamine (DA) systems has received some attention. Naloxone has been found to block d-amphetamine-induced increases in motor activity (Holtzman, S., J. Pharmacol. Exp. Ther., 189, 51, 1974; Dettmar et al, Neuropharmacol. 17, 1041, 1978) and to potentiate the effects of low doses of apomorphine and ET-495 on conditioned behavior (Harris et al, Eur. J. Pharmac., 43, 243, 1977). In the present study we have confirmed and extended the observations on the interactions between naloxone, amphetamine and apomorphine in Sprague-Dawley rats. Naloxone in doses of 0.3 to 10mg/kg, s.c. antagonized the increase in ambulation and rearing induced by 1mg/kg of d-amphetamine. When the dose of d-amphetamine was increased to 3mg/kg, naloxone (3mg/kg) specifically antagonized rearing activity. No dose (0.3 to 10mg/kg) of naloxone significantly affected the quality of d-amphetamine or apomorphine induced stereotyped behavior. However, naloxone (3mg/kg) significantly augmented the apomorphine (1mg/kg) induced increase in ambulation. Naloxone (3mg/kg) decreased ambulation in a novel environment and enhanced the rate decreasing effects of apomorphine (0.03mg/kg) in the same paradigm. Naloxone was administered chronically via pellet-implantation. Tolerance developed to the inhibition of d-amphetamine induced rearing but not ambulation. Naloxone in concentrations as high as 10^{-5}M had no significant effect on [^{3}H] spiroperidol binding in both the nucleus accumbens and caudate nucleus. Similarly, naloxone did not effect [^{3}H]-DA synaptosomal uptake in the nucleus accumbens and caudate nucleus. However, naloxone (10^{-6} to 10^{-5}M) was found to block the d-amphetamine (10^{-6} to 10^{-5}M) induced release of [^{3}H]DA from caudate slices. At 10^{-7} to 10^{-6}M naloxone decreased the spontaneous release of [^{3}H]DA but increased release at 10^{-5}M. The significance of these and additional findings in terms of the naloxone effects on d-amphetamine and apomorphine induced behaviors will be discussed. This study was supported in part by grant MH-25487.

16.11 FAILURE OF MORPHINE TO PRODUCE PHYSICAL DEPENDENCE FOLLOWING CENTRAL ADMINISTRATION DURING HIBERNATION. Alexander L. Beckman and Carmen Llados-Eckman*. Alfred I. duPont Institute, Wilmington, DE 19899.

Previous work in our laboratory demonstrated that non-hibernating (ie., euthermic) ground squirrels (Citellus lateralis), treated with subcutaneously implanted morphine (M) pellets, developed a strong and characteristic abstinence syndrome following naloxone (Nx) administration. By contrast, animals failed to show any abstinence syndrome when treated with M in this way during deep hibernation and given Nx immediately after arousal to the euthermic state. In considering the possibility that peripherally administered M did not enter the brain during deep hibernation, we have examined the development of physical dependence on M following intracerebroventricular (icv) infusion during this natural state of CNS depression.

Euthermic C. lateralis of either sex were implanted under light ether anesthesia in the interscapular region with a miniature osmotic pump. The pump delivered M (55µg/µl) through a previously implanted cannula guide tube to the lateral cerebral ventricle at a rate of 50-55µg/µl/hr for periods of 24, 48, or 72 hr. Hibernating animals received the same administration of M, with osmotic pump activation provided by sustained immersion in a container of 0.9% saline (in place of interscapular implantation). In all animals, morphine abstinence was precipitated by Nx (1mg/kg, s.c.) during euthermia and was characterized by quantifying the occurrence of signs displayed in the 30 min period following Nx injection.

Animals exposed to M during euthermia displayed a strong abstinence syndrome consisting of exploratory behavior, nesting, grooming, vocalization, shakes, yawning, chewing, chromodacryorrhea, digging, dyspnea, eyetwitch, flattened posture, ptosis, and forward-extended tail. Animals exposed to M during deep hibernation, however, displayed no abstinence signs following arousal to the euthermic state and immediate testing with Nx. Some hibernating animals displayed M-induced stereotypy following arousal to the euthermic state. Euthermic and hibernating saline control experiments were without effect.

These data support our earlier findings (Soc. Neurosci. Abstr. 5:599, 1979) and demonstrate that, whereas exposure of the brain to M during euthermia results in physical dependence, similar exposure to M during deep hibernation does not. It therefore appears that CNS mechanisms underlying the development of physical dependence on M are altered during this depressed state. (Supported by the A.I. duPont Institute and NIDA grant DA 02254).

17.1 FINE STRUCTURAL ANALYSIS OF AXONS ENTERING THE EPIDERMIS WITHIN THE RECEPTIVE FIELD OF HIGH-THRESHOLD MECHANORECEPTIVE MYELINATED AFFERENTS. L. Kruger, E. R. Perl and M. J. Sedivec.* Depts. of Anatomy, Anesthesiology and Brain Research Institute, UCLA Center for Health Sciences, Los Angeles, CA and Dept. of Physiology, University of North Carolina, Chapel Hill, NC.

Myelinated hindlimb afferents in the cat conducting at velocities of 10-35 m/sec. and subserving high-threshold mechanoreceptors, possess receptive fields consisting of a number of spot-like responsive areas separated by regions unresponsive to the same stimuli. It has been suggested that these receptors constitute a class of myelinated 'nociceptor' partially involved in the sensitivity to mechanically induced pricking pain (Burgess and Perl, '67).

Following electrophysiological mapping of receptive field 'spots,' fine steel pins were inserted perpendicularly into the epidermis at a distance of $\sim$ 0.5 mm in order to bracket the responsive points. The cats were then aldehyde perfused, each receptive field spot was carefully blocked, osmium treated, embedded in plastic and trimmed. Semi-thin sections were cut within the delineated area for orientation and tracing of single thinly-myelinated axons penetrating into the papillary layer of the dermis. Axonal profiles enter the epidermal basal lamina displaying clear pleomorphic and a few dense-core vesicles at the penetration site. They are surrounded by Schwann cell processes that lose their basal lamina within the epidermis; the axon ultimately becoming completely enveloped by basal keratinocytes. These axonal profiles can generally be distinguished from Langerhans cell 'dendrites.' The low density of this pattern in cat hindlimb hairy skin and its consistent presence in the marked areas of focal responsiveness suggest that nociceptors with thinly myelinated fibers possess unmyelinated terminal branches penetrating the basal epidermis where the Schwann cell loses its basal lamina in the zone where vesicles are found and displays marked pinocytotic activity. This pattern forms a receptive apparatus within the basal epidermis which appears to correlate with the distribution of delta nociceptor spots.
(Supported by grants NS-5685 and NS-10321 from NINCDS of the U.S. Public Health Service.)

17.2 SYNAPTOLOGY OF PHYSIOLOGICALLY IDENTIFIED, HRP-STAINED, NEURONS IN THE SUPERFICIAL DORSAL HORN OF CATS. A. R. Light, M. Réthelyi*, E. R. Perl. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, N.C. 27514.

The activity of neurons in the marginal zone (MZ) and substantia gelatinosa (SG) of the cat's spinal cord was recorded with HRP-filled microelectrodes. On the basis of physiological characteristics (localization and size of the receptive field, nature of cutaneous stimuli activating the neurons), the neurons could be subdivided into three broad groups: group 1 - neurons activated by noxious mechanical stimuli; group 2 - neurons activated by innocuous mechanical stimuli; group 3 - neurons activated by both noxious and innocuous stimuli (Neurons most commonly observed were of groups 1 or 2). Following physiological identification, the neurons were stained by iontophoretic injection of HRP. A histological analysis was carried out on 50 µm thick Vibratome sections cut in the sagittal plane and subsequently serially cut into ultrathin sections. The location and shape of perikarya and the arborization pattern of dendrites and axons varied considerably among the neurons. Nevertheless, relations were consistently seen: group 1 neurons were mostly confined to the MZ and to the outer layer of the SG, group 2 neurons to the inner SG and to the dorsal part of the nucleus proprius; group 3 neurons showed widespread dendritic and axonal arborizations. With few exceptions the dendrites of the neurons were richly supplied with long, often complicated appendages. Presynaptic axon terminals were found to make contact with a labeled neuron all along its dendritic shafts, and consistently on the enlarged heads of the dendritic appendages. Although these axon terminals varied in size, vesicle content and synaptic connections, an axon terminal of a particular ultrastructure was often found to synapse repeatedly with the dendrites of the same neuron. It appears probable that these repeated connections provide a structural basis for the occurrence of a large number of neurons in the superficial dorsal horn with no, or minor, convergence of various sensory modalities. In addition to axon terminals, vesicle containing, dendritic-like profiles often made synaptic contact with labeled dendrites of all three groups in MZ and SG. Similar profiles were also both pre and postsynaptic to axon terminals that synapsed with labeled neurons. All three functional groups displayed vesicles inside labeled dendritic appendages. These were common in the inner substantia gelatinosa, and less numerous in the marginal zone. Thus, dendritic interactions may modify the input of MZ and SG neurons at, or before, the first synapse.

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17.3 CARDIOPULMONARY INPUTS ONTO THORACIC SPINOTHALAMIC NEURONS IN THE PRIMATE. R.W. Blair, R.N. Weber*, and R.D. Foreman. O.U. Hlth. Sci. Ctr., Dept. of Physiology & Biophysics, Oklahoma City, OK. 73190.

During angina pectoris or the onset of a myocardial infarction in man, pain is often felt in the left arm and shoulder, a phenomenon known as "referred pain." Previous work from this laboratory has demonstrated that thoracic cells of origin of the spinothalamic tract (ST) receive convergent input from the cardiopulmonary region and the left forelimb. This viscerosomatic convergence was suggested as the mechanism whereby referred pain could occur. The present study was performed to quantify the visceral input onto ST cells in the thoracic spinal cord. Monkeys (Macaca mulatta and Macaca fascicularis) were initially anesthetized with ketamine and chloralose, and were maintained at a constant level of anesthesia with an infusion of pentobarbital. They were paralyzed with gallamine. Neurons of the ST in the T_3-T_5 segments of the thoracic gray matter were antidromically activated from the contralateral ventral posterior lateral thalamic nucleus. All cells reported were antidromic, and all received visceral and somatic inputs. Visceral input to the ST neurons was elicited with electrodes placed on the left ansa subclavia and sympathetic chain between the T_2 and T_3 rami communicantes. A total of 32 ST cells were examined; their antidromic conduction velocity (CV) was 23.4 $\pm$ 2.1 (SE) m/s. The spinal cord location for 25 of the cells was histologically determined; 8% of the ST neurons were found to be in lamina I, 36% in lamina IV, 32% in lamina V, and 24% in lamina VII. The mean antidromic CV of the neurons in the different laminae was not significantly different. The classification of visceral fibers activating the ST neurons was inferred from both the minimum afferent conduction velocity (MACV) and the threshold for cell activation. MACV was determined for 28 cells; the average MACV was 9.0 $\pm$ 1.3 (SE) m/s, which indicates that the ST neurons received input from Aδ fibers. For 21 cells the stimulus intensity was increased sufficiently to activate C-fibers if they were present. Ten ST neurons were found to receive C-fiber input; MACV was 0.65 $\pm$ 0.16 m/s, and the stimulus threshold for cell activation by C-fibers was 28.3 $\pm$ 6.3 times greater than for activation by Aδ fibers. One additional ST cell received only C-fiber input. Preliminary data also suggests that some ST cells in the T_3-T_5 segments receive Aδ and C input from afferents coursing in the greater splanchnic nerve. This study demonstrates the following: (1) ST spinal neurons in the T_3-T_5 segments receive input from both Aδ (primarily) and C-fibers, and (2) thoracic ST neurons are found about equally in laminae IV, V, and VII, with a fewer number in lamina I.
Supported by NIH grants HL 22732, HL 00557, and HL 07430.

17.4 RESPONSES OF PRIMATE SPINOTHALAMIC TRACT NEURONS TO NATURAL STIMULATION OF THE TESTICLE AND URINARY BLADDER. R.J. Milne, G.J. Giesler, Jr. and W.D. Willis, Jr. Marine Biomed. Inst. and Depts. of Physiol. & Biophysics and of Anatomy, Univ. TX Med. Branch, Galveston, TX 77550

Compression of the testicle and overdistension of the urinary bladder produce intense pain. One characteristic of this visceral pain is its pattern of referral to the skin of the flank and inguinal area, suggesting possible convergence of inputs transmitting nociceptive information from skin and viscera. We report spinothalamic tract neurons in the primate which respond to graded natural stimuli to the testicle and/or urinary bladder, and also to the cells' cutaneous receptive fields.

Male monkeys (Macaca fascicularis) were anesthetized with α-chloralose (80 mg/kg) and an infusion of sodium pentobarbital (4 mg/kg/hr), paralyzed with gallamine triethiodide and artificially ventilated. Extracellular single unit activity was recorded with glass capillary microelectrodes from lower thoracic or upper lumbar spinothalamic tract neurons identified by antidromic activation of the contralateral ventral posterolateral nucleus of the thalamus. Each unit responded with an increased firing rate to innocuous stimuli (brushing the hair or skin) and more vigorously to noxious stimuli (pinching or noxious heat) applied within its cutaneous receptive field. In addition, each unit received excitatory input from the urinary bladder and/or the ipsilateral testicle.

Distension of the urinary bladder under constant pressure (0 to 60 cm H_2O) or constant volume (10 to 50 cc) conditions evoked an increase in firing rate which was graded with stimulus strength. Compression of the testicle with forces in the range 200 to 1200 gm-wt likewise induced responses which were graded with the applied force. Application of noxious heat to the testicle induced a vigorous response. Activity elicited by either somatic or visceral stimuli reached its peak rapidly and declined, usually within 1 minute, close to background levels. We conclude that spinothalamic tract cells which display a wide dynamic range of responses to somatic stimuli also have powerful excitatory visceral inputs. Such viscero-somatic convergence may provide a neural substrate for pain referred from visceral structures.

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17.5 ELECTROPHYSIOLOGICAL ANALYSIS OF THE RESPONSES OF SPINAL NEURONES IN RATS TREATED WITH CAPSAICIN. J.A. Pearson, M.C. Green*, W.A. Staines and H.C. Fibiger. Department of Physiology and Division of Neurological Sciences, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

A reduction in responsiveness to thermal noxious stimuli, and a 50% depletion of spinal substance P levels, has been demonstrated in rats tested 12 weeks after neonatal treatment with capsaicin (Nagy, J.I. et al. Brain Res. 186, 1980, 435-444). It is suggested that this analgesia is a consequence of the neurotoxic effect of capsaicin on nociceptive substance P-containing sensory neurones. The present study was undertaken to determine whether treatment with capsaicin influences the responses of spinal neurones to cutaneous stimulation.

Two-day old, male, Wister rats were given subcutaneous injections of either capsaicin (50 mg/Kg) or vehicle (10% ethanol, 10% Tween-80 in 0.9% saline). Electrophysiological experiments were carried out 4-5 months later. Rats were anaesthetized with Urethane (1.5 g/Kg) and a laminectomy was performed to expose the lumbar enlargement of the spinal cord. Neurones, situated in the dorsal horn, were classified according to their responses to mechanical stimuli applied to their cutaneous receptive fields. Type I cells were driven only by weak, presumably non-noxious, stimuli. Type II cells could only be activated by intense stimuli and neurones of Type III responded to both non-noxious and noxious mechanical inputs. In rats which had received injections of vehicle, the number of cells which were classified as Types I, II and III were 47, 17 and 77 respectively (32, 12, 55%). After capsaicin treatment, the proportion of cells in each category (43, 16, 50 ie 39, 15, 46%) did not differ significantly ($p>0.05$) to that seen in control animals. In contrast to its lack of effect upon the sensitivity to mechanical stimuli, capsaicin treatment resulted in a reduced ability to respond to noxious thermal stimuli ($p<0.01$). Heat pulses (45^{o}C for 15 secs) applied to the skin excited 26 out of 51 cells (51%) in control animals but only 10 out of 49 (20%) in capsaicin treated rats.

It is concluded that the neurones which are disrupted by neonatal administration of capsaicin are involved in thermal rather than mechanical nociception.

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17.6 AFFERENT AND EFFERENT CONNECTIONS OF PERIAQUEDUCTAL GREY IN THE MONKEY. Patrick W. Mantyh*. (SPON. S. Hockfield) Dept of Anat., University of California, San Francisco, CA 94143.

Combined horseradish peroxidase (HRP) and tritiated amino acid injections ranging from .01-.1ul were stereotaxically placed in various regions of the periaqueductal grey (PAG) of 10 squirrel monkeys. After a 2-4 day survival time the monkeys were perfused and alternate frozen sections processed for either HRP histochemistry (Mesulam '78) or autoradiography (Cowan et. al. '72).

From this study it appears the PAG receives wide ranging afferents from the rostralmost pole of the frontal cortex to the sacral spinal cord. HRP positive cells were found in the following areas: Frontal granular cortex; Amygdala; central and basal lateral nuclei; Substantia innominata; Hypothalamus: ventromedial, supraoptic, suprachiasmatic, dorsomedial, lateral, paraventricular, periventricular, posterior, peri-arcuate; Zona incerta; Mesencephalon: superior colliculus, cuneiformis, PAG; Pons: raphe magnus, locus coeruleus; Medulla: parvo-, gigantocellular reticular, spinal trigeminal nuclei; Spinal cord. Of all these areas the hypothalamus and zona incerta have the heaviest projection to the PAG. The afferent connections revealed here implicate the PAG as a visceral, nociceptive, and cognitive integrator.

Efferent projections were observed in the following nuclei after tritiated amino acid injections into the PAG: Bed nucleus of the stria terminalis; Hypothalamus: ventromedial, supraoptic, suprachiasmatic, dorsomedial, lateral, periventricular, posterior, supramammilary, preoptic area; Dorsal thalamus: centromedian, parafascicularis, medialis pars medialis, paraventricular thalami, centralis medialis, reuniens, rhomboidalis; Ventral tegmental area of Tsai; Zona incerta; Mesencephalon: cuneiformis, PAG, superior colliculus; Pons: raphe magnus, raphe pallidus, locus coeruleus; Medulla: parvo- and giganto-cellular reticular formation. Efferent and afferent connections of the PAG show a significant degree of overlap. This reciprocity is especially evident in the hypothalamic and brainstem areas.

Afferent and efferent connections remain quite constant from one animal to another even though the area of the PAG injected varied. These findings, along with Nissl, Weil, and Golgi analysis of the PAG (Abols and Mantyh, Neurosci. Abst. '80) do not support the division of the PAG into distinct cell groups, each with its own cytoarchitecture and connectivity. (Supported by H.J. Ralston NS-11614 and W.R. Mehler NASA task 199-05-02-07.)

17.7 RESPONSE OF NUCLEUS RAPHE MAGNUS NEURONS TO IONTOPHORETICALLY APPLIED SUBSTANCE P. Scott L. Pomeroy and Michael M. Behbehani. Dept. of Physiology, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

The medullary nucleus raphe magnus (NRM) appears to be an important location of neurons which inhibit nociception when the periaqueductal gray matter (PAG) is stimulated. Stimulation of PAG can influence the activity of NRM neurons; little is known about the neurotransmitter which mediates these responses. Substance P is present in nerve terminals within the NRM and produces analgesia when injected intracerebrally.

To test the hypothesis that substance P is the neurotransmitter of the PAG to NRM projection, we compared the response of NRM neurons to PAG stimulation with response to iontophoretically applied substance P in urethane anesthetized Sprague-Dawley rats. Post-stimulus time histograms were recorded from NRM neurons while electrically stimulating PAG with bipolar tungsten electrodes (parameters: 40μsec, .5-.10 mA, 1Hz). It was found that NRM neurons responding to PAG stimulation with either excitation or inhibition were more likely to be excited by substance P than neurons which did not respond to PAG stimulation. Thirty-three of the sixty-three NRM neurons studied were excited by PAG stimulation; twenty-three of these neurons were excited by substance P. Eight NRM neurons were inhibited by PAG stimulation; seven of these neurons were excited by substance P. Twenty-two neurons did not respond to PAG stimulation; five of these were excited by substance P. A Chi-square test for inhomogeneity found this distribution of responses to have a significant interaction ($X^2 = 15.6$, $df = 2$, $p<.001$). No NRM neurons were inhibited by substance P. Substance P does not appear to be the neurotransmitter of this projection since neurons inhibited by PAG stimulation were excited by the putative transmitter.

To test the hypothesis that substance P may modulate the response of NRM neurons to PAG stimulation, substance P was iontophoretically applied to NRM neurons while stimulating PAG. Thirteen responding neurons were studied: the responses of three were inhibited by substance P. One neuron was inhibited at low currents, but had a potentiated response at higher currents. Substance P may, therefore, have a modulating role in the NRM.

17.8 THE ROLE OF NOREPINEPHRINE IN THE INTERACTION BETWEEN THE PERIAQUEDUCTAL GRAY (PAG) AND NUCLEUS RAPHE MAGNUS (NRM). M.M. Behbehani and S.L. Pomeroy. Dept. of Physiology, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

In recent years the involvement of a descending system in analgesia produced by morphine and by electrical stimulation of the brain has been well documented. It has been shown that interaction between the PAG and NRM is an important functional aspect of this descending system. Several neurotransmitter substances are known to be involved in analgesia produced by morphine and electrical stimulation but it is not known which one of these transmitters are involved in the interaction between the PAG and NRM. Histochemical experiments have shown that norepinephrine containing cell bodies and terminal are found in the PAG and NRM. The object of the experiments reported here was to determine how those cells in the NRM that respond to PAG stimulation respond to iontophoretically applied norepinephrine. Male Sprague-Dawley rats weighing 250-300 grams were used. After surgical procedure under urethane anesthesia, activity of single cells in the NRM was recorded using a recording electrode glued to a five barrel drug electrode. The PAG was stimulated monopolarly with 40μsec, 0.5 to 1.5 mA constant current pulse. Norepinephrine HCL (100mM), Sotolol HCl 500 mM, piperoxan chlorhydrate (PIP) 100 mM and sodium acetate were iontophoresed as needed.

Seventy percent of the cells in the NRM responded to PAG stimulation; 51% responded by excitation and 19% responded by inhibition. Of those cells that responded to PAG by excitation, application of NE produced 30% excitation, 35% inhibition and no response in 35% of the cells. Of the cells that responded to PAG stimulation by inhibition, NE application caused 28% excitation, 42% inhibition and no response in 30%. The onset of response to NE was slow and at current of 30 nA usually the response became noticeable after at least 10 seconds of drug application. The inhibitory effect of NE had a slower time course than the excitatory effect. The response to NE could be partially blocked by sotolol but seldom could be affected by PIP. In 15% of the cells application of sotolol alone caused an increase in the firing rate. Statistical analysis showed no correlation between response to PAG stimulation and NE response. It is concluded that although the cells in the NRM can respond to NE by excitation the more dominant response is inhibition. Furthermore, it seems unlikely that the NE is the transmitter which mediates the interaction between the PAG and NRM.

17.9 A PHARMACOLOGICAL STUDY OF INHIBITION OF PRIMATE SPINOTHALAMIC TRACT CELLS FROM PERIAQUEDUCTAL GRAY AND NUCLEUS RAPHE MAGNUS. R.P. Yezierski, T.K. Wilcox and W.D. Willis. Marine Biomedical Institute and Departments of Physiology & Biophysics and of Anatomy, University of Texas Medical Branch, Galveston, TX 77550

It has been previously shown by our laboratory that activity of primate spinothalamic tract (STT) cells is inhibited by stimulation in nucleus raphe magnus (NRM). We have now shown that a comparable inhibition is obtained by stimulation in the periaqueductal gray (PAG). The present experiments were undertaken to study the pharmacology of descending inhibitory pathways activated by stimulation in these brain regions.

Monkeys (Macaca fascicularis) were anesthetized with α-chloralose and pentobarbital, paralyzed and artificially ventilated. STT cells were identified by antidromic activation from the contralateral thalamus and functionally characterized. Cells used in the present study were classified as wide dynamic range or high threshold. Stimulating electrodes were placed in both NRM and PAG. Pre-drug inhibitory effects of NRM and PAG stimulation on evoked activity of STT cells were determined. Stimulus parameters consisted of long and short duration trains delivered at 333 Hz with currents of 50-200 microamps. Inhibition was observed following NRM or PAG stimulation of spontaneous activity as well as STT cell activity evoked by non-noxious and noxious cutaneous stimulation, and responses to C-fiber volleys from the ipsilateral sural nerve. A tendency was noted for PAG inhibition to be greater on STT cell activity evoked by noxious than by non-noxious cutaneous stimulation.

The results of this study showed PAG inhibition to be antagonized by intravenous administration of the serotonin antagonists methysergide (2-5 mg/kg) and metergoline (2-4 mg/kg). This effect was seen when stimulating electrodes were located in the PAG at the level of the third or fourth cranial nerve nuclei. The opiate antagonist naloxone (0.2-0.4 mg/kg) and the α-blocker phentolamine (1-3 mg/kg) had no antagonistic effects on PAG inhibition. NRM inhibition was not antagonized by these various monoaminergic or opioid antagonists. The results of these experiments will be discussed in relation to the involvement of endogenous opiates and monoamines in descending inhibition.

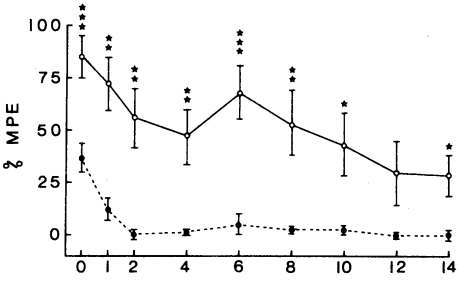
This work was supported by research grants NS 09743 & NS 11255, by postdoctoral fellowship NS 06193 to R.P.Y. from the National Institutes of Health and by a grant from the Moody Foundation.

17.10 DORSOLATERAL FUNICULUS (DLF) LESIONS BLOCK FOOT-SHOCK PRODUCED OPIATE ANALGESIA. LR Watkins*, DA Cobelli* & DJ Mayer (SPON: JH Johnson) Dept Physiol, Med Coll Va, Richmond, VA 23298.

The analgesic effects of systemic morphine, intracerebral morphine & electrical stimulation of the periaqueductal gray are greatly attenuated following lesions of the DLF. In contrast, bilateral DLF lesions do not reduce footshock produced analgesia (FSA) elicited by shocking rats on all 4 paws. Recently we suggested that FSA involves both opiate & non-opiate mechanisms since naloxone antagonizes FSA produced by front paw (FP) but not hind paw shock. In order to provide further support for the involvement of an endogenous opiate mechanism in FSA, the effect of DLF lesions on this analgesia was examined.

Two groups were tested: bilateral DLF lesioned rats (vertebral T2) & sham operated rats. Lesions were made with a carbon dioxide laser. Six days after surgery rats were tested for baseline tail-flick (TF) latencies & FP shocked 90 sec (1.5 mA, rms).

Immediately post-shock, TF latencies were significantly elevated above baseline for both DLF & sham operated rats. At 1 min post-shock, the TF latency of DLF rats was no longer significantly above baseline ($p>0.05$). In contrast, the TF latency of sham operated rats remained significantly elevated throughout testing.



Bilateral DLF lesions greatly reduce the effects of FSA produced by FP shock. We conclude that the DLF plays an important role in this FSA. It is of interest that DLF lesions do not completely eliminate the increased TF latency measured immediately post-shock. This is in agreement with the observation that naloxone also fails to prevent significant analgesia from FP shock for the first 2 min following shock. These results strongly indicate that the same endogenous opiate analgesia system is activated by morphine & electrical stimulation of the brain. PHS Grant DA-00576.

17.11 A STUDY OF THE RESPONSE PROPERTIES OF MEDIAL BRAINSTEM CELLS PROJECTING TO THE SPINAL CORD IN MONKEYS. T.K. Wilcox, R.P. Yezierski, K.D. Gerhart and W.D. Willis. Marine Biomedical Institute and Departments of Physiology & Biophysics and of Anatomy, University of Texas Medical Branch, Galveston, TX 77550

Electrical stimulation within the medial brainstem has been shown to inhibit responses of spinothalamic tract (STT) cells to noxious and innocuous cutaneous stimuli. A similar inhibition of STT cells by periaquaductal gray (PAG) stimulation has been demonstrated by our laboratory. The present study examines the effect of PAG stimulation on and the receptive field properties of cells in the medial brainstem which have projections to the lumbrosacral spinal cord.

Monkeys (M. fascicularis) anesthetized with α-chloralose and pentobarbital were paralyzed and artificially ventilated. Stimulation sites in the PAG were selected on the basis of inhibition of evoked activity in STT cells (50-500 μA pulses, 100 ms trains, 333 Hz). Spinothalamic tract cells were antidromically activated from the ventral posterior lateral nucleus of the thalamus. Medial brainstem cells were antidromically activated by stimulation of the left dorsolateral fasciculus of the spinal cord (L4) and 100 μsec current pulses not exceeding 1 mA. The conduction velocities of these cells ranged from 7-81 m/s.

The cutaneous receptive fields of medial brainstem cells were large and often included the entire body. Both excitatory and inhibitory fields were found. Most cells responded only to noxious mechanical and/or thermal stimuli. Some brainstem cells were unresponsive to cutaneous stimulation. Stimulating electrodes were placed in contact with the left sural nerve to examine further the response properties of these cells. Stimulation of A and C fibers in the sural nerve elicited noticeable changes in the activity of those medial brainstem cells having cutaneous input from the sural region. Responses of cells to electrical stimulation in the PAG were mostly excitatory, although inhibition of spontaneous and evoked activity was sometimes observed. These responses were obtained using stimulus parameters similar to those used to inhibit evoked responses in STT cells.

Results of this study of the response characteristics of medial brainstem cells projecting to the spinal cord will be discussed in relation to the mechanism of descending inhibition.

This work was supported by research grants NS 09743 & NS 11255, by postdoctoral fellowship NS 06193 to R.P.Y. from the National Institutes of Health and by a grant from the Moody Foundation.

17.12 THE EFFECTS OF NUCLEUS RAPHE MAGNUS AND ADJACENT RETICULAR AREAS ON THE RESPONSES OF NUCLEUS CAUDALIS NEURONS TO NOXIOUS AND NON-NOXIOUS STIMULI IN CATS. J.O. Dostrovsky and Y. Shah*, Department of Physiology, Univ. of Toronto, Toronto, Canada M5S 1A8.

The nucleus raphe magnus (NRM) and possibly the nucleus reticularis magnocellularis (RMC) have been implicated as components of a descending pathway involved in the inhibition of nociceptive transmission in the spinal cord and trigeminal subnucleus caudalis. Raphe neurons have been shown to project via the dorsolateral funiculus and terminate in the dorsal horn of the spinal cord and in nucleus caudalis. There is also evidence that the dorsolaterally located nucleus gigantocellularis (NGC), which has a descending projection by a different pathway, the ventral lateral funiculus, is involved in the inhibition of sensory input. Here we report a comparison of the effects of these areas on the neuronal responses of caudalis neurons to noxious and non-noxious stimuli.

Experiments were done on chloralose anesthetized cats. Concentric bipolar electrodes were stereotaxically positioned in the NRM, NGC and RMC and histologically verified. Bipolar electrodes were also implanted in the teeth. Single units were recorded with glass-coated tungsten or carbon fibre microelectrodes. Units were categorized by sensory examination according to standard criteria as low threshold mechanoreceptive (LTM), wide dynamic range (WDR) and nociceptive specific (NS). Units were excited by just-suprathreshold stimulation intensities delivered to the skin (electrically or mechanically) or tooth pulps. The effects of stimulating the NRM, NGC or RMC (100 ms, 500 Hz, train 0.1 ms pulse width, delivered 130 ms prior to the test stimulus) on these peripherally elicited responses were determined.

Conditioning stimuli were tested on the cutaneous responses of 22 LTM, 14 WDR and 5 NS,and on the tooth pulp evoked responses of 24 units. 95% of these units were inhibited by NRM, NGC or RMC at stimulation currents less than 250 μA. No consistent difference was found between the stimulation thresholds necessary for NRM, NGC, or RMC induced inhibition. Furthermore there was no consistent difference between the thresholds required to inhibit cells in the various categories studied.

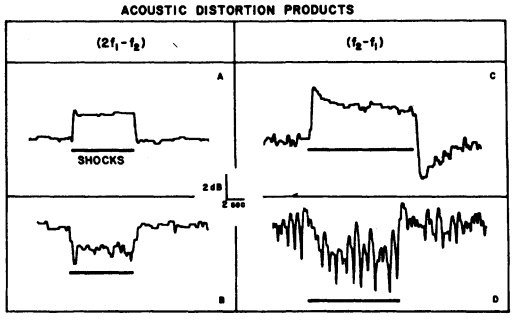
Thus the NGC, RMC and NRM appear to be equally effective in inhibiting responses to both noxious and non-noxious stimulation. These results do not support the idea of a nociceptive specific inhibitory pathway from the NRM.

This project was supported by grant #1R01 DE05404 from NIDR, DHEW.

18.1 EFFERENT SYNAPTIC ACTIVITY MODIFIES COCHLEAR MECHANICS SEEN IN EAR-CANAL ACOUSTIC DISTORTION PRODUCTS. J.H. Siegel and D.O. Kim. Dept. of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO. 63110.

The intermodulation-distortion products (f_2-f_1) and ($2f_1-f_2$), believed to originate in the cochlea, were monitored in the ear-canal sound pressure with a two-tone stimulus as previously described (Kim, Hearing Res. 2, 1980). Mountain (J. Acoust. Soc. Amer. 67: S89(A), 1980) has reported that shocking the crossed olivo-cochlear bundle (COCB) reduces the amplitude of the acoustic distortion product (f_2-f_1). In our experiments chinchillas were deeply anesthetized with Dial (70mg/kg) in urethane. Both middle ear muscles were completely severed from the ossicles to eliminate their influence during the shocks. 400-Hz shock trains were delivered through bipolar electrodes on the dorsal surface of the brainstem in the 4th ventricle. Depending on the primary stimulus frequencies, (f_2-f_1) or ($2f_1-f_2$) was increased, decreased, or unchanged during the shocks. For ($2f_1-f_2$) panel "A" below shows a 3dB increase (f_1=4500Hz, f_2=6000Hz, with SPL (re 20μPa) L_1=63dB, L_2=62dB) while panel "B" shows a 2dB decrease (f_1=2170Hz, f_2=2790Hz, L_1=64dB, L_2=66dB). For (f_2-f_1) panel "C" shows a 3dB increase (f_1=2170Hz, f_2=2790Hz, L_1=64dB, L_2=66dB) while panel "D" shows a 2dB decrease (f_1=2700Hz, f_2=4200Hz, L_1=69dB, L_2=62dB). The effect was completely blocked by perfusing the cochlea with artificial perilymph containing 10 μM d-tubocurarine, which did not itself alter the distortion amplitude. This clearly mechanical change is almost certainly mediated by efferent synaptic activity, presumably through the outer hair cells.

(Supported by NIH grants NS00162, NS07498, NS05839, and RR00396).



18.2 INCORPORATION OF ^{14}C-2-DEOXYGLUCOSE IN THE COCHLEA: EFFECTS OF ACOUSTIC STIMULATION. A. F. Ryan*, P. Goodwin*, N. K. Woolf and F. Sharp. Otolaryngology Research Laboratory, University of California at San Diego Medical School, San Diego, CA 92103.

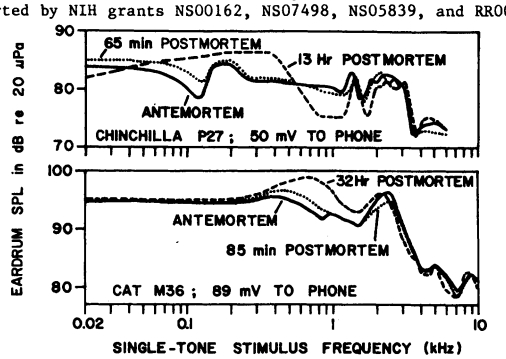
Activation of cellular structures of the central auditory pathway can be demonstrated by the increased incorporation of ^{14}C-2-deoxyglucose (2-DG). However, this technique as employed in previous investigations does not lend itself to use in the cochlea. The bony capsule and large fluid spaces of the inner ear make it difficult to obtain and preserve frozen sections. We have developed an alternative technique which permits the visualization of 2-DG uptake in cochlear tissues.

2-DG (New England Nuclear) was suspended in tissue culture medium and injected intracardially (16 μCi/100 gm, in 0.1 ml) into adult mongolian gerbils. Animals were immediately placed in a double-walled, sound-attenuated chamber (IAC 1200A) with an anechoic lining, in the dark for one hour. Experimental subjects were exposed free-field to wide-band (0-100 kHz) noise delivered through a mid-range driver (JBL 2482) for the one-hour period, at an intensity of 25, 45, 65, 85 or 105 dB SPL. Control subjects were treated identically, but received no noise exposure. All animals were then sacrificed by decapitation. The brains were removed and frozen in isopentane at -20°C. The inner ears were dissected whole and frozen in Freon 12 cooled in liquid nitrogen to its freezing point (-160°C). The brains were sectioned at 20 μm, dried and exposed for 5-7 days on x-ray film. Inner ears were freeze-dried at -40°C for 72 hr, and the round and oval windows opened. Some specimens were vapor-stained over a 4% solution of osmium tetroxide for 3-10 min. Inner ears were then embedded in Epon or Spurr resin. No dehydration sequence was necessary, and only organic solvents (absolute alcohol, acetone and propylene oxide) were employed in the embedding procedures. Evaluation of the solvents employed indicated no loss of 2-DG during embedding. Once polymerized, the cochleas were cut in mid-modiolar section utilizing a 100 μm jeweler's saw and exposed on LKB Ultrofilm for 7-12 days. Exposures of brain sections revealed increased uptake of 2-DG during acoustic exposure in all of the structures of the central auditory pathway, although relatively modest activity was detected in medial geniculate nucleus and cortex. 2-DG uptake was maximal at exposure intensities of 85 and 105 dB SPL. Control inner ears showed maximum uptake of 2-DG in stria vascularis, with visualization of organ of Corti, spiral ganglion and VIIIth nerve possible. Vapor staining with osmium tetroxide appeared to have no effect on 2-DG distribution. Preliminary data from experimental inner ears indicate that 2-DG uptake increased during exposure to 85 and 105 dB, in spiral ganglion and VIIIth nerve.

18.3 POSTMORTEM EFFECTS AND SPECIES DIFFERENCE FOR ACOUSTIC INPUT CHARACTERISTICS AT THE EARDRUM OF THE CHINCHILLA AND THE CAT. D.O. Kim, J.H. Siegel and C.E. Molnar*. Box 8101, Washington University School of Medicine, St. Louis, MO 63110

A closed acoustic system containing a Beyer DT48 earphone and a probe microphone (Kim, Hearing Res. 2, 1980) was used in this study. The chinchillas and the cats were anesthetized with Dial (70 to 75 mg/kg) in urethane, and the bulla and septum were widely opened. In the chinchilla, both of the middle ear muscles were completely severed; in the cat, they were left intact. A notch at 115Hz, as shown in the figure, was seen in the antemortem chinchillas whether or not the middle ear muscles were severed, but not seen in the cat. This difference is not due to experimental apparatus—an identical set of apparatus was used for both species. Pronounced postmortem changes occurred over several hours after death. For example, the 115Hz notch is absent in the 13 Hr postmortem chinchilla curve. Prominent harmonic distortion products as large as -22 dB relative to the primary component, with primary level in the range of 55 to 90 dB SPL, were observed at this notch in the live chinchilla. The 115Hz notch may correspond to a resonance in the cochlear input impedance. The postmortem changes reported here may in part reflect changes in cochlear mechanics occurring over several hours after death, as observed by Rhode (Basic Mech. Hear., Möller, Ed., 1973) which are interpreted by Kim et al. (Psychophys., Physiol. & Behav. Stud. Hear., van den Brink and Bilsen, Eds., 1980) as possibly due to a loss of active mechanical energy source in the cochlear partition soon after death and to a later decrease in the partition stiffness.

(Supported by NIH grants NS00162, NS07498, NS05839, and RR00396).



18.4 Effects of kanamycin on the auditory system of the leopard frog (Rana pipiens). G. Rose* and R.R. Capranica, Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

It is well known that the ototoxic drug kanamycin leads to irreversible loss of hair cells in the mammalian cochlea. To determine whether similar effects occur in cold-blooded vertebrates, kanamycin (12.5%) was infused (10 nl/g) directly into the fluid-filled inner ear of leopard frogs (Rana pipiens); a group of control animals received injections of equivalent amounts of amphibian Ringer's solution. Subsequently, multi-unit recordings from the midbrain (torus semicircularis) and recordings from single auditory nerve fibers were conducted. In recordings from the midbrain, large threshold elevations (up to 40 decibels) were encountered one to two days following a single injection of the drug. This elevation was most pronounced in the frequency range of 200 to 700 Hz, corresponding to the region of maximum sensitivity of the amphibian papilla. Single unit recordings from the eighth nerve confirmed that injection of kanamycin leads to a pronounced elevation in thresholds of the low- and mid-frequency sensitive fibers; high-frequency (1,000 - 1,800 Hz) sensitive fibers presumably from the basilar papilla showed a significant, although less marked, elevation in threshold. Furthermore, there was a clear correlation between threshold and Q_{10} value: fibers with high threshold in our treated animals had very broad tuning curves. Injection of Ringer's solution in our control group of animals failed to alter the response properties of the auditory system.

Our most recent results suggest that the deleterious effects of kanamycin on auditory function in anurans may be completely reversible. Threshold sensitivity and tuning sharpness recover to normal values within one to two weeks following a single drug injection. These results will be discussed with regard to the presence of a physiological vulnerable frequency filtering mechanism at the level of the auditory periphery.

This research was supported by NIH grant NS-09244.

18.5 POTENTIATION OF KANAMYCIN'S OTOTOXICITY BY AMINOOXYACETIC ACID. R. Cronin-Schreiber*, G. Bryant*, B. Melamed*, C. Norris and P. Guth. Dept. Pharmacology and Otolaryngology, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Aminooxyacetic acid (AOAA) has been shown to produce a reversible loss of hearing acuity, presumably by diminishing the endocochlear potential (Bobbin, R.P., et al., Nature, 223:70, 1969). Based on this finding Bobbin, et al. (Trans. Amer. Acad. Ophthal. Otolaryng., 82:299, 1976) investigated the possible protective effect of AOAA against noise-induced loss of cochlear hair cells, and found that AOAA did, indeed, protect against this type of hearing loss. We decided to study the possible protective effect of AOAA against kanamycin-induced loss of cochlear hair cells and found that rather than a protective effect AOAA potentiated kanamycin's ototoxicity. Single subcutaneous doses of AOAA at 8, 11, 15, and 25 mg/kg, which normally do not damage the cochlea, markedly potentiated kanamycin's ability to cause cochlear damage in the guinea pig. Kanamycin generally is given in subcutaneous doses of 400 mg/kg daily for 10-14 days to produce ototoxicity. When combined with AOAA, a single 400 mg/kg dose of kanamycin is sufficient to cause cochlear damage as measured electro- and histocochleographically. Twenty-one days after an injection of one dose each of AOAA and kanamycin, it is not possible to detect hearing thresholds electrocochleographically at 2, 4, 8, 12 and 16 kHz. The pattern of histological damage produced is somewhat different from that produced by kanamycin alone. The combination caused widespread destruction of both inner and outer hair cells, whereas kanamycin generally causes the loss of outer hair cells before many of the inner hair cells are destroyed. The mechanism of this unexpected interaction of AOAA and kanamycin is unknown but may involve the interference of glutamate metabolism by AOAA and the interference of glucose transport by kanamycin (Garcia-Quiroga, J., et al., Res. Commun. Chem. Pathol. Pharmacol., 22:535, 1978), thus depriving the cochlea of its chief energy sources.

Supported in part by NSF #11647 and NSF #07058-03.

18.6 ONTOGENY OF FREQUENCY SELECTIVITY AND SENSITIVITY OF THE PERIPHERAL AUDITORY SYSTEM OF THE POST-METAMORPHIC BULLFROG, RANA CATESBEIANA. William P. Shofner and Albert S. Feng. Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801

During the post-metamorphic development of the bullfrog, there is a dramatic increase in the size of the tympanic membrane, middle ear bones, and middle ear cavities. Changes in the size of these structures presumably influence the efficiency of sound transmission at various frequencies. This raises the question as to whether the frequency selectivity and sensitivity of the bullfrog peripheral auditory system change during development.

Auditory responses to pure tone bursts were studied on over 200 single auditory fibers of the VIIIth cranial nerve of juvenile bullfrogs ranging in snout-vent lengths of 37-46 mm (2.5-12 g body weight) and compared to those of fully developed adults. The distribution of best excitatory frequencies (BEFs) from the juveniles indicated that at least three populations of auditory fibers exist: a low-frequency population centered around 100-300 Hz, a mid-frequency population had BEFs distributed between 900 Hz and 1600 Hz, and a high-frequency population had BEFs ranging from 1800 Hz to 2400 Hz. It is unknown at the present time as to which auditory papillae these populations innervate. The distributions of BEFs of the mid- and high-frequency populations found in the adult were lower than those observed in the juvenile. In the adult, the mid-frequency population had BEFs distributed between 500 Hz and 900 Hz, and the BEFs of the high-frequency population ranged from 1100 Hz to 1800 Hz. Furthermore, the majority of high-frequency sensitive fibers in the juvenile could be excited by tones as high as 4000-4500 Hz, whereas all high-frequency fibers in the adult could not be excited by tones beyond 3000 Hz even at intensities of 100 dB SPL. Thus, there appeared to be a downward shift in the frequency selectivity with development. A comparison of the distribution of thresholds at BEFs indicated that the sensitivity of the low-frequency fibers in the juvenile generally were poorer than those fibers found in the adult. The mechanisms responsible for these changes are currently being investigated.

Supported by NSF grant 79-12271, RIAS Study Grant SER 78-18244 from NSF, and NIH training grant HEW PHS GM 07283-05.

18.7 NEURAL DELAY IN THE AUDITORY SYSTEM DETERMINED USING AMPLITUDE MODULATED SOUNDS. A. R. Møller. Division of Physiological Acoustics, University of Pittsburgh School of Medicine, *Pittsburgh, PA 15213.*

Gross potential responses from the auditory nerve and the cochlear nucleus of anesthetized rats to tones and noise which were amplitude modulated with pseudorandom noise were compared with the responses to transient sounds such as tone and noise bursts. The responses to several periods of the amplitude modulated sounds were averaged and syncronized to the periodicity of the pseudorandom noise. The averages were cross correlated with one period of the pseudorandom noise. The wave morphologies of the cross correlation functions resembled those of the averaged responses to tone and noise bursts. However, the latencies of peaks obtained from cross correlation functions showed smaller decreases as the stimulus intensity increased than did the latencies of the peaks from responses to tone and noise bursts. The latencies of the peaks in the cross correlation function obtained from the gross potential responses had values that were close to those of single unit responses to the same types of stimuli. This indicated that the latencies obtained from the responses to amplitude modulated sounds were more accurate measures of synaptic and axonal delay than were the latencies of the responses to transient sounds such as tone or noise bursts.

18.8 THE FINE STRUCTURE OF STELLATE CELLS IN THE ANTERIOR DIVISION OF THE ANTEROVENTRAL COCHLEAR NUCLEUS (AVCN) OF THE CAT. N. Cant. Department of Anatomy, Duke University Medical Center, Durham, *N. C. 27710*

The anterior division of the AVCN contains at least two types of large neurons defined on the basis of their dendritic morphology in Golgi preparations--the bushy cells and the stellate cells. The fine structure and synaptic organization of the bushy cells has been described previously (e.g., Cant & Morest, '79), but no detailed description of the stellate cells is available. In the present study, stellate cells were observed with the electron microscope and the fine structure of their somas and proximal dendrites and of the synaptic terminals contacting them was described. Their synaptic organization was then compared to that of the bushy cells.

The stellate cells are identified by their somatic and dendritic size and shape, their distribution within the AVCN and the organization of their cytoplasmic organelles. They are distinguished from bushy cells by the absence of stacks of granular endoplasmic reticulum encircling the nuclear envelope. Although only one type of stellate cell has been identified in light microscopic preparations, two distinct types are found in electron micrographs. The type I stellate cells are large neurons with relatively pale cytoplasm and very few synaptic endings contacting the somatic or proximal dendritic surface. In contrast, their more distal dendrites are contacted by many terminals. The type II stellate cells are large neurons with many somatic spines and numerous axosomatic and axodendritic synapses. Both neuronal types receive synaptic endings from the cochlea as well as several types of non-cochlear endings, most of which resemble those that contact the bushy cells. However, the fine structure of a few of the non-cochlear terminals contacting the type II neurons appears to be different from that of terminals described previously. In addition, large end-bulbs of Held have not been seen contacting either type of stellate cell.

In summary, the anterior division of the AVCN contains at least three types of large neurons defined on the basis of their fine structure and synaptic organization. Since distinctive arrangements of cochlear and non-cochlear synaptic terminals could result in different response patterns to acoustic stimuli, each of these neuronal types may correspond to a different type of single unit, defined physiologically.

Supported by US PHS grant 1R01NS14655.

18.9 TYPES OF GIANT NEURONS IN DEEP DORSAL COCHLEAR NUCLEUS (DCN) OF CATS. Eileen S. Kane. Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605

Giant neurons (average diameter > 22.0 μm) of the deep DCN (layers 3 & 4 of Lorente de Nó) have been identified by somatic shape, location, orientation and basal dendritic numbers and orientations in Nissl material. The major types (elongate bipolar, elongate multipolar, radiate, oriented and globular giants) correspond to distinct giant neurons studied in Golgi-impregnated cat deep DCN. Both types of elongate giant cells have smooth somata, but bipolars have a cylindrical dendritic field and smooth, long dendrites, parallel to the striae. Elongate multipolars have bowl-shaped dendritic fields and highly branched, appendage-covered dendrites. Radiate giants have ovoid-to-spherical, smooth somata and thin, appendage-covered, radiating dendrites. Oriented giants, with smooth, spherical somata, have stout, long dendrites extending a cone-shaped field into the superficial DCN. Globular giants have smooth, ovoid somata vertical to the DCN surface and one or two very thick, short primary and long secondary dendrites. Each giant cell type was first recognized in thick plastic sections and then identified in thin sections. All giant cell basal and primary dendrites receive very dense synaptic inputs. Somata of elongate multipolar, radiate and oriented giants have very dense synaptic coverage while elongate bipolar cell somata have less and globular giants have the sparsest coverage. Many large, primarylike terminals are found on basal dendrites of all giant cell types and notably, upon somata of elongate multipolar and radiate giant cells. Nauta preparations (after complete ipsilateral cochlear ablations and 2-14 day survival times) show primary inputs to all giant cell types. Elongate bipolar cells are always surrounded by peridendritic (not perisomatic), medium-caliber preterminal fragments but elongate multipolars have perisomatic and peridendritic fragments, as do radiate giant cells. Preterminal fragments are predominantly associated with basal and primary dendrites of both oriented and globular giant cells, but both have occasional perisomatic degeneration. Thus, giant cell types receive cochlear inputs of different densities and distributions; elongate multipolars and radiate giant cells receive the densest inputs. Since all giant cell types have observable (Golgi-impregnated) axons directed toward or into the striae, we conclude that all of these large neurons can transform and convey differently encoded primary information to higher auditory centers.

(Supported by The Deafness Research Foundation and NIH Grants NS-14260 and NS-02900 (RCDA) to ESK.)

18.10 LOCALIZATION OF LABEL FROM H^3-GABA IN PARALLEL FIBERS IN THE OUTER MOLECULAR LAYER IN THE CAT DORSAL COCHLEAR NUCLEUS. I.R. Schwartz, Division of Head and Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

Light and electron microscopic autoradiography was performed on sections from fresh slice preparations of the cat dorsal cochlear nucleus following 20 minute room temperature incubation in oxygenated Ringers bicarbonate solutions containing micromolar amounts of different putative neurotransmitter amino acids. Some parallel fibers in the outer molecular layer were heavily labeled following incubation with H^3-GABA. Some terminals in the outer molecular layer were labeled following incubation with H^3-glutamic acid and H^3-glycine. However, after H^3-glutamic acid the labeled terminals were distributed deeper in the outer molecular layer and generally appeared to be larger than the parallel fiber endings. Labeled terminals were mainly found on cell bodies and dendrites after H^3-glycine. Virtually no endings were labeled after incubation with H^3-aspartic acid, H^3-taurine or H^3-alanine. Granule cell bodies were unlabeled after all the amino acids.

The localization of label derived from H^3-GABA in parallel fibers is consistent with its presumed role as an inhibitory neurotransmitter in the dorsal cochlear nucleus and the biochemical evidence of its higher concentration in the molecular layer (Godfrey et al., 1977). The label localization could be due either to an exchange with high endogenous concentrations or to a high affinity uptake. Moreover, given the level of preservation of the slice preparation produced by the tissue chopper, the label localization could reflect synaptosomal-like properties rather than properties of intact neurons. Further studies are underway to examine label localization in hand sliced sections in which there is improved preservation.

Supported by NS 09823 and 14503.

19.1 EFFECTS OF RESTRAINT ON DOPAMINE TURNOVER AND TYROSINE HYDROXYLASE ACTIVITY IN RAT FRONTAL CORTEX. Linda Toth Kennedy and Michael J. Zigmond. Department of Pharmacology, School of Pharmacy, and Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Dopamine (DA) turnover in rat frontal cortex (FC) has been shown to increase following footshock. We wished to confirm these results using a second stressor, restraint, and to determine whether the increased turnover was accompanied by an increase in tyrosine hydroxylase (TH) activity.

Adult, male Sprague-Dawley rats (Zivic-Miller Labs) were housed one per cage and were handled daily for at least one week. Rats were then subjected to 15 minutes of restraint, and DA turnover estimated by measuring dihydroxyphenylacetic acid (DOPAC) levels or the rate of DA depletion following synthesis inhibition. Fifteen minutes of restraint increased DOPAC levels in FC by 30% (control = .046 ug/g; restraint = .060 ug/g; $p<.05$), suggesting an increase in DA turnover in that structure. In a second experiment, rats were subjected to 15 minutes of restraint immediately prior to the administration of the DA synthesis inhibitor alpha-methyltyrosine (AMT; 250 mg/kg, i.p.), and were sacrificed 15 minutes post-injection. The turnover rate of DA in FC of non-stressed rats was 0.035 ug/g/h, while that of stressed rats was 0.118 ug/g/h. Similar results were obtained if rats were treated with AMT prior to restraint, and sacrificed immediately after the 15 minute stress period.

We next examined the ability of restraint to increase TH activity in FC. Tissue was homogenized in 50 mM Tris-HCl, pH 6.0, and the 20,000 x g supernatant assayed for TH activity in a Tris-acetate buffer (pH 6.2) in the presence of subsaturating concentrations of tyrosine and cofactor. No significant change in TH activity was observed using either the synthetic cofactor, 6MPH4, or the presumed natural cofactor, biopterin. Moreover, no difference in TH activity was observed between samples from control and restrained animals incubated in the presence of the phosphatase inhibitor, NaF. TH activity could be increased *in vitro* by incubating the tissue under phosphorylating conditions (cAMP, ATP, magnesium, and protein kinase), implying that TH activity was not maximally active in crude homogenates.

These data thus support previous suggestions that stress increases DA turnover in rat FC. However, although TH from FC could be activated *in vitro*, *in vivo* activation could not be detected following restraint-induced stimulation of DA turnover. (Supported in part by USPHS Grants MH-29670 and MH-00058.)

19.2 ACUTE HEAT STRESS LOWERS BRAIN STIMULATION REWARD THRESHOLD. R.W. Phelps* and M.J. Lewis. Dept. of Psychology, Tufts University, Medford, MA. 02155.

The effects of acute heat stress were investigated on brain stimulation reward (BSR), and food and water intake in adult male albino rats. All rats were implanted in the medial forebrain bundle(MFB) with bipolar platinum electrodes. Each received extensive training to press a lever for stimulation under a concurrent fixed-ratio/ continuous schedule of reinforcement. This schedule was used to determine BSR **threshold** according to the methods similar to Huston and Mills (1969). After reliable BSR performance was established, all rats were subjected to 3 hrs of 22°C(control) and then either 30°C or 40°C heat in an insulated temperature controlled chamber. Rats were immediately removed at the end of the period of heat application and BSR threshold, rate of response and brain impedance were determined in an operant chamber. Heat stress of 40°C, but not 30°C, lowered BSR threshold levels and increased brain impedance over control values. No change in overall response rate for the sessions was observed. Other rats subjected to the 40°C heat stress showed a decrease in food and, unexpectedly, water intake during a 40 minute period after the heat stress. The effects of acute heat stress on BSR are discussed in terms of known effects of acute heat stress on brain monoamine systems.

(Supported in part by grants from Tufts University Faculty Research Committee, and the Dean of Faculty and NIDA 02176-01).

19.3 BEHAVIORAL AND HISTOLOGICAL ANALYSIS OF PSYCHOSOCIALLY STRESSED MICE. W.P. Meehan* and J.N. Naranjo.(SPON: J.C. Shih). Department of Physiology,University of Southern California School of Medicine,Los Angeles, CA 90033 and Department of Anatomy,Harvard Medical School,Boston,MA 02178

The highly accellerated rate of neuron loss in stressed animals may be related to increased levels of corticosteroids. The adrenal glands of psychosocially stressed CBA/USC male mice are enlarged as compared to aged-matched controls, indicative of increased corticosteroid synthesis and release. In addition, stressed male mice have been shown to have increased plasma levels of corticosterone. Recent evidence suggests that high levels of corticosteroids lead to pathologic changes in the brain including neuron loss.

The brains of stressed and non-stressed same age control mice were compared histologically using a silver degeneration stain (Naranjo and Greene,1977).Numerous degenerating fiber tracts were noted in the stressed animals while minimal degeneration was found in the non-stressed animals.

To test the behavioral ramifications of the neuronal loss, especially loss in the fornix and hippocampus, two additional groups of animals were compared in their ability to learn a delayed alternation task. The stressed animals displayed a significant deficit ($p<.01$) in spatial learning ability, and were significantly ($p<.01$) slower than the controls.

After behavioral testing, the brains of the animals were removed, stained, and examined in a blind fashion by three trained observers. The hippocampus of each stressed animal as well as the fiber tracts communicating hippocampal afferent and efferent imput (fornix, fimbria, and mammillothalamic tract) contained degenerating neuronal material. Examination of the non-stressed animals revealed only occassional degenerating fibers.

The histopathology of the stressed animals' hippocampus and fiber tracts strongly supports the possibility that memory impairment in these animals was a result of stress related degenerative changes in the central nervous system.

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19.4 CORRESPONDENCE OF BEHAVIORAL THERMAL SELECTION PREFERENCES WITH AUTONOMIC THERMONEUTRAL ZONE. M.L. Laudenslager, D. Tomback*, University of California at Santa Barbara, 93106.

Previous studies of Chukar partridges suggested that the preferred ambient temperature (PAT) defined within a lab environment overlapped the autonomic thermoneutral zone (TNZ) of that species. The present study determined the PAT in three additional species (mourning doves, domestic pigeons, and Clark's nutcracker) and compared these with the TNZ for these species. PAT was determined in a two-compartment environmental chamber. One chamber was maintained at 0°C; the other could be varied between 20 and 55°C. The bird could move between the two chambers at any time. For any test, only one warm chamber temperature was presented. PAT was determined from the warm chamber temperature at which the bird would not escape into the cold. Two domestic pigeons demonstrated PATs of 32.1 and 32.3°C. A single mourning dove selected a PAT of 34.5°C. Two nutcrackers showed PATs of 21.5°C and 28.7°C. The PATs demonstrated for columbiformes fell within TNZs previously reported in the literature. TNZ for the nutcrackers was found by measuring rates of oxygen consumption ($\dot{V}O_2$) over an ambient temperature range of -5 to 44°C. Minimum $\dot{V}O_2$ was observed between 25 and 30°C for winter-adapted birds and between 20 and 30°C for summer-adapted birds. The lack of perfect correspondence between the PATs and TNZs for the nutcracker may be related to the fact that the behavioral tests were made in laboratory-acclimated birds. In general, these additional tests further support the common sense notion that when a choice is possible, birds will select ambient temperatures at which metabolic rate is minimized. (Supported by University of California, Santa Barbara Faculty Grant #412 and National Science Foundation Grant #PCM-7825238.)

19.5 SYMPATHO-ADRENAL AND CARDIOVASCULAR CORRELATES OF AGGRESSIVE BEHAVIOR IN THE AWAKE CAT. S.L. Stoddard-Apter, A. Siegel, C.H. Block* and B.E. Levin. Dept. of Neurosciences, College of Medicine & Dentistry of New Jersey, New Jersey Medical School, Newark, N.J. 07103, and Dept. of Neurology, V.A. Medical Center, East Orange, N.J. 07019.

The hypothalamus of the awake, intact cat was stimulated at multiple sites to determine those regions which could elicit integrated behavioral responses and/or activation of the cardiovascular (CV) and sympatho-adrenal (SA) systems. Cats were restrained in a headholder, and CV parameters (blood pressure and heart rate) were monitored continuously from an intra-arterial cannula. Activation of the sympathetic nervous system and adrenal medulla were determined simultaneously by radioenzymatic assay of plasma norepinephrine (NE) and epinephrine (E), respectively. Blood samples were withdrawn from an indwelling venous cannula prior to stimulation of the hypothalamus at 0.4 mA for 5 sec, and immediately, at 30 sec, and at 1, 2, 3, 4, 5, 10, and 15 min after stimulation (30 min intertrial interval). Preliminary trials showed that the method of restraint used produced plasma catecholamine levels intermediate between those in the unrestrained and those in the anesthetized cat. Sites which elicited maximum CV and SA activation were generally located along a continuum throughout the hypothalamus, extending from the perifornical area rostrally, through the dorsolateral hypothalamus, to the mammillary bodies caudally. The CV and SA activation sites were, for the most part, identical; they differed only at the level of the ventromedial nucleus, where the effective CV sites were situated more dorsally than the effective SA sites, and at the level of the caudal extent of the mammillary bodies, where the effective CV sites were located lateral to the effective SA sites. Inasmuch as these responses could be elicited throughout the rostro-caudal extent of the hypothalamus, these data suggest that hypothalamic sites which activated the CV and SA systems represented stimulation of descending fiber bundles originating at more rostral levels. Those sites which elicited integrated behavioral responses, however, were localized in more circumscribed regions, which were not necessarily contiguous with the descending CV and SA pathways. Escape behavior was generally associated with the greatest CV and SA activation; a smaller response was elicited from stimulation of sites producing affective defense behavior. Predatory attack sites tended either to inhibit the sympathetic system (NE), or to activate it only after an initial delay.

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19.6 INFLUENCE ON CARDIOVASCULAR FUNCTION BY PREFRONTAL CORTEX AND ITS THALAMIC RELAY NUCLEUS. Charles H.K. West and Robert M. Benjamin. Dept. of Neurophysiology, Univ. of Wisconsin Medical School, Madison, Wisconsin 53706.

Influence of prefrontal cortex on emotions and autonomic functions has long been known. Prefrontal cortical modulation of autonomic parameters was re-emphasized by the report that electrical stimulation of orbitofrontal cortex in awake rhesus monkeys evoked cardiovascular changes (Hall and Cornish, Exp. Neurol. 56: 289-297, 1977). One dramatic finding of this study was the occurrence of severe multifocal cardiac necrosis in most of the stimulated animals, implying a relationship between prefrontal cortex and emotionally induced cardiopathy. We decided to investigate the neural pathways that might mediate this phenomenon in the rabbit, where much of the neuroanatomy of the prefrontal cortex system has been studied by our laboratory. Only small (<5%) changes in heart rate to stimulation of sulcal prefrontal cortex were observed in anesthetized animals. In contrast, electrical stimulation (20-300 μA, 60 Hz sine wave, 3 sec duration) of the thalamic mediodorsal nucleus (MD), the primary relay nucleus for prefrontal cortex, elicited highly significant decreases in heart rate proportional to the stimulating current. At the higher current intensities used (100-300 μA), heart rates were reduced by as much as 75-85% from pre-stimulus levels. This stimulus-produced bradycardia appeared quickly after stimulus onset (delay < 1 sec, peak within 5 sec), and the heart rate usually returned to baseline within 30 sec after stimulation. The cholinergic innervation of the heart by the vagus was implicated in the generation of the response since either administration of atropine (sulfate or methyl nitrate, 0.15 mg/kg iv) or bilateral vagotomy abolished the majority of the response. Administration of a β-adrenergic receptor blocker (propranolol, 3 mg/kg iv) did not affect the heart rate response. The effective area for evoking a response within the medial thalamus was localized to MD, the midline nuclei between both MD's, and a region posterior to MD (e.g., parafascicular nucleus). The descending effector pathway from MD to autonomic centers may involve these neighboring midline areas since a knife cut along the medial border of MD blocked the responses elicited from areas farther lateral in M.D. Furthermore, complete bilateral ablation of prefrontal cortex did not diminish the stimulus - produced bradycardia evoked from MD. Previous work on the rabbit has shown that the midline nuclei medial to MD (but not MD itself) project to various subcortical regions including the medial (periventricular) portion of the hypothalamus (West, et al., Neuroscience, 4: 1977-1988, 1979), thus suggesting a potential output pathway for autonomic responses.

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19.7 BRAIN AREAS IMPLICATED IN CHOLINERGIC MEDIATION OF SEXUAL BEHAVIOR. G. P. Dohanich* and L. G. Clemens. Department of Zoology, Michigan State University, East Lansing, MI 48824.

Sexual behavior in female rats was measured following stimulation of cholinergic receptors in several brain areas. Ovariectomized female rats were pretreated with .13 μg estradiol benzoate 72, 48, and 24 hr before behavioral testing. This estrogen regimen does not induce sexual behavior in female rats. However, carbamylcholine chloride (carbachol), a muscarinic receptor agonist, significantly increased sexual behavior, as indicated by the incidence of lordosis, in these estrogen-primed females within 15 min after bilateral infusion (.5 μg/cannula) into the medial preoptic area (POA) or ventromedial hypothalamus (VMH). Carbachol failed to induce sexual behavior at this dose of estrogen priming following infusion into the mesencephalic reticular formation (MRF) or frontal cortex. Infusion of the artifical cerebrospinal fluid vehicle (aCSF) was ineffective in all sites.

			MEAN LORDOSIS QUOTIENT ± SEM			
SITE	TREATMENT	RESPONDING	Pretest	15 min	45 min	90 min
POA	aCSF	0/12	2 ± 1	3 ± 3	1 ± 1	2 ± 1
	Carbachol	9/12	4 ± 3	58 ± 9	37 ± 8	7 ± 3
VMH	aCSF	0/12	8 ± 4	5 ± 4	4 ± 3	6 ± 4
	Carbachol	10/12	2 ± 1	58 ± 8	38 ± 9	26 ± 9
MRF	aCSF	1/9	2 ± 2	8 ± 8	9 ± 9	12 ± 9
	Carbachol	1/9	0 ± 0	8 ± 5	6 ± 6	11 ± 8
Cortex	aCSF	1/11	4 ± 2	8 ± 5	13 ± 7	11 ± 5
	Carbachol	4/11	5 ± 3	21 ± 8	18 ± 8	17 ± 8

Results indicate an estrogen-dependent cholinergic mechanism in the POA and VMH which facilitates the occurrence of sexual behavior in female rats. In addition, the rapid but transient nature of the behavioral response suggests that carbachol induced sexual behavior by directly stimulating postsynaptic cholinergic receptors, rather than by a secondary action.

19.8 EFFECTS OF TESTOSTERONE ON BODY WEIGHT AND PROTEIN INTAKE. L. I. Siegel*, A. A. Nunez* and G. N. Wade*. (SPON: J. Meyer). Dept. Psychology, Univ. Massachusetts, Amherst, MA 01003.

Previous work from this laboratory has shown that testosterone propionate (TP), administered peripherally or centrally, affects both food intake (FI) and body weight (BW) in gonadectomized (GX) male rats. To determine the influence of TP on intake of dietary components, GX male rats, each receiving a daily injection of either 0.2 mg TP, 2.0 mg TP or the injection vehicle, were given access to one of two pairs of diets. One set of diets was isocaloric and equal in fat content, varying only in percent compo- of carbohydrate and protein; in the other pair both diets had equal percentages of protein and of fat but varied in carbohydrate and caloric content. Injections of the low dose of TP resulted in increased caloric intake and rate of weight gain in GX males compared to control rats. The increase in FI was due to increased consumption of all dietary components. Males given long-term treatment with the high dose of TP, however, ate fewer calories and had lower body weights than did rats given 0.2 mg TP. Although FI was decreased in rats receiving the 2.0 mg TP dose, protein intake was selectively increased such that it did not differ significantly from that of the low TP-treated males.

We have previously shown that the lowered BW of males given high TP doses may be due, in part, to metabolic effects of estrogenic metabolites of the hormone, although at lower doses the estrogenic effects may be masked by anabolic actions of TP. The results of the present study show that TP stimulates protein consumption whether or not it is given in amounts sufficient to be converted to estrogens. In addition, the decrease in FI and BW in rats receiving the high dose of TP is similar to that observed in intact, sexually active male rats who show a rise in circulating testosterone titers and a concomitant decrease in FI and BW.

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19.9 VASOPRESSIN-DEFICIENT RATS (BRATTLEBORO STRAIN) ARE IMPAIRED IN ADAPTATION AND POSITIVELY REWARDED DISCRIMINATION LEARNING. S. I. Gingold*, G.N.O. Brito, G. J. Thomas and D. M. Gash*. Center for Brain Research, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Vasopressin has recently been implicated in memory processes. However, most studies which have suggested a role of vasopressin in memory processes have used negatively reinforced behavioral tasks (i.e., shock). It is unclear then whether the effects observed are due to associative (memory) or nonassociative (performance) factors. If vasopressin is really a "memory molecule" then it should also be involved in positively reinforced behavioral tasks.

Six Brattleboro rats from our colony and six normal rats (Long-Evans) obtained commercially were used in this study. The Brattleboro animals were characterized as homozygous diabetes insipidus (DI) by water intake (24 hr.) and osmolarity of urine. The animals were housed in individual wire-mesh cages and given food and water ad lib. After 7 days they were started on a reduced food-regimen that lasted for the duration of the experiment. Seven days later maze adaptation began. The animals were given eight daily sessions (12T/day) of adaptation in the T-maze. On each trial, only the door to one of the two arms was raised (Fellows' schedule) 2 sec after raising the start-box door. After the animals were running fast, visual brightness discrimination was begun. The rats were given 15 daily sessions (12T/day) and the position of the positive stimulus (a 7.5W light bulb) was located in either arm according to a Fellows' schedule.

DI rats were significantly slower than normals in the maze adaptation sessions, although by the 8th session they were running as fast as normals. DI animals made significantly more errors than normals in the visual discrimination task. Only one of the six DI animals reached criterion (92% correct for two consecutive sessions) within 15 sessions whereas every normal animal reached criterion within that time.

The data suggest that vasopressin-deficient rats differ from controls in adapting to a new environment and in the performance of a positively rewarded visual discrimination task. Whether this represents a defect in associative mechanisms or a performance defect at the sensory level (visual) remains to be determined.

19.10 ESTRADIOL IMPLANTS IN THE AMYGDALA FACILITATE MATERNAL BEHAVIOR IN THE FEMALE RAT. A.S. Fleming*, G. Orpen* and C. Luebke.* (SPON: E. Dudek). Psychology, Univ. of Toronto, Mississauga, Ontario.

Five groups of day 18 pregnant, ovariectomized Caesarian-delivered Wistar female rats were tested for maternal behavio to four 5-10 day old foster pups proffered 48 hours after pregnancy termination and daily until animals became maternal, or for 10 days. Four groups sustained intracerebral implants of estradiol 17-β in 1) the amygdala, 2) the lateral hypothalamus, and 3) other control sites. Two groups received implants of cholesterol in 4) the amygdala and 5) other control sites. Implants were inserted through chronically implanted cannulae at the time of pregnancy-termination and were removed 48 hours later on the day of the first test.

Animals with estradiol implants in the amygdala (Group 1) became maternal more rapidly than did groups sustaining estradiol implants in other brain areas (Groups 2 and 3) and Groups 2 and 3 became maternal more rapidly than did Groups 4 and 5 who received cholesterol implants.

This study suggests that the high levels of endogenous estradiol found at parturition may promote the rapid onset of maternal behavior at this time by acting on estrogen receptors in the amygdala (Fleming, Vaccarino and Luebke, Physiol. and Behav., in press) as well as by acting on the medial preoptic area (Numan, 1974).

19.11 CENTRAL EFFECTS OF ESTRADIOL ON FOOD INTAKE IN FEMALE RATS. A. A. Nunez*, J. M. Gray* and G. N. Wade* (SPON: R. M. Gold). Department of Psychology, Univ. Mass., Amherst MA 01003

Implants of estradiol benzoate (EB) in the ventromedial hypothalamus (VMH) reduce food intake in ovariectomized rats. Recent findings indicate that in addition to possible central effects, estrogens may modulate food intake by acting directly on peripheral sites to alter metabolism (Phys. Behav. 1979, 22:583-593). In this experiment, we investigated the effects of VMH implants of EB on food intake in ovariectomized rats. After 24 hrs of intrahypothalamic hormone stimulation, vaginal smears were obtained and adipose tissue lipoprotein lipase (LPL) activity and cytoplasmic progestin receptors were measured.

The hormonal treatment resulted in a significant reduction in food intake without cornification of the vaginal epithelium. Furthermore, the reduction in food intake was observed in the absence of changes in adipose tissue LPL activity or progestin receptors. Systemic administration of EB reduces LPL activity and induces the appearance of progestin receptors in adipose tissue. These data suggest that estrogenic stimulation of the VMH may be sufficient to produce the changes in food intake seen after systemic administration of EB to ovariectomized rats.

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20.1 CHRONIC LITHIUM ADMINISTRATION IN RATS: PHARMACOKINETICS, DISTRIBUTION AND EFFECTS ON DOPAMINE RECEPTORS. W.J. Shoemaker, D.A. Staunton, P.J. Magistretti, F.B. McCoy and F.E. Bloom. Arthur V. Davis Ctr. for Behav. Neurobiol., The Salk Inst., La Jolla, CA 92037.

Most published procedures for chronic lithium dosing yield highly variable food and water intake with corresponding variations in blood and brain Li^+ values, weight gain and state of hydration. We have devised a diet containing 40 mmoles/Kg Li^+ that allows weight gain without evidence of toxic symptoms when ingested over several weeks. The diet is formulated into a pellet form containing 1.696 g/Kg LiCl, 210 g/Kg protein, 690 g/Kg carbohydrate sources, 50 g/Kg lipid; rats are allowed to eat ad libitum and have access to water at all times. The lithium dose per day during the first week on the diet ranges from 86-96 mg/L; during the second week the range is 92-136 mg/L. The animals require 5-6 days to reach a steady-state level of brain and blood Li^+. Blood levels in a series of 75 rats ranged from 0.5 to 0.7 mEq/L, although the average brain levels were always higher (range 0.6 to 0.8 mEq/L). However, Li^+ does not accumulate evenly throughout the brain. When specific brain regions were analyzed, the corpus striatum accumulated Li^+ to the greatest extent (1.15 mEq/L) compared to cerebellum (.69 mEq/L) and pons medulla (.632 mEq/L). The great accumulation of Li^+ in the corpus striatum led us to study the binding properties of dopamine receptors in rats maintained on the lithium diet. Antagonist binding to dopamine receptors in crude, resuspended membranes prepared from the corpus striatum was determined with ^{3}H-spiroperidol in a group of animals fed the lithium diet (brain levels = 1.08 mEq/L) for 3 weeks. Consistent with previous reports, Scatchard analysis revealed no alteration in the affinity (K_D: 84.6 ± 18 pM, treated; 81.0 ± 19 pM, control) of ^{3}H-spiroperidol for the receptors or the density (B_{max}: 577 ± 38 fmoles/mg protein, treated; 590 ± 35 fmoles/mg, control) of dopamine receptors. The ability of the dopamine agonists, apomorphine and ADTN, to compete for ^{3}H-spiroperidol binding was also determined in striatal membranes prepared from these animals. The K_is for both apomorphine ($p < 0.01$) and ADTN ($p < 0.05$) were elevated in lithium-fed animals. These preliminary results suggest that lithium selectively reduces the affinity of dopamine agonists, but not antagonists, for dopamine receptor sites in the neostriatum. Further studies may reveal the behavioral and therapeutic relevance of this action of lithium. Supported by NIMH grants MH 29466; MH 08080 for D.A.S. P.J.M. is a recipient of a Swiss National Sciences Foundation Fellowship.

20.2 BEHAVIORAL EFFECTS OF EARLY POSTNATAL LEAD EXPOSURE IN THE RHESUS MONKEY: FIXED INTERVAL RESPONDING AND INTERACTIONS WITH DRUGS. P.J. Bushnell, P. Mele and R.E. Bowman. Dept. Biochemistry and Dept. Psychology Primate Laboratory, Univ. of Wisconsin, Madison, WI 53706.

Operant behavior was studied in a group of adolescent monkeys as a part of a test battery designed to assess the behavioral sequelae of early postnatal Pb intoxication. During the first year of life, a high-Pb group (n=4) received Pb in milk at 0.88 mg/kg·day, a low-Pb group (n=4) at 0.29 mg/kg·day, and a control group (n=4) received no added dietary Pb. Pb levels in whole blood, obtained biweekly from each animal throughout treatment, averaged 65, 32, and 4 ug/dl for the high-Pb, low-Pb, and control groups, respectively, over the year of treatment. Blood Pb levels in experimental animals fell after Pb ingestion stopped, and were all less than 10 ug/dl by 33 months of age, when operant testing began (Bushnell & Bowman, Pharm. Biochem. Behav. 10:733, 1979). One monkey in each experimental group responded at very low rates throughout these tests; their data were excluded from analysis. Otherwise, the performance of the experimental animals did not differ from control on measures of barpress response acquisition, response characteristics on FR schedules, or on resistance to extinction. However, the performance of the experimental groups on an FI60" schedule showed a flatter scallop in cumulative records and significantly smaller indices of curvature, compared to controls. In challenges with pentobarbital (0 - 20 mg/kg, sc) and scopolamine (0 - 40 ug/kg, sc)during FI responding, the FI scallop flattened at lower doses of both drugs for controls than for experimental animals. Thus a small but persistent behavioral deficit, resulting from early postnatal exposure to Pb, was observed in an operant paradigm, complementing results in the same animals on a reversal learning task (Bushnell & Bowman, J. Toxicol. Envir. Health 5:1015, 1979). Supported by NIEHS grant 2-R01-ES01062 and funds from the Food Research Institute, Univ. of Wisconsin.

20.3 INTRAVENOUS SELF-ADMINISTRATION OF ETHANOL MAINTAINS PHYSICAL DEPENDENCE IN RATS. R. Numan. Psych., U. Santa Clara, CA 95053

In a previous report (Numan & Gilroy, Pharmac. Biochem. Behav. 9:279, 1978) it was shown that the intravenous (IV) method is a rapid and reliable model for the induction of physical dependence upon ethanol in rats. The present research now reports that rats will also self-administer (IV) ethanol, and that these self-infusions maintain previously induced physical dependence.

In Exp.1, male hooded rats (N=10) were implanted with jugular cannulae and housed in sound attenuated operant chambers with food and water freely available. They were maintained under these conditions 24h/day. Following habituation, the rats were exposed to periodic cycles of forced ethanol infusions that induced physical dependence. Each of these induction cycles was administered as previously described (ibid). Briefly, ethanol (30% V/V) was administered over a 5-day period at a dose of 10-16g/kg/day. Following each cycle, forced infusions were discontinued, but the rats were allowed access to a lever for self-administration of ethanol (CRF). Each lever press infused 0.2ml of ethanol (20% V/V). The rats were maintained on self-administration for at least 24h. If a rat did not develop self-administration behavior (SAB) within 24h, the next forced induction cycle was initiated.

Of the 10 rats tested, 2 failed to show any signs of SAB, even after 5 cycles of forced dependence. In contrast, 8 rats did develop SAB on CRF after a mean of 4 (S.E. 0.38) forced dependence cycles. These rats were maintained on CRF for a mean of 6.4 days (S.E. 0.75) and self-infused a mean of 11.3g/kg/day (S. E. 0.87). These rats were then advanced to Fixed Ratio (FR) schedules of reinforcement (FR2-FR5). Results on FR were more variable; 5 of the rats increased lever presses and maintained daily ethanol intake comparable to CRF. The other 3 rats also increased lever presses, but did not maintain ethanol doses comparable to CRF.

Following extended testing under the various schedules, the rats were placed on withdrawal. All rats that maintained high rates of SAB showed mild to severe withdrawal symptoms, suggesting that the self-infusions maintained physical dependence.

In Exp.2, rats (N=6/group) were allowed to self-infuse either saline or ethanol (20% V/V). These rats had no prior exposure to either saline or ethanol, and forced infusions were never administered. The rats remained in their operant chambers for 21 days under CRF. Each lever press led to a 0.2ml infusion. None of the rats developed SAB, but the saline controls made more self-infusions than the ethanol rats ($P<.05$). These results suggest that the ethanol paramaters yielding significant self-administration in Exp.1 are neutral or even aversive to ethanol naive rats. (supported by Grant AA 03451)

20.4 THE EFFECT OF ORAL ALCOHOL ADMINISTRATION ON BRAIN γ-GLUTAMYL TRANSPEPTIDASE. E. Reyes*, J. M. Rivera and L. J. Lewis. Dept. Pharmacology, Sch. of Medicine, University of New Mexico, Albuquerque, N.M. 87131

The effect of oral alcohol administration on γ-glutamyl transpeptidase (γGTP) was studied. Wistar rats were pair-fed nutritionally adequate liquid diets containing either alcohol (36% of total calories) or isocaloric carbohydrates (dextrin) for 6 weeks as described by Teschke et al (1977). Blood samples were obtained from the tail vein of each animal to monitor blood alcohol levels throughout the experiment. Blood alcohol levels were determined by measuring the increase in NADH according to the method described by Lundquist (1959). Weights of the animals were also determined daily. At the end of the 6 week experimental period, the animals were found to have an average blood alcohol level of 765 mg/dl. The animals were decapitated and the brains dissected according to the method described by Glowinski and Iverson (1966). Each brain region was homogenized in Tris buffered saline and assayed for protein and γGTP activity. γGTP activity was increased significantly in the cerebellum, pons-medulla and striatum. Serum samples were applied to a concanavalin A column and eluted as described by Reyes and Barela (1980). Serum γGTP activity was found to be increased in the alcohol treated animals. This work was supported by MBS program grant 081-39.

20.5 CHRONIC ETHANOL EFFECTS ON SYNAPTIC FUNCTION AND DISTRIBUTION IN THE CA1 FIELD OF RAT HIPPOCAMPUS. W.C. ABRAHAM, B.E. HUNTER, P.B. MANIS, S.F.ZORNETZER and D.W. WALKER. Dept. Neuroscience, Univ. Florida Coll. Med. and VA Med. Ctr., Gainesville, FL 32610.

This study explored the neurotoxic effects of chronic ethanol exposure through electrophysiological analyses of the Schaffer collateral-commissural (SC-COM) input to CA1 stratum radiatum in the rat hippocampus.

Long-Evan rats were maintained on an ethanol-containing liquid diet (35-39% ethanol-derived calories; 8.1-9.4% v/v ethanol) for 20 weeks. Control groups were pair-fed a liquid diet with sucrose isocalorically substituted for ethanol or given free access to standard laboratory chow and water. Two to four months following removal from the special diets, the animals were prepared for acute electrophysiological study. Concentric bipolar electrodes were placed in stratum radiatum of anterodorsal CA1 to stimulate the Schaffer collateral and commissural fibers coursing through this region. Extracellular field potentials were recorded with micropipettes (1-2μm tip) placed in field CA1 1mm posterior to the stimulating electrode.

Laminar profiles were conducted in 25μm steps perpendicular to the pyramidal cell layer. Analysis of the field potentials by current-source density (CSD) techniques revealed a statistically significant shrinkage (45μm) of the SC-COM synaptic field in the stratum radiatum of ethanol-exposed rats. The CSD also unexpectedly revealed the apparent existence of two overlapping but spatially distinct synaptic fields (current sinks) in the stratum radiatum of control animals. Chronic ethanol treatment led to a significant reduction in the spatial extent of the synaptic field proximal to stratum pyramidale with a corresponding expansion of the more distal synaptic field. The anatomical basis of these two synaptic fields is currently under investigation.

The ethanol treatment had little effect on population EPSP or population spike (PS) responses to single or multiple shocks of the SC-COM fibers. The treatment groups had virtually identical input-output curves. Furthermore no differences between groups were found in the post-tetanic effects of 1, 5, 10 or 100Hz stimulus trains. However during paired-pulse stimulation stimulation, the ethanol group exhibited greater facilitation of the test pulse PS. In addition the ethanol group showed a trend toward greater facilitation of the PS during 5 and 10Hz tetani. The overall pattern of results suggests that although chronic ethanol treatment reduces the extent of the SC-COM terminal zone in stratum radiatum, there are no dramatic changes in the normal physiology of this pathway. However ethanol treatment may produce a reduction of recurrent inhibitory processes, thus leading to greater responses during repetitive stimulation.

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20.6 CONTINGENT TOLERANCE TO AMPHETAMINE ANOREXIA IS DIET SPECIFIC. David L. Wolgin. Dept. Psychol., Florida Atlantic University, Boca Raton, Fla. 33431.

When rats are injected with amphetamine and given sweetened milk on a repeated basis, they gradually recover from the initial drug-induced anorexia and ingest more and more each day. However, if the drug is injected after the rats have had access to milk, a similar loss of sensitivity to amphetamine is not observed; i.e., there is no attenuation of anorexia when the drug is subsequently given prior to the milk (Carlton & Wolgin, Physiol. Behav. 7:221, 1971). It is generally assumed that such "contingent tolerance" is mediated by some form of learning. To help specify more clearly what, precisely, is learned, amphetamine tolerant rats were given transfer tests in which they were offered substances different from the ones they had ingested while becoming tolerant.

In the first experiment, rats made tolerant to amphetamine with sweetened milk as the diet showed no tolerance when subsequently offered a variety of other foods (sucrose, Nutrament, Noyes pellets, Purina pellets) or water. In the second experiment, rats were made tolerant to amphetamine with either sweetened milk or milk adulterated with a small amount (.06 mg/cc) of quinine as the diet. Control groups treated identically but injected with saline did not differ in the amount of diet ingested during this phase of the experiment, demonstrating that the diets were equally palatable to undrugged animals. Following the development of tolerance, each of the groups was switched to the other diet for six additional tests. Tolerant rats switched from milk to quinine-adulterated milk reverted to anorexia throughout the six trials. The corresponding control group drank less of the adulterated diet for 1-2 days but then recovered to the previous level of milk consumption. Conversely, tolerant rats switched from adulterated to unadulterated milk showed an immediate and sustained increase in consumption. The corresponding control group showed no change, maintaining the same high level of intake it had previously shown with the adulterated milk.

These results demonstrate that tolerance is not due to adaptation to drug-induced cues within a specific behavioral task. They are also inconsistent with a conditioning theory of tolerance. Such a theory would predict a loss of tolerance whenever the conditional stimuli associated with the test differ from those present during the acquisition of tolerance. Switching rats from quinine-adulterated to unadulterated milk, however, did not result in a loss of tolerance.

20.7 MOUSE STRAIN DIFFERENCES IN THE RESPONSE TO CHRONIC BROMOCRIPTINE TREATMENT. P.K. Randall*and J.F. Stern* (SPON: J.E. Holmes). Andrus Gerontology Center, USC, Los Angeles, CA. 90007.

Chronic treatment with the dopamine agonist, bromocriptine results in diminution of striatal DA receptor number and DA-activated adenyl cyclase in striatal slices(Quik, M. and Iverson,L. L., Naunyn-Schmiedeberg's Arch. Pharmacol., 304: 141,1978). Following a 7 day (15mg/kg/day,subcutaneous) regime of bromocriptine stereotypic behavior resulting from 2.0 mg/kg apomorphine was significantly reduced in the C57BL/6J mouse, paralleling a 15% reduction in ^{3}H-spiroperidol binding in the striatum. These data suggest that bromocriptine produces a functionally significant reduction in the sensitivity of striatal DA receptors in this strain.

In BALB/cJ mice,on the other hand the classic stereotypic behavior pattern totally disappeared as a coherent behavioral syndrome. Instead, these mice showed increased motility and an oro-buccal abnormality consisting of repeated and extreme tongue protrusion when tested with apomorphine. Since this behavior was blocked by the DA receptor blocker, haloperidol, it is likely that it results from DA receptor activation.

Additional experiments confirmed that acute combinations of bromocriptine and apomorphine do not elicit the oro-buccal behavior in any strain tested and the the dose response curve with respect to the chronic bromocriptine in the BALB/cJ mice is biphasic. Increases in the incidence of the behavior were observed between 3.75 and 15 mg/kg/day with a subsequent decline to 30mg/kg/day.

These data suggest that DA-related oro-buccal abnormalities may be potentiated by chronic drug treatment with the induction of receptor supersensitivity in some strains of mice. Further, chronic bromocriptine treatment may produce a relatively greater subsensitivity of the DA "auto-receptor" than of the striatal post-synaptic receptor in the BALB/cJ mouse. The disappearance of the normal decrease in locomotor activity following apomorphine treatment in this strain is consistent with this interpretation.

20.8 DEVELOPMENT OF TOLERANCE TO PHENCYCLIDINE (PCP)-INDUCED STEREOTYPED BEHAVIOR AND ATAXIA. R. G. Fessler, R. D. Sturgeon*, S. F. London*, and H. Y. Meltzer. Lab. of Biological Psychiatry, Illinois St. Psychiatric Inst., Chicago, IL 60612.

The development of tolerance to chronic, once daily administration of PCP (5 or 10 mg/kg ip) was quantified in male rats on independent scales designed to assess locomotor activity, stereotyped behavior and ataxia. Blind ratings of these PCP-induced behaviors were compared on days 1, 3, 5 and 10 of the experiment. Tolerance to the stereotypy-inducing effect of PCP was apparent by the third day of administration (5 mg/kg, $p < .05$; 10 mg/kg, $p < .01$). By day 5 the stereotypy-inducing effects of PCP were reduced by approximately 30% at either dose. Tolerance to PCP-induced ataxia was also apparent by day 3 for the 5 mg/kg dose of PCP ($p < .02$), but was not significant until day 5 for 10 mg/kg of PCP ($p < .01$). By day 5 PCP-induced ataxia was reduced by 31% and 37% for the 5 and 10 mg/kg doses of PCP, respectively. Although not statistically significant, locomotor activity was observed to increase during chronic PCP (10 mg/kg) administration as ataxia decreased. This observation agrees with previous reports of an inverse relationship between PCP-induced locomotor activity and ataxia. At 5 mg/kg, PCP-induced locomotion increased on day 3 compared to day 1 in a manner similar to that found for 10 mg/kg PCP, but then decreased at days 5 and 10. This subsequent decrease in locomotor activity may represent the development of tolerance to the locomotor activating effects of PCP. This research was supported by USPHS DA 02081.

20.9 BEHAVIORAL AND PHYSIOLOGICAL RESPONSES TO CONCURRENT FOOD-DEPRIVATION AND ETHANOL CHALLENGES. James L. Tramill* and Andrea L. Wesley, Univ. of Southern Mississippi, Hattiesburg, MS 39401.

Studies of the behavioral and physiological responses to ethanol administration may present differences which hold in common some causative factors. Facilitation of aggressive responding has been reported by some studies, while others report inhibition after ethanol exposure. Significant reductions in aggressive responding have been observed in fasted rats, but not in nonfasted controls. Homeostatic factors regulating the organism's nutritional state could influence aggressive responses to ethanol administration as well as physiological responses. Some findings indicate that fasted humans, rats, and dogs exhibit ethanol-induced hypoglycemia. Other findings indicate ethanol-induced hyperglycemia in both fasting and nonfasting states. These studies propose that a fasting period is necessary, but not sufficient to produce the effect. Repeated differences in findings may reflect species and/or methodological variables and, thus, emphasize the need for clarification of the possible interactions of nutritional state and ethanol exposure. Female, Sprague-Dawley rats were maintained on a 12:12 LD cycle at 80% of free-feeding body weight. Intraperitoneal injections of either saline or 50% ethanol were administered for 14 days. Ethanol injections resulted in a significant decrease in blood glucose levels (t (10) = 2.29, $p < .05$). Aggression as the behavioral measure was then tested in a single-restrained animal, shock-elicited aggression apparatus (Azrin, et al., 1968). There was a significant reduction in the shock-elicited biting response for the ethanol group (t (10) = 3.65, $p < .01$). Results support the need to study nutritional state as a factor in developing an animal model for chronic alcoholism.

20.10 EFFECTS OF NALOXONE ON DEVELOPMENT OF ETHANOL DEPENDENCE IN RATS. Robert F. Berman, Julia A. Lee*, Karen L. Olson*, and Mark S. Goldman*. Dept. of Psychology, Wayne State University, Detroit, Michigan 48202.

A link between ethanol dependence and opiate addiction is suggested by several lines of evidence, including a cross-tolerance between ethanol and morphine and alleviation of ethanol withdrawal by morphine. Recently, Ho and Ho (Pharm. Biochem. Behav., 11:111, 1979) reported reduced ethanol narcosis by the opiate antagonist naloxone; and Blum, et al (Nature, 265:49, 1977) reported reduced withdrawal symptoms in naloxone-treated mice made ethanol-dependent by exposure to ethanol vapor. In the present study the effects of naloxone on the development of ethanol dependence were further examined in rats, using an ethanol-containing liquid diet previously shown to produce ethanol dependency (Baker, Cannon, Berman, Atkinson, Addict. Behav., 2:35, 1977). Thirty adult male Long Evans rats were reduced to 75% of their free-feeding body weight and made ethanol-dependent by maintenance on a liquid diet consisting of Sustacal (Mead Johnson & Co.) and ethanol. The percentage of total calories derived from ethanol in the diet was increased daily by 1%, from 35% to 40%, where it remained for the duration of the 21 day addiction phase. Half of the animals (Groups NS and NN) were injected with 2 mg/kg naloxone (ip) daily, and the remaining animals (Groups SS and SN) were injected with saline. After 21 days on the ethanol diet, all animals were withdrawn from ethanol, injected (ip) with either naloxone (10 mg/kg, Groups SN and NN) or saline (Groups SS and NS), and observed for the occurrence of withdrawal symptoms. Withdrawal symptoms were rated using a scale developed by Hunter, et al (Pharm. Biochem. Behav., 3:619, 1975). Rats that received daily injections of naloxone during the addiction phase consumed significantly more of the ethanol diet than did saline-injected rats. As a consequence, naloxone-injected rats showed significantly greater weight gains on the diet compared to the controls. During acute withdrawal (1st 24 hours), rats injected with naloxone both during addiction and during withdrawal (NN) showed significantly fewer withdrawal symptoms when compared to all other groups. However, audiogenic seizures were reliably elicited from almost all animals, regardless of naloxone treatment history. In addition, animals treated with naloxone during addiction (Groups NS and NN) showed greatly attenuated postwithdrawal hyper-excitability and hyper-irritability compared to animals injected with saline during the addiction phase (Groups SS and SN). These results indicate that naloxone can modify alcohol intake and withdrawal symptomatology. The exact mechanism remains to be determined.

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20.11 NEURONAL LOSS IN HIPPOCAMPUS INDUCED BY PROLONGED ETHANOL INGESTION IN RATS. David E. Barnes and Don W. Walker. VA Medical Center and Dept. of Neuroscience, Univ. of FL College of Medicine, Gainesville, FL 32610.

Ninety male Long-Evans hooded rats (Charles River) were used. At 75 days of age, they were weighed and divided into weight-matched groups (N=10). One group received unrestricted access to the ethanol-containing diet. A second group was pair fed an identical diet except that sucrose was substituted isocalorically for ethanol. A third group received unrestricted access to standard pelleted laboratory food. The liquid diets contained 35-39 percent of the total caloric content as ethanol or sucrose-derived calories. The ethanol containing diet was 8.1 to 9.4 percent ethanol (by volume). Groups were maintained on the diets for 10, 20, or 30 weeks. After the liquid diets were discontinued, all animals were on Lab Chow for at least two months before sacrifice. Following intracardiac perfusion with 10% neutral buffered formulin and post-fixation in Bouin's solution, the brains were coded and embedded in Paraplast. Both sagittal and coronal 4 µm sections (1 in 10) were saved and stained with either cresyl violet or hematoxylin-acid fuschin. Quantitative determination of the total number of hippocampal pyramidal cells per section were made by counting at a magnification of 675X each soma containing a clearly defined nucleolus throughout the entire stratum pyramidale from CA1 through CA4.

Ethanol exposure resulted in a 16-28% loss in hippocampal pyramidal cells with the 10 week group being least affected and the 30-week animals most severely affected (-28%, $P < .001$). In addition, the majority of neuronal loss was in region CA1 suggesting a preferential susceptibility of these cells to ethanol exposure. Qualitative observations suggest that the dendritic field of remaining CA1 pyramidal cells may be attenuated by ethanol exposure. (Supported by the Veterans Administration and NIAAA Grants AA-00200 and AA-03965).

20.12 POTENTIATION OF THE STIMULANT EFFECTS OF D-AMPHETAMINE AFTER CHRONIC DESIPRAMINE TREATMENT. C. Spyraki and H.C. Fibiger, Div. Neurol. Sci., Univ. British Columbia, Vancouver, B.C. Canada.

A characteristic feature of tricyclic antidepressants (TCA) is their ability to potentiate various central effects elicited by amphetamine-like drugs. However, this effect has only been observed after acute administration whereas the clinical efficacy of TCA's are observed after chronic (10-20 days) administration. The present study examined the effect of chronic administration of desmethylimipramine (DMI) on amphetamine-induced motor stimulation, a constellation of dopamine-mediated behaviors (Brain Res. 1975, 93, 441).

Male Woodlyn rats were injected intraperitoneally twice daily, for 14 days with 5 mg/kg of DMI per injection. Control rats received injections of an equivalent volume of vehicle. Twenty-four hours after the last injection the rats were placed individually in the photoactometers and their activity recorded for one hour. They were then injected with vehicle or d-amphetamine sulphate (0.5 or 1.5 mg/kg) i.p. and immediately replaced in the activity cages for another three-hour period.

Chronic DMI treatment did not alter spontaneous locomotor activity during the one hr habituation phase. However, the motor stimulation produced by 1.5 mg/kg d-amphetamine was greatly enhanced in the chronic DMI treated group ($P < .01$). No differences were observed between the DMI and vehicle groups after 0.5 mg/kg d-amphetamine. In another experiment, DMI pretreatment for 2 days did not affect the amphetamine-induced motor behavior. The potentiation of the stimulant effect of amphetamine after two weeks of DMI treatment was not due to any anticholinergic effects of DMI because this regimen did not affect either hypothermia or other effects of oxotremorine.

These results demonstrate that 14-day but not 2-day chronic pretreatment of DMI potentiates the stimulant effects of d-amphetamine. Inasmuch as this is a dopamine-mediated phenomenon, these results point to an influence of chronic DMI treatment on central dopaminergic mechanisms. The results are consistent with a growing body of evidence implicating dopamine in the mechanism of action of TCA's, and indirectly, in the pathogenesis of depression.

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21.1 THE EFFECTS OF PONTINE TEGMENTAL LESIONS ON NUCHAL MUSCLE ACTIVITY IN THE RABBIT: A QUANTITATIVE STUDY. C. Bratin*, and R.T. Pivik. Lab. Neurophysiology, School of Psychology and Department of Psychiatry, Faculty of Health Sciences, University of Ottawa, Ottawa K1N 5C8, Canada.

Lesions in the region of the pontine tegmental nucleus locus coeruleus (LC) are reported to eliminate tonic EMG inhibition which normally accompanies paradoxical sleep (PS) and increase activity during wakefulness. In a previous communication (Bratin, C. and Pivik, R.T., 20th Annual Meeting of the Association for the Psychophysiological Study of Sleep, Abstract, 1980) we reported the absence of PS and the presence of agitated behavior sequences following slow wave (SW) sleep in rabbits with bilateral LC lesions $\geq$ 80%. The present analyses of quantified nuchal EMG activity were undertaken in these animals and a second group with partial, primarily posterior, LC lesions (<50%) to assess possible changes in EMG activity subsequent to these brainstem lesions.

Adult male New Zealand White rabbits (n=13) were implanted for chronic recording of EEG, EOG and nuchal EMG activity, and bilateral guide tubes were stereotaxically aimed at the medial pontine tegmental and central regions of the brainstem. Recordings (9:00-15:00 hrs) were taken one week after implantation (baseline) and at weekly intervals for at least two weeks post-lesion. Lesions, effected via a temperature controlled radio-frequency probe inserted through the guide tubes, were histologically verified (28µ serial sections). Data reported are based on initial analyses comparing baseline and two week post-lesion periods. EMG activity was quantified using resetting integrator techniques. Frequency counts of quantified EMG activity corrected for system noise were correlated with independently scored state determinations (1 minute samples).

For the LC group a slight increase in post-lesion EMG activity occurred during SW and pre-PS periods, with significant ($p<.01$) increases post-lesion for active wakefulness (AW) and PS. Post-lesion PS data consisted primarily of phasic motor activation whereas pre-lesion PS data included both tonic and phasic PS episodes. Pre-post lesion comparisons for the partial LC group showed, for every comparison, i.e., AW ($p<.01$), QW ($p<.01$), SW ($p<.05$), pre-PS ($p<.05$) and PS ($p<.05$), significant post-lesion decreases. The total LC lesion data do not indicate a tonic post-lesion increase in EMG activity across states. The partial LC lesion data are more difficult to interpret. The post LC has been implicated in suppression of motor behavior during PS in the cat. The present data may reflect a species difference in this phenomenon, but are also consonant with the suggestion that neurons other than noradrenergic, e.g., cholinergic, located in the LC region may mediate motor phenomena during PS and related states.

21.2 ASYMPTOMATIC ALPHA-DELTA SLEEP. Carl P. Browman and James K. Walsh. Department of Psychiatry, State University of New York at Stony Brook, Stony Brook, NY 11794 and Sleep Disorder Center, University of Cincinnati Medical Center, Cincinnati, OH 45267.

The intrusion of alpha activity during slow-wave sleep (SWS) had been described as a characteristic waveform associated with certain behavioral, psychological and physiological disorders. However, we observed alpha-delta sleep in two asymptomatic young adults, a male rotating shift worker (age 32) and a female college student (age 22). Medical histories were unremarkable, as was a cardiovascular examination for the female. Personality test results were within normal limits; projective techniques failed to reveal any affective disorder. Both were socially well adjusted. Polysomnograms were obtained for three to four consecutive, drug-free nights. The EEG was recorded from standard C3-A2 or C4-A1 placements. All polysomnographic results exclude first-night data and are based on five nights. The mean sleep latency was 13.2 min. The average amount of REM sleep was 21.2% and the mean SWS was 15.1%. The sleep efficiency index was .951 for the male and .988 for the female. Alpha-delta sleep was evident to varying degrees on all nights for each person. The alpha frequency during SWS was slightly slower than that observed during wakefulness. Postsleep questionnaires and subjective evaluations of typical sleep were comparable to normal controls without alpha-delta sleep. Neither subject reported symptoms of any sleep-wake pathology.

We conclude that the superimposition of alpha and delta frequencies in the sleep EEG is not necessarily an indicant of pathology. The significance of this waveform and the etiologic basis, in either asymptomatic subjects or patients with concomitant disorders, are yet to be determined.

21.3 TONIC AND PHASIC INFLUENCES DURING REM SLEEP UPON UNIT ACTIVITY OF DORSAL LATERAL GENICULATE NUCLEUS IN THE RAT. G.A.Marks*, J. Farber*, J.K.Chapin, H.P.Roffwarg. Sleep Neurophysiology Lab, Department of Psychiatry, University of Texas Health Science Center, Dallas, Texas 75235.

Evidence derived mainly from the cat indicates that there is a central phasic event system operating during REM sleep. One measure of its output is the Ponto-Geniculo-Occipital (PGO) wave. We have recently reported on a REM sleep associated waveform, recorded from the dorsal pontine tegmentum of the rat, that shares many of the properties of PGO waves (Farber et.al. Science, in Press, Marks et. al. Exp. Neurol., in Press) The failure of studies to reveal the presence of PGO waves at the level of the thalamus in the rat raises the question as to whether the rat pontine waves are isolated local events or components of a system of propagated phasic influences.

In the present investigation, etched tungsten microelectrodes were used to record the activity of single cells extracellularly in unanesthetized freely moving rats. The presence of both tonic and phasic REM sleep influences were examined in 27 identified dorsal lateral geniculate (dlgn) units during at least one entire sleep-wake cycle. In all units spontaneous discharge rates were increased in REM sleep (median increase=150%) as compared to slow wave sleep (SWS). During SWS, units exhibited a characteristic burst-pause pattern. With the transition to REM sleep, the units assumed a more continuous high frequency pattern at or above waking levels. Visual inspection of the recordings did not reveal an obvious temporal relationship between dlgn unit discharge and the occurrence of REM sleep pontine waves. However, perievent time histograms, averaged over at least 15 waves, revealed three distinct populations of dlgn units with: 1) no relationship to pontine waves (30%); 2) a significant increase in discharge rate within 250 msec after the peak of pontine waves (48%) and, 3) a decrease preceding a significant increase at approximately 600 msec after the pontine waves (22%).

These data support the conclusion that there are both tonic and phasic influences upon the dlgn during REM sleep. Tonic influence is evidenced by a sustained increase in unit discharge rate and phasic influence is evidenced by the more subtle changes in discharge rate temporally associated with a pontine central phasic event. The latter further supports the presence of a REM sleep phasic event system in the rat.

21.4 DIFFERENTIAL RESPONSIVITY OF RATS TO A CONDITIONED AVERSIVE STIMULUS DURING REM AND SLOW WAVE SLEEP. J.M. Halperin, D. Miller* and L.C. Iorio*. Schering-Plough Corp., Bloomfield, N.J. 07003.

This study investigated whether conditioned aversive and neutral stimuli differentially affect arousal from rapid eye movement (REM) and slow wave sleep (SWS) by measuring the incidence of arousal from each state in response to a tone prior to and following conditioning.

Eight rats, chronically implanted with electrodes for recording EEG and EMG, were housed individually in soundproof chambers throughout the experiment. After 24 hr adaptation to the recording leads, a 2-sec tone was played into the chamber 14-20 times within an 8-hr period while the rat was in either REM or SWS for a minimum of 30 sec. The number of times that the rat awakened within 5 sec of the tone was determined using standard EEG and EMG criteria. During each of the next 5 days, 4 rats received a delayed conditioning paradigm in which a 5-sec., 0.5 ma, electric shock was delivered through the floor grid of the chamber 1 sec after the termination of the tone; the others received backwards conditioning, during which the shock preceded the onset of the tone. During both procedures there were 40 trials with a 60-sec intertrial interval. Rats received the same exposure to the two stimuli with both conditioning paradigms, however, the tone should have become associated with the shock only as a result of delayed conditioning. The day after the fifth conditioning session consisted of presentation of the tone alone, as was done prior to conditioning.

Prior to conditioning the tone awakened the rats significantly ($p<.001$) more from SWS than REM. Delayed conditioning significantly increased the incidence of arousal following the tone during SWS ($p<.05$), while there was a small, non-significant, reduction of arousal from REM ($p>.10$). Backwards conditioning resulted in small, non-significant, reductions in arousal following the tone from both states of sleep ($p>.05$). No significant differences occurred in SWS or REM time between pre- and post- conditioning arousal sessions.

The addition of an aversive component to the tone, through conditioning, increased its awakening potency during SWS, but not during REM. This suggests that REM may be a period that maintains sleep even in the presence of environmental duress.

21.5 INCREASED ^{3}H-LYSINE INCORPORATION IN DIFFERENT FRACTIONS OF BRAIN DURING SLEEP. G. Chakraborty* and A. K. Sinha. Dept. Physiol. & Biophys. College of Medicine & Dentistry of NJ-Rutgers Medical School, Piscataway, NJ 08854.

A number of indirect observations and some experimental evidence suggest that sleep may be a period of increased protein synthesis in brain. We are investigating to determine what area of brain and which subcellular fractions are related to this increased synthesis. We have used an experimental protocol that produces uninterrupted non-rapid eye movement sleep (no rapid eye movement sleep during this time) in hamsters (40 gm) for about an hour (Macho and Sinha, Life Sc. 26:291, *1980*). Ten min after an animal fell asleep, we injected 0.5 mCi (in 0.5 ml saline) of ^{3}H-lysine (sp. activity 80.5 Ci/mmol) through a chronically implanted femoral venous catheter. Fifty min after that injection we killed it by decapitation. Same amount (mCi/gm bd wt) of ^{3}H-lysine was injected in awake animals that were kept awake till decapitation (50 min after injection). The presence of incorporated ^{3}H-lysine was determined in the protein powder prepared from liver and mitochondrial, synaptosomal, microsomal and soluble supernatant fractions of cerebral cortex and brain stem. In 6 awake and 6 sleeping hamsters no difference in ^{3}H-lysine incorporation was detected in any fraction of liver and in the mitochondrial and microsomal fractions of cerebral cortex and brain stem. The synaptosomal fraction prepared from cerebral cortex and brain stem, showed respectively 30% and 37% increased incorporation during sleep. The soluble supernatant from cerebral cortex showed 26% increased incorporation during sleep. Currently we are electrophoretically fractionating the proteins to determine if the increased incorporation is taking place in all proteins or in some specific groups of proteins. [This project was supported by grants from NIH (NS 13118) and Air Force Office of Scientific Research (78-3532A)].

21.6 ^{14}C-DEOXYGLUCOSE AUTORADIOGRAPHY OF THE RAPHE NUCLEI DURING SLEEP-WAKE ACTIVITY IN THE RAT. P.Ramm* and B.J. Frost (Spon: M.W. Donald). Dept. of Psychology, Queen's University, Kingston, Ontario. Canada K7L 3N6.

Investigations of the role played by the raphe nuclei in sleep-wake activity have involved lesions, pharmacological treatments or electrophysiological recordings. As an adjunct to these methods, and to direct subsequent electrode penetrations in the diffuse raphe system, we report state-dependent variation in functional activity revealed by ^{14}C-2-deoxyglucose (^{14}C-2DG) autoradiography.

^{14}C-2DG was injected into waking (n=4) or sleeping (n=4) rats via a chronic in-dwelling jugular cannula. During the following 45 min, EEG and EMG records were obtained. Autoradiographs were analyzed using a computerized image analysis system to read optic densities within the films. Optic densities of the dorsal raphe (dr), nucleus centralis superior, and raphe magnus, obscurus, pallidus and pontis were compared to surrounding areas of the ponto-mesencephaic reticular formation. A significant relative difference in optic density was observed in an area app. 1 mm posterior to the interaural line, comprising part of the dr and extending laterally into the periaqueductal central gray (SG). The ratio of density in this area/density in the reticular surround was 0.84 in sleeping animals, and 0.77 in waking animals. The area exhibits relatively increased regional metabolic activity in the waking state. These findings support a recent report that lesions involving the dr and lateral SG are among the only raphe lesions which are followed by long-lasting and specific slow wave sleep deficits. We are presently placing lesions within the area delineated by the autoradiographs, to further examine its relation to sleep-wake activity.

21.7 FENFLURAMINE, FLUOXETINE AND QUIPAZINE SUPPRESS SLEEP IN RATS. C. Fornal* and M. Radulovacki (SPON: E.M. Burns). Dept. of Pharmacology, Univ. of Ill. Med. Ctr., Chicago, IL 60612.

The aim of the present study was to investigate the effects of pharmacological agents which increase serotonin (5-HT) receptor activity by various mechanisms on the sleeping behavior in rats. For this purpose we administered fenfluramine (FEN) (1, 5, 10 mg/kg, i.p.), which releases 5-HT from the nerve terminal, fluoxetine (FLU) (5, 10 mg/kg, i.p.), which inhibits reuptake of 5-HT into the nerve terminal, and quipazine (QUIP) (1, 3, 5, 10 mg/kg, i.p.) which directly stimulates post-synaptic 5-HT receptors, to rats previously implanted with EEG and EMG electrodes for a 12-hr recording session.

Our results show that all agents significantly altered sleep-wake patterns in a dose related manner, suppressing sleep and prolonging wakefulness. Slow wave sleep (SWS) and rapid eye movement (REM) sleep latencies were greatly increased. The suppressant effect on SWS seen with the 5 mg/kg dose of FEN was effectively blocked by pretreatment with a serotonergic receptor antagonist, methergoline (MET) (2.5, 5.0 mg/kg, i.p.) 1 hr prior to FEN administration. In contrast, pretreatment with a dopaminergic receptor antagonist, α-flupenthixol (.2 mg/kg, i.p.) had no effect on the action of FEN. Pretreatment with FLU (5, 10 mg/kg, i.p.) 24 and 1 hr, to prevent FEN from entering the serotonergic nerve terminal and releasing 5-HT, did not counteract the effects of FEN. These results suggest that FEN may effect sleep through direct 5-HT receptor stimulation. Similarly, the suppressant effects on SWS induced with the 5 mg/kg dose of QUIP was reversed by pretreatment with MET (2.5 mg/kg, i.p.) 1 hr prior to QUIP administration.

We conclude that increased availability of 5-HT at post-synaptic receptors or direct 5-HT receptor stimulation suppresses sleep and increases wakefulness.

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21.8 ATROPINE ABOLISHES REM SLEEP REBOUND AND INCREASES WAKEFULNESS IN RATS. W.C. McDaniel* and M. Radulovacki (SPON: K.R. Unna). Dept. of Pharmacol., Univ. of Il. at the Med. Ctr, Chicago, Illinois 60680.

Laboratory studies of animals and studies of humans suggest cholinergic modulation of rapid eye movement (REM) sleep. The anticholinergic agent atropine delays the onset and decreases the amount of REM sleep while physostigmine, which enhances cholinergic activity, when injected during slow wave sleep (SWS), induces REM sleep. The purpose of this work was to determine whether the decrease of REM sleep caused by atropine represents a suppression of REM sleep followed by REM rebound or reduction of REM sleep without subsequent REM sleep rebound.

Adult male rats were implanted with EEG and EMG electrodes and were deprived of REM sleep by a flower pot method for 24hrs. After REM sleep deprivation animals were given saline (control group), atropine sulfate (50mg/kg. i.p.) or atropine methyl nitrate (53mg/kg. i.p.). Atropine methyl nitrate was given in order to differentiate between the central and peripheral anticholinergic effects on REM sleep rebound since the drug is a cholinergic antagonist with predominant peripheral effects.

TABLE I

	HOURS	CONTROL	ATROPINE SULFATE	ATROPINE METHYL NITRATE
WAKE-	0-12	265 ± 56	425 ± 72*	267 ± 49
FULL NESS	12-42	593 ± 55	704 ± 83	704 ± 37
	0-42	857 ± 96	1128 ± 150	971 ± 57
	0-12	95 ± 17	21 ± 10*	99 ± 29
REM	12-42	174 ± 25	156 ± 31	172 ± 8
	0-42	269 ± 34	178 ± 31*	271 ± 32

The results are means SD (minutes) for 4 animals in each group. *$p<0.025$

From the Table 1 it can be seen that administration of atropine sulfate significantly increased wakefulness during 0-12h period which remained increased during the entire 42h recording period. The results also show significant reduction of REM sleep during the initial 0-12h which was not followed by REM sleep rebound during the next 12-42h period. Administration of atropine sulfate did not significantly affect SWS.

Our data show that unlike caffeine, which is a suppressant of REM sleep (see Yanik, Walovitch and Radulovacki, this meeting) atropine reduces REM sleep without subsequent appearance of REM sleep rebound. According to the postulate of Stern and Morgane (Behav. Biol. 11: 1, 1974) elimination of REM sleep rebound by atropine may be indicative of the drug's role in fulfulling the REM "need".

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21.9 α-FLUPENTHIXOL ABOLISHES THE EFFECTS OF d-AMPHETAMINE AND CAFFEINE ON WAKEFULNESS AND SLOW-WAVE SLEEP BUT NOT ON REM SLEEP IN RATS. R. Walovitch*, G. Yanik*, W. J. Wojcik, C. Fornal* and M. Radulovacki. University of Illinois at the Medical Center, Dept. of Pharmacology, Chicago, Illinois 60680.

CNS stimulant effects of amphetamine and caffeine have been attributed to their action on brain catecholamines. Since brain dopamine (DA) has been proposed in the regulation of wakefulness and brain norepinephrine (NE) in the regulation of rapid eye movement sleep (REM), we decided to use in rats a DA receptor blocker, α-flupenthixol (α-Flu), in order to investigate the contribution of dopaminergic system in the effects of amphetamine and caffeine on wakefulness slow-wave sleep (SWS) and REM sleep.

Adult male rats were implanted with the EEG and EMG electrodes and selectively deprived of REM sleep by a flower pot method for 24h. One hour before termination of REM sleep deprivation animals were pretreated with either the drug vehicle (propylene glycol, i.p.) or α-Flu (0.2 mg/kg or 0.4 mg/kg i.p.). Upon termination of REM sleep deprivation both propylene glycol and α-Flu pretreated rats received saline (i.p.), d-amphetamine (1 mg/kg, i.p.) or caffeine (25 mg/kg i.p.). The results show that administration of amphetamine and caffeine alone significantly increased wakefulness and reduced SWS and REM sleep during the first 3h of EEG recording. They also indicate that administration of 0.2 mg/kg α-Flu had no effect on wakefulness or sleep while administration of 0.4 mg/kg α-Flu significantly reduced wakefulness and increased SWS. Pretreatment of animals with 0.2 mg/kg α-Flu did not affect amphetamine's action on wakefulness or sleep. In contrast, pretreatment with 0.4 mg/kg α-Flu abolished amphetamine's effect both on wakefulness and SWS but not on REM sleep. However, pretreatment of animals with either 0.2 mg/kg or 0.4 mg/kg α-Flu abolished effects of caffeine on wakefulness and SWS. Unexpectedly, 0.2 mg/kg but not 0.4 mg/kg α-Flu antagonized caffeine's effect on REM sleep, although the mean REM sleep minutes between the two groups differed by only 2 minutes during the 3h recording period.

In conclusion, our findings show that the actions of amphetamine and caffeine on wakefulness and SWS can be abolished by blocking DA receptors. This indicates that stimulation of DA receptors is directly related to increase in wakefulness and decrease in SWS. However, DA blockade did not antagonize amphetamine and caffeine reduction of REM sleep suggesting that these agents do not affect REM sleep by a dopaminergic mechanism.

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21.10 SPECIFIC INCREASE IN BRAIN DOPAMINE, BUT NOT NOREPINEPHRINE OR SEROTONIN, TURNOVER DURING REM SLEEP REBOUND IN RATS. W.J. Wojcik and M. Radulovacki. Dept of Pharmacol., Univ. of Il. Med. Ctr., Chicago, Il. 60612

Increased brain norepinephrine (NE) turnover has been reported during rebound of REM sleep in rats which were deprived of REM sleep by a flower pot method (Pujol et al Sci.159:112,'68). Since the method, using small platforms on inverted flower pots, cannot discriminate between the effect of stress and REM sleep loss, modification was made by introducing larger platforms on which animals can have REM sleep but are still subjected to immobilization stress as on small platforms. We have implanted rats with electrodes for EEG and EMG recording and deprived them of REM sleep for either 24 or 96 hrs by placing them on small (8 cm. dia./400g rat) or large (17 cm. dia./400g rat) platforms. The results showed that only the 96 hr protocol produced a selective REM rebound in the rats placed on small platforms. Both the small and large platform rats lost 18% of their initial body weight in 96 hrs.

Turnover of brain monoamines, DA, NE and serotonin (5HT) was determined by measuring the brain content of monoamine metabolites using the flurometric method of Meek and Neff (Br. J. Pharm 45:435,'72) for the NE metabolite, MOPEG-SO_4, and the HPLC method using reverse phase chromotography with electrochemical detection for the DA metabolites, DOPAC and HVA, and the 5HT metabolite, 5HIAA. Probenecid (50mg/kg), a dose which blocks the egress of these metabolites from the brain without suppressing REM rebound, was administered to all groups 4 hrs after termination of the 96 hr deprivation and all rats were decapitated 2 hrs later.

	CONTROL	SMALL PLATFORM	LARGE PLATFORM
MOPEG-SO_4	528 ± 30(6)	708 ± 27(8)*	693 ± 35(7)*
5HIAA	2181 ± 143(6)	2966 ± 200(6)	3123 ± 278(6)*
DOPAC	808 ± 47(6)	1081 ± 42(6)*	931 ± 51(6)
HVA	378 ± 12(6)	539 ± 59(6)*	421 ± 25(6)

*$p<0.05$, means ± SE (pmole/g tissue), () indicate the number of animals.

The data show that brain MOPEG-SO_4 and 5HIAA levels were elevated in rats on both small and large platforms and could not be correlated to REM rebound which appears only in rats on small platforms. However, DOPAC and HVA levels were selectely elevated only in the REM rebounding rats indicating an increase in DA turnover during REM sleep rebound.

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21.11 BROMOCRIPTINE REDUCES SLEEP IN RATS. M. S. Brodie*, R. Walovitch*, G. Yanik* and M. Radulovacki (Spon: H. L. Jackman), Dept. of Pharmacology, Univ. of Ill. at the Medical Center, Chicago, IL. 60680.

The aim of the study was to test the hypothesis that repeated administration of bromocriptine, a dopamine receptor stimulant, to rats would reduce sleep in these animals. The hypothesis was based on our previous findings that administration of bromocriptine (5 mg/kg, i.p.) to rapid eye movement (REM) sleep deprived rats increased wakefulness and abolished REM sleep rebound (Radulovacki et al, Life Sci., 24, 18, 1705, 1979).

Bromocriptine (7.5 mg/kg, i.p.) was administered at 9h intervals 4 times within 27h to adult male rats implanted with EEG and EMG electrodes which were polygraphically recorded for 84h. The results show that slow wave sleep (SWS) was most significantly affected by the administration of the drug. From Table 1 it can be seen that there was a significant reduction of SWS during the entire 0-84h polygraphically recorded period. However, the effects of bromocriptine administration were more pronounced on wakefulness and REM sleep than on SWS during 0-36h period.

Table 1

	N	Time period (Hours)	Wakefulness	% Change SWS	REM
Bromocriptine 7.5 mg/kg	8	0-36	+43^C	-35^B	-57^C
	8	37-84	+ 4	- 7	+14
	8	0-84	+21	-21^A	-25

N-number of animals. A($P<0.05$), B($P<0.001$), C($P<0.0005$), by one-way ANOVA with multiple comparisons performed by Scheffe' Test.

It is of interest to note that the reduction of SWS or REM sleep during 0-36h period was not followed by a rebound of SWS or REM sleep during the next 36-84h period. The question that our data raise is the nature of bromocriptine's effect on sleep. According to Stern and Morgane (Behav. Biol. 11, 1, 1974) absence of REM sleep rebound (in this case also SWS rebound) upon withdrawal of a drug treatment which decreases REM sleep could be interpreted as a fulfillment of a possible neurochemical function of REM sleep. In agreement with that postulate, it appears that the effects of repeated administration of bromocriptine on wakefulness and sleep in rats may be indicative of fulfillment of sleep "need" in the animals.

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21.12 REM SLEEP DEPRIVATION DOES NOT AFFECT B-ADRENERGIC RECEPTOR SENSITIVITY IN RAT BRAIN. N. Micovic* and M. Radulovacki (Spon: C. Anderson). Dept. of Pharmacol., Univ. of Ill. at the Medical Center, Chicago, Illinois 60680.

Repeated administration of electroconvulsive shock (ECS) or chronic administration of tricyclic antidepressants has been accepted to be an effective therapy for patients with depression. Both treatments probably augment the postsynaptic availability of norepinephrine (NE) and were reported to decrease the density of B-adrenergic receptor binding sites in rat brain.

Recently, REM sleep deprivation has been suggested as a successful treatment for patients with endogenous depression (Vogel et al. Arch. Gen. Psychiat. 37, 247, 1980). In order to evaluate the effect of REM sleep derprivation on B-adrenorectors, we decided to compare the effects of REM deprivation with those of desipramine (DI), a tricyclic antidepressant, on the density of B-adrenoreceptors in the brains of rats.

Adult male rats were subjected to REM deprivation by flower pot technique for 7 days, while the other group of animals received DI (10mg/kg, i.p.) once a day for 7 days. Animals in the control group were kept in their individual cages and were administered with saline (i.p.) once a day during 7 days. We have used the radiolabelled ligand ^{3}H-dihydroalprenolol (^{3}H-DHA) for identification and characterization of B-adrenergic receptors in the brain and measured the binding of ^{3}H-DHA to particulate fractions prepared from rat cerebral cortex. The method used was that of Bylund and Snyder (Molec. Pharmac. 12, 568, 1976). The effects of DI and REM deprivation treatments on B-adrenergic receptor sensitivity (Table 1) were assesed by determining the maximum number of ^{3}H-DHA binding sites by Scatchard analyses. The results show that DI caused a 25% reduction in receptor density ($p<0.05$), while REM deprivation treatment showed only a tendency for the receptor density to decrease.

Table 1

Treatment	Maximum binding (pmol/gm tissue)
Control	7.88 ± 0.25
REM Deprivation	6.98 ± 0.25
Desipramine (10mg/kg)	5.90 ± 0.67*

*$p<0.05$. Results are represented as means ± s.e.m.

In conclusion, our data indicate that REM sleep deprivation did not significantly affect the sensitivity of B-adrenergic receptors in rat brain. If changes in B-adrenergiv receptor sensitivity could be taken as an indicator of successful antidepressant therapy, then REM sleep deprivation method compares less favorably than chronic ECS or chronic administration of tricyclic

21.13 CAFFEINE PRODUCES REM SLEEP REBOUND IN RATS. G. Yanik*, R. Walovitch* and M. Radulovacki (Spon: M. Buschman). Dept. of Pharmacology, Univ. of IL. at the Medical Center, Chicago, IL. 60680.

Caffeine, a CNS stimulant, produced a dose-related decrease in total sleep time in humans without evidence to indicate the presence of carry-over or rebound effects from one night to the next (Karacan et al. in Sleep Res., Eds. Chase, Stern and Walter, BIS/BRI, 3, 56, 1974). In view of the findings that administration of bromocriptine (Radulovacki et al. Life Sci., 24, 18, 1705, 1979), α-adrenoreceptor blockers phenoxybenzamine and phentolamine (Radulovacki et al. Pharmacol. Physiol. Behav. in press), or atropine (McDaniel and Radulovacki, this meeting) abolishes REM sleep rebound in rats, we administered caffeine (25 mg/kg, i.p.) to adult male rats deprived of REM sleep for 24h to investigate its effect on REM sleep rebound. The animals were implanted with EEG and EMG electrodes and were continuously monitored by the EFG for 36h immediately following REM sleep deprivation (RD) and administration of caffeine. The results show significant increase in wakefulness and decrease in slow wave sleep (SWS) during the first 12h following administration of caffeine. In agreement with the above mentioned human study, there was no SWS rebound effect during the entire recording period. However, there was a significant REM sleep rebound during the 12-18h period after initial suppression of REM sleep during the first 6h following RD and caffeine administration.

	Hours after RD	RD Control	Caffeine (25 mg/kg)	Diff. from control (min)
	0- 6	69 ± 8	10 ± 3	-59^{X}
	6-12	64 ± 3	56 ± 7	- 8
REM	12-18	42 ± 4	66 ± 6	+24^{X}
Sleep	18-24	42 ± 4	53 ± 3	+11
	24-30	36 ± 4	43 ± 4	+ 7
	30-36	37 ± 3	40 ± 3	+3

The results are means ± S.E. (minutes), for 8 rats in each group. *P<0.01

These data show that caffeine produces a significant suppression of REM sleep which is followed by REM sleep rebound. The effect is at variance with those of bromocriptine, α-adrenoreceptor blockers or atropine on REM sleep rebound and indicates that caffeine is a mere suppressant of REM sleep.

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22.1 GABA UPTAKE BY ISOLATED CELLS OF THE GOLDFISH RETINA.
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In the goldfish retina, H1 horizontal cells, which receive input predominantly from red-sensitive cones, possess a high-affinity uptake mechanism for the neurotransmitter γ-aminobutyric acid (GABA). Additionally, GABA accumulation into these neurons is enhanced by light stimulation of the retina (Lam and Steinman, PNAS 68:2777; Marc et al., J. Comp. Neurol. 182:221). To examine the cellular mechanisms regulating this stimulation-dependent process, isolated cells enriched with horizontal cells that contain few or no synaptic endings were prepared from goldfish retinas using a procedure described previously (Lam, Nature 254:345). These cells were placed on a transparent Nucleopore filter (5 μm pore size) which allows visualization of cellular morphology by interference optics as well an incubation and perfusion of the cells with different media. When isolated cells were incubated with isotonic Ringer's solutions containing 1 to 60 mM K^+ and 1 μM ^{3}H-GABA, H1 horizontal cells still retained a Na^+- and temperature-dependent mechanism for ^{3}H-GABA accumulation which was inhibited by 100 μM nipecotic acid, indicating that somas of these cells possess high-affinity uptake sites for GABA. The ^{3}H-GABA accumulated was not significantly degraded during a 10 minute incubation and was not released by increasing the K^+ concentration in the medium. Thus, ^{3}H-GABA accumulation under our experimental conditions is predominantly due to uptake rather than release of GABA. Under these conditions, ^{3}H-GABA uptake is maximal at about 6 mM extracellular K^+ and decreases to less than 15% of the maximum at 60 mM extracellular K^+. Kinetic analyses of both initial and steady rates of GABA uptake indicate that our findings are consistent with the hypothesis that GABA uptake into somas of H1 horizontal cells increases with hyperpolarization and decreases with depolarization.

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22.2 IN VITRO ASSESSMENT OF NOREPINEPHRINE (NE) UPTAKE IN RAT CORTICAL-HIPPOCAMPAL SYNAPTOSOMES FOLLOWING DEPOLARIZATION.
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A number of investigators have reported that the rate of choline (CH) uptake is directly related to the level of nerve impulse activity and release of acetylcholine (ACh). In contrast to these data are several reports of a decreased uptake of norepinephrine (NE) following treatments which increase the activity of catecholamine neurons. The implication that the uptake mechanism of NE nerve terminals may be subject to qualitatively different regulatory influences than the mechanism for CH uptake was investigated in the present study by comparing the effects of K^+ depolarization on the in vitro uptake of NE and CH into synaptosomes isolated from rat hippocampus and cortex.

Synaptosomes were initially incubated in a buffered media containing 4.7mM K^+-no Ca^{++}, 55mM K^+-no Ca^{++} or 55mM K^+-1.8mM Ca^{++}. Duplicate aliquots of each condition were subsequently incubated in the presence of 10^{-8}M tritiated CH and NE. Preincubation of synaptosomes in 55mM K^+ alone resulted in a 37% increase in the uptake of CH but did not affect the uptake of NE. When Ca^{++} was present in the incubation media, depolarization with elevated K^+ levels produced a 107% increase in CH uptake and an 18% increase in the uptake of NE. When rats were anesthetized 30 min prior to sacrifice, CH and NE uptake were decreased by 35 and 15 per cent respectively. We interpret these data to indicate that the uptake of NE is not regulated to the same extent as CH by changes in neural activity. This difference may be accounted for by the differences between the means available to the nerve terminal for maintaing releasable pools of neurotransmitter. While replenishment of ACh pools relies predominantly on the uptake and conversion of choline into ACh, maintenance of NE stores occurs as the result of both the re-uptake of released transmitter and the synthesis of new NE from tyrosine. The absence of marked changes in NE uptake following depolarization is thus suggested to reflect the ability of the nerve terminal to either maintain or increase its rate of NE synthesis as necessary to compensate for the loss due to release.

22.3 INHIBITION OF CATECHOLAMINE TRANSPORT INTO CHROMAFFIN GRANULES BY PHENYTOIN.
Jean D. Deupree, David A. Downs*, Carl F. Gessert*, and James A. Weaver*. Dept. of Pharmacology, University of Nebraska College of Medicine, Omaha, NE 68105.

The anticonvulsant drug, phenytoin, has been reported to inhibit the transport of norepinephrine into synaptosomes, but the effects of this drug on the transport of catecholamines into storage granules has not been studied. In order to determine whether phenytoin would alter the storage of catecholamines, we examined the effects of phenytoin on the uptake of catecholamines into bovine chromaffin granules.

Relatively pure chromaffin granule ghosts were isolated from bovine adrenal glands. The ghosts contained less than 10 nmoles of epinephrine per mg of protein and less than 0.5 nmoles/mg/min of monoamine oxidase activity. Uptake of catecholamines was measured by incubating the granules for 10 min with 1 μM [^{3}H](-) norepinephrine in 50 mM Hepes buffer, pH 7.5, 4 mM ATP, 4 mM $MgCl_2$, and 0.1 mM EDTA. The transport process was terminated by adding 1 μM reserpine. The granules were isolated by centrifugation, solubilized with Protosol and counted for radioactivity. Nonspecific binding of [^{3}H](-)norepinephrine was eliminated by conducting control assays in the presence of 1 μM reserpine.

Phenytoin (0.4 mM) inhibited [^{3}H](-)norepinephrine transport by 47% whereas 1 mM phenobarbital inhibited transport less than 10%. Maximal inhibition of uptake appeared to occur at 0.4 mM, although it was not possible to test higher concentrations of the drug due to its limited aqueous solubility at this pH. The concentration of phenytoin required to produce 50% of this maximal inhibition was 50 μM, which is within the anticonvulsant range of the drug in blood plasma. The inhibition by phenytoin appeared to be noncompetitive with respect to norepinephrine since phenytoin reduced the Vmax but not the Km for norepinephrine. Catecholamines are transported into chromaffin granules by a carrier-mediated transport mechanism which utilizes a Mg ATPase as the driving force. Phenytoin (0.1 mM) did not inhibit the Mg ATPase activity in the chromaffin granules, suggesting that the inhibition of transport was not due to an inhibition of the driving force for the transport process.

These results indicate that phenytoin inhibits the transport of catecholamines into chromaffin granules. This action of phenytoin was not shared by phenobarbital, but it may be related to the anticonvulsant properties of the drug since it occurred at therapeutic doses of the drug.
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22.4 MEASUREMENT OF UPTAKE SITES FOR GLUTAMATE AND OTHER NEUROTRANSMITTERS IN PREVIOUSLY FROZEN RAT AND HUMAN BRAIN TISSUE.
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Glutamate uptake appeared to be stable when measured in striatal synaptosomes from rat brains stored for up to 4 hours postmortem at 25°C. Uptake decreased sharply between 4 and 8 hours at room temperature. Freezing of tissue on dry ice, storage at 4°C for up to 7 days and at -80°C for 5 days resulted in 20-25% residual glutamate uptake. Quantitatively similar data were obtained in eight extrastriatal brain areas.

Kinetic analysis of glutamate uptake sites in stored + frozen tissue revealed the loss of the majority of both sodium-dependent high-affinity- and temperature-sensitive low-affinity sites (V_{max} values) while the respective K_m values were not altered. Pharmacological properties of the high-affinity site versus a number of specific and unspecific uptake inhibitors remained unchanged by the storage- and freezing procedure.

The striatal reuptake systems for GABA, choline, serotonin and dopamine were differentially labile to storage and freezing: whereas 39% of the GABA-sites were still measurable, choline- and monoamine uptake processes decreased to 12% or less as compared to control values. Experiments using fresh and frozen kainic acid lesioned striata (Schwarcz and Coyle, Brain Res., 127,235,1977) showed that this differential vulnerability to freezing of the various neurotransmitter uptake sites was not due to differential localization on surviving non-neuronal elements.

The tissue treatment chosen for parts of the present study roughly corresponds with the preparation of human post-mortem brain tissue for enzyme-, neurotransmitter- or receptor binding assays. Our animal data suggested that uptake could be demonstrated in human autopsy material, thus adding an important parameter to the battery of neurochemical markers already available for post-mortem in vitro examination; and studies on rapidly processed human tissue showed that sodium-dependent glutamate uptake could indeed be measured in various brain regions including caudate nucleus, putamen, pallidum, cerebellum, thalamus, hypothalamus and cortical areas.

This study was performed largely to test the feasibility of a direct experimental approach to the glutamate uptake hypothesis of the human neurodegenerative disorder, Huntington's disease (Coyle et al., Prog. Neuro-Psychopharmacol., 1,13,1977). Our present data suggest that such an undertaking may be more realistic and meaningful than hitherto assumed.

This work was supported by a grant from the Wills Foundation.

22.5 INHIBITION OF NOREPINEPHRINE UPTAKE IN RAT SUPERIOR CERVICAL GANGLIA BY XYLAMINE. J.B. Fischer*, A.K. Cho* (SPON: B. Howard). Brain Research Institute and Pharmacology Department, UCLA, Los Angeles, CA 90024

Xylamine, (N-2'-chloroethyl-N-ethyl-2-methylbenzylamine)(XYL), an alkylating analog of bretylium synthesized in this laboratory, is an irreversible inhibitor of cortical norepinephrine (NE) uptake at concentrations that hardly affect striatal dopamine uptake in studies with rat brain synaptosomes. When administered to rats in vivo XYL produced an inhibition of cortical NE uptake and a depletion of cortical NE content that persisted for several days. Rat superior cervical ganglia (SCG) maintained in organ culture in a chemically defined medium (BGJ_b, with penicillin and streptomycin) have been used to examine the actions of this compound on adrenergic neurons. The ability of SCG to take up NE by a cocaine sensitive and Na^+ dependent mechanism increases about sixfold after 2 days in culture. This increased uptake capacity is maintained through 6 days of culture, so that the preparation is exceedingly stable. SCG were preincubated in Kreb's Ringer bicarbonate solution (KRBS) for 1 hr with different concentrations of XYL, placed in fresh KRBS for 30 min to wash away unbound drug, and then in fresh KRBS containing 0.2 µM [^{3}H]NE for 30 min. [^{3}H]NE in the SCG was extracted with 15% 1 N formic acid in acetone, and the protein content of each ganglion was determined. NE uptake was calculated as pmol NE taken up per mg of protein per 30 min. XYL inhibited NE uptake in cultured SCG with an IC_{50} of about 0.1 µM, a value similar to that found in rat brain synaptosomes and rabbit aorta, and inhibited NE uptake into freshly removed, uncultured SCG with an IC_{50} near 0.3 µM. XYL, at 1 and 10 µM, inhibited NE uptake into cultured SCG by 62 and 89%, respectively. Cultured SCG recovered their NE uptake ability, so that with 2 days of culture following 1 and 10 µM XYL treatment, the SCG showed only 12 and 21% inhibition of NE uptake, respectively. The protein content of the SCG did not decrease significantly during 2 days of culture after 10 µM XYL treatment, having 63 $\pm$ 3 and 56 $\pm$ 4 µM per ganglion for control and XYL treated SCG. Fresh SCG treated with 1 µM XYL and then cultured for 2 or 4 days showed no decrease in NE uptake ability or in protein content. As XYL is thought to act by covalently binding to a site or sites involved in NE uptake, the reversal of its inhibition of NE uptake in the SCG after culture may be due to the synthesis of new sites. Therefore the recovery of SCG but not cortical uptake may reflect the proximity of the noradrenergic cell bodies. The fact that 10 µM XYL does not decrease the protein content of the SCG indicates that this compound is not acting in a non-specific, cytotoxic manner. Thus XYL shows potential as a useful tool for studying noradrenergic systems.

23.1 EFFECTS OF VALPROIC ACID AND γ-ACETYLENIC GABA ON METABOLIC ENZYMES IN PRIMARY DISSOCIATED CELL CULTURE OF MOUSE FOREBRAIN. R.C. Frere*, A.B. Young, and R.L. Macdonald. Neurosciences Program and Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

The enzymes glutamic acid decarboxylase (GAD), γ-aminobutyric-α-oxoglutarate transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH) regulate the levels of γ-aminobutyric acid (GABA) in mammalian brain. Alteration of GABA-mediated inhibition has been implicated in the action of many convulsants and anticonvulsants and some of these drugs have been shown to have effects on the GABA regulating enzymes. We have investigated the actions of the anticonvulsant valproic acid (VPA) and γ-acetylenic GABA (GAG) on GABA metabolic enzymes in primary dissociated cell (PDC) culture of mouse forebrain.

We have found that these cultures show measurable enzymatic activity for GAD, GABA-T and SSADH. The respective activities of these GABA regulators are as follows: 16 nmole CO_2/mg/hr protein, 81 nmole/mg/hr, and 265 nmole/mg/hr.

The effects of VPA and GAG on these enzymes were measured following a 20 minute preincubation. Different drug doses were used with a minimal concentration of substrate necessary to produce activity. VPA (10-40 mM) reduced GAD activity with an IC_{50} of 22 mM in forebrain cultures, but did not affect GABA-T or SSADH. Using the same methods with extracts of adult rat brain, we found that VPA had an IC_{50} of 25mM for GAD and an IC_{50} of 6mM for SSADH. GAG (1 μM-10 mM) had an IC_{50} of 200 μM for GAD, 30 μM for GABA-T and no effect on SSADH in forebrain cultures.

These studies have demonstrated the presence of the primary GABA metabolic enzymes in PDC cultures of forebrain. Previous work has shown that VPA has an inhibitory effect on SSADH and GABA-T, but this was not found in our in vitro preparation. The inhibition of GABA-T and GAD by GAG correlates with findings in other preparations. The effect of VPA and GAG on convulsant-induced paroxysmal activity will be correlated with these neurochemical results. The culture model lends well to simultaneous biochemical and neurophysiologic studies of drug action and permits direct correlation of physiological data with pharmacological effect. Supported by The United Cerebral Palsy Foundation and NIH grants NS 15140-02, NS 00420-01, NS 15225-01 and RCDA NS 00408.

23.2 ERYTHROSIN B: SPECIFIC INHIBITOR OF HIGH AFFINITY [^{3}H]-OUABAIN BINDING AND ION TRANSPORT IN RAT BRAIN. E.K. Silbergeld and S.M. Anderson*. Section on Neurotoxicology, NINCDS, NIH, Bethesda, MD 20205.

The U.S.F.D.& C. dye Red No. 3 (erythrosin B (EB), tetraiodofluorescein) has been recently investigated for possible neurotoxic properties (c.f. Science (1980) 207: 1485, 1497, 1489). EB appears to affect sodium-sensitive processes, such as neurotransmitter uptake by synaptosomes. Its inhibition of high affinity dopamine uptake by rat caudate synaptosomes is restricted to sodium-dependent uptake. An important regulator of sodium metabolism in neurons, and other cells, is provided by Na, K-ATPase. The effects of EB on Na, K-ATPase were studied in rat cortical tissue by using three approaches: (1) displacement of specific [^{3}H]-ouabain binding to synaptic membranes; (2) inhibition of ^{86}Rb transport into intact synaptosomes; and (3) enzyme activity (catalysis of ATP) in synaptosomes. Two binding sites for [^{3}H]-ouabain were observed under conditions of 135 mM NaCl, 1 mM $MgCl_2$, and 1 mM Na_2ATP in a 50 mM Tris-HCl buffer. The two sites had Kd values of 1-2 nM and 80-100 nM, and Bmax values of 50 and 110 fmol/mg protein respectively. EB, in concentrations as low as 100 pM, significantly inhibited binding of [^{3}H]-ouabain to the high affinity site; at 100 nM, EB completely inhibited binding to this site. Concentrations of EB as high as 100 μM did not inhibit [^{3}H]-ouabain binding to the low affinity site. In submicromolar concentrations, EB also inhibited synaptosomal uptake of ^{86}Rb and activity of ATPase. EB was inactive in inhibiting ATPase activity in red blood cell ghosts. In a structure-activity study (binding and enzyme activity) of ten other fluorescein analogs, only rose bengal (tetrachloro-tetraiodofluorescein), eosin Y (tetrabromofluorescein) and diiodofluorescein possessed any activity in brain tissue. The base compounds fluorescein and xanthene were inactive in concentrations as high as 100 μM.

As recently proposed on the basis of binding studies and electrophoretic separation, two Na, K-ATPases appear to exist in mammalian brain. One enzyme may be associated with those neural functions involving rapid ion fluxes. These experiments suggest that EB is a specific probe for one ouabain binding site (or one Na, K-ATPase), in brain. This hypothesis is supported by the lack of activity of EB or its congeners in red blood cells. The results may be of interest in investigating possible neurotoxic effects of fluorescein-based food and cosmetic dyes. However, there is at present no information on penetrability of these compounds into brain after peripheral administration or ingestion.

23.3 INACTIVATION OF GLUTAMATE DECARBOXYLASE. Mary P. Meeley* and David L. Martin. Department of Chemistry, University of Maryland, College Park, MD 20742.

Glutamate promoted dissociation of pyridoxal-P from glutamate decarboxylase (GAD), which results in inactive apoenzyme, has been implicated in the regulation of the enzyme. Previous studies also suggest that inorganic phosphate and adenine nucleotides may be involved in GAD regulation. We have examined the roles of glutamate, ATP and Pi in the inactivation process using partially purified GAD from hog brain. The time course of apoenzyme formation was measured by preincubating holoenzyme with unlabelled glutamate for various times at 30°C, followed by a 5 min assay with labelled substrate to determine the activity remaining. An initially rapid inactivation was observed which was still ongoing even after 90 min of incubation with 10 mM glutamate. The overall rate of apoenzyme formation was dependent on the glutamate concentration. α-Methyl glutamate promoted inactivation of holoenzyme at least twice as effectively as glutamate. The inactivation was not first order but appeared to be the sum of two exponential decay processes. With 10 mM glutamate, the half-lives were approximately 6 min for the first component and greater than 50 min for the second. The potent inhibition of GAD exhibited by ATP can be relieved by Pi, but only in the presence of the cofactor, pyridoxal-P. 500 μM ATP accelerated glutamate-promoted inactivation more than two-fold, but had little or no effect on GAD in the absence of substrate. The ATP stimulation of inactivation by glutamate was unaffected by Pi, which was consistent with the finding that Pi only relieves inhibition by ATP when pyridoxal-P is present. This result indicated that Pi and ATP are not merely directly competitive. GAD regulation may involve a balanced cycle of apoenzyme formation and its reactivation to holoenzyme by pyridoxal-P. Reactivation by pyridoxal-P is stimulated by Pi, whereas the opposing inactivation with glutamate is enhanced by ATP.

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23.4 CYSTEINE SULFINIC ACID DECARBOXYLASE ISOENZYMES IN RAT BRAIN - COMPARISON WITH LIVER CYSTEINE SULFINIC ACID DECARBOXYLASE D.H. Ransom*, W.H.Oertel, V.K.Weise*, D.E.Schmechel*, H.Krutzsch* and I.J. Kopin. Laboratory of Clinical Science,NIMH, Bethesda, MD 20205

The biosynthetic enzyme for taurine is considered to be cysteine sulfinic acid decarboxylase [CSD(CSAD/CAD)4.1.1.29]. Recent work has demonstrated the presence of two CSD-enzymes in bovine brain (Wu et al., 1978; Heinämäki and Piha, 1979) one of which seems identical to glutamic acid decarboxylase (GAD). As CSD-activity is also present in non-neuronal systems, we compared the two brain enzyme activities with liver CSD activity in rat. Brain- and liver-CSD were partially purified by homogenization, centrifugation and ammonium sulfate precipitation (27%-68%) and subsequently chromatographed in parallel on amino-ethyl-Sepharose. Brain CSD was separated into two peaks, termed CSD I and CSD II. Brain CSD I eluted exactly in the same fraction as liver CSD at a potassium phosphate (KP)(pH = 6.9) concentration of 15-20 mM, CSD II coeluted with GAD at 50 mM KP. Using cysteine sulfinate as substrate, the apparent Kms of the partially purified preparations were 6 mM for brain CSD II, 55-100 μM for brain CSD I and 50 μM for liver CSD. pH-dependency for brain CSD I and liver CSD was virtually identical with an activity maximum between pH 7.4 to 7.8, however, brain CSD II exhibited a distinct pH-maximum at 7.4 to7.5.

Liver CSD and brain CSD I were not effected, whereas, brain CSD II was inhibited to 75% by 8mM glutamic acid. Liver CSD and brain CSD I were not effected by a specific anti-rat GAD-antiserum No. 1440, which precipitates GAD and brain CSD II.

The evidence obtained suggests the existence of two CSD isoenzymes in brain: CSD I appears to be very similar or identical to liver CSD and CSD II is indistinguishable from GAD.

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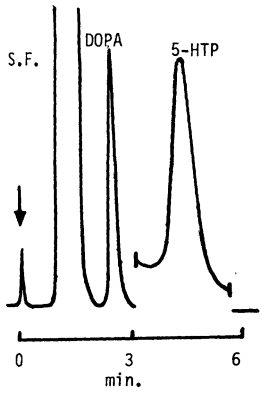
23.5 SIMULTANEOUS DOPA AND 5-HTP ANALYSIS FROM RAT BRAIN UTILIZING HPLC WITH ELECTROCHEMICAL DETECTION. S. P. Arneric*, D. B. Goodale, J. R. Flynn*, and J. P. Long* (SPON: W. W. Kaelber). Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

We have developed a rapid one step assay for simultaneous measurement of picogram amounts of 5-hydroxytryptophan (5-HTP) and nanogram amounts of dihydroxyphenylalanine (DOPA) by high performance liquid chromatography with electrochemical detection (HPLC-EC). Methodology, importance of sample storage and applications to pharmacologic alteration of DOPA and 5-HTP synthesis in rat brain is presented.

Tissue (~10 mg) from olfactory tubercle and caudate nucleus was homogenized in 0.1 N $HClO_4$ containing 0.02% ascorbic acid. Suspensions were incubated on ice for 30 min, centrifuged at low speed, and 30 µls of supernatant assayed. Samples were injected onto strong cationic exchange resin (Bondapak, CX/Corasil, Waters Associates Inc.) in a 750 mm x 2 mm i.d., microbore glass column. A Bioanalytical Systems, Inc. electrochemical detector was used. Sensitivities of 15 ng/ml for DOPA and 3 ng/ml for 5-HTP were obtained. Extraction efficiency of DOPA and 5-HTP from tissue was 95% ± 7%. Time and temperature dependent storage experiments suggest that 5-HTP samples may be stored a maximum of 24 hours at 4°C. Freezing (-10°C) is detrimental to 5-HTP samples. DOPA may be stored at 4°C or -10°C for at least 96 hours. Pharmacologic alteration of DOPA and 5-HTP synthesis was measured following para-chlorophenylalanine (PCPA, 100 mg/kg, i.p.) or apomorphine (APO, 1 mg/kg, s.c.); gamma-butyrolactone (GBL, 750 mg/kg, i.p.); and an aromatic L-amino acid decarboxylase inhibitor (NSD 1015, 100 mg/kg, i.p.). APO and PCPA selectively decreased DOPA and 5-HTP, respectively, in striatal brain as expected. Rapid and easy sample preparation, excellent tissue recoveries, and low equipment cost make this method suitable for routine measurement of DOPA and 5-HTP. (Supported by NIH Grant NS-4431.)



23.6 INFLUENCE OF DEVELOPMENT AND RESERPINE ON CATECHOLAMINE BIOSYNTHETIC ENZYMES IN BRAIN AND ADRENALS OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR). Ranbir K. Bhatnagar, M. Squillante*, M. O'Neil*, S. Kasik* and G. Rios*. Dept. of Pharmacol., Univ. of Iowa, Iowa City, IA 52242.

Catecholamines in the adrenal glands and hind brain, and elevated sympathetic activity have been implicated in the ontogenesis and maintenance of hypertension. This study was undertaken to assess the developmental pattern of phenylethanolamine-N-methyltransferase (PNMT) in the adrenals and pons-medulla of SHR and normotensive Wistar-Kyoto rats (WKYR). In addition, the effects of reserpine induced increase in sympathetic activity on PNMT and other biosynthetic enzymes was determined in adult rats. In young rats whole adrenals and medulla oblongata were removed at 3, 10, 15, 21 and 37 days of age. Adult rats were treated with reserpine for 4 days and adrenals, pons + medulla and blood samples were removed for study.

In young rats, PNMT activity/per pair of adrenals was significantly lower at all ages in SHR except 3 days. In contrast PNMT activity was significantly higher in the medulla of SHR at 15 and 21 days of age. The activities of tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DβH) and PNMT (per pair of adrenals) were not different between adult SHR and WKYR whereas PNMT activity of pons + medulla was significantly higher (44%) in SHR. Norepinephrine (NE)/Epinephrine (E) ratio was significantly higher in the adrenals of SHR at 3, 10, 15, 21 and 37 days of age but not in adults (300-350 gm).

After reserpine, NE and E were reduced by 57 and 82% respectively in the adrenals of SHR and WKYR. Adrenal TH and DβH activities increased by 160% and 110% respectively in both SHR and WKYR whereas PNMT activity remained unaltered. In pons + medulla, TH activity increased by 79 to 86% in both SHR and WKYR and again PNMT activity remained unaltered. The increase (100-150%) in serum DβH activity was similar in both strains of rats. The adrenal weight of adult SHR (45.9 mg/pair) was significantly greater than that of WKYR (36.4 mg/pair). Reserpine produced a further significant increase in the adrenal weight of both SHR (54.8 mg/pair) and WKYR (47.9 mg/pair).

In conclusion: (1) the epinephrine system in the pons-medulla but not the adrenal glands may be involved in the ontogenesis and maintenance of hypertension in SHR, (2) based on adrenal TH and DβH and plasma DβH activities the sympathoadrenal activity does not appear to be elevated in adult SHR and (3) PNMT, inducible by glucocorticoids, is not activated by stimuli which increase sympathetic outflow as measured by enhanced activity of TH and DβH. Supported in part by Grants HLB-14388 and NS-12121.

24.1 DEMONSTRATION OF MOTOR CIRCUITS WITH CORTICAL STIMULATION AND 14-C-DEOXYGLUCOSE AUTORADIOGRAPHY. R.C. Collins, T. Der*, E.W. Lothman, and E.F. Vastola*, Neurology, Wash. Univ., St. Louis, MO 63110

In order to learn which neuroanatomic structures are active during a particular movement we have used the method of 14-C-deoxyglucose autoradiography (DG) in combination with intracortical bipolar stimulation of motor cortex. 300 g rats were anesthetized with halothane for insertion of vascular catheters for later infusion of DG. A side by side or concentric bipolar electrode (0.2 mm diameter; 0.5 mm tip separation) was inserted into forelimb motor cortex 2 mm anterior and 3 mm lateral to bregma and cemented to the skull. Stimulation was begun 1 to 4 hours after halothane, or after several days recovery. Stimulation parameters were varied in order to determine the necessary and best conditions for autoradiographic labeling. In each case DG (60 μCi/kg) was given intravenously during steady stimulation and animals killed at 45 min for processing. Brains were perfused-fixed, frozen, cut serially at 20 μm and exposed to SB-5 film for autoradiographic development. Single shocks (.25 msec, 0.5/sec, 40V) which caused a barely detectable movement caused no consistent increment in DG uptake. Train stimuli (0.25 msec, 250 hz, 40 msec, 0.5/sec, at 5 to 15V) caused discrete contralateral forelimb movements and DG uptake in ipsilateral subcortical structures: caudate/putamen, GP, N. reticularis, VA-VL, VL, VM, CL, Pf, STN, and SN. Metabolism was also increased in ipsilateral and transcallosal cortical columns. Train stimuli caused a local evoked potential but no seizure. The stimulus site was characterized by 1.5 to 3 fold increment in DG uptake. The lateral borders of the stimulus site were sharpley defined by a *surrounding field* where DG metabolism was slightly depressed. "Metaboic" columns appeared beyond this. Barbiturate raised the voltage threshold for stimulation induced movement 5 to 10 fold, but resulted in a generally similar pattern of metabolic activation.

24.2 TURNING BEHAVIOUR AND 2-DEOXYGLUCOSE AUTORADIOGRAPHY FOLLOWING UNILATERAL KAINIC ACID LESIONS OF THE GLOBUS PALLIDUS IN RAT.
J. S. Yeomans and D. van der Kooy. Depts. of Anatomy and Psychology, U. Toronto, Toronto, Ontario, M5S 1A1, Canada.

Unilateral injection of 1.5 n moles of kainic acid into the rat globus pallidus produces intense turning to the contralateral side for several hours post injection, weaker ipsilateral turning for about 2 more days, followed by no apparent turning bias. To determine the neural correlates of these turning biases, ^{14}C-2-deoxy-D-glucose was injected at the peaks of the turning (3 hr., 20 hr. post injection) and 7 days post injection, and Sokoloff's autoradiographic procedures were applied. At the injection site a region of relatively little label was seen within about 1 mm of the cannula tip 3-4 hr. post injection. Patches up uptake were found 20-22 hr. and 7 days post injection in loci which correlated with concentrations of glial cells in Nissl stained sections.

Farther away from the injection site intense consistent increases in ipsilateral vs. contralateral ^{14}C-2DG uptake were seen in caudate-putamen and substantia nigra. Dramatic, but inconsistent changes were found in many other brain structures.

24.3 PRESYNAPTIC INPUT FROM THE VENTRAL TEGMENTAL AREA OF TSAI TO HYPOTHALAMIC NEURONS. F. C. Barone, M. J. Wayner, W. H. Tsai, S. L. Scharoun*, H. U. Aguilar-Baturoni and R. Guevara-Aguilar. Brain Research Lab, Syracuse Univ, 601 Univ Ave, Syracuse, NY 13210 and Depto de Fisiologia, Facultad de Medicina, U.N.A.M., Mexico.

Electrical recordings from single neurons were made in the hypothalamus of urethane anesthetized male hooded rats. A concentric bipolar stimulating electrode was placed in the ventral tegmental area of Tsai (VTA) and the effects of electrical stimulation, 0.5 msec single rectangular pulses of 0-500 μA, on ipsilateral lateral preoptic and lateral hypothalamic (LPA-LH) neural activity were determined. Reliable current dependent increases and decreases in neural activity were observed. An analysis of response latencies indicated that the effects were mediated mono and polysynaptically. Significant effects occurred with relatively short latencies and at low current intensities. Antidromic invasion was also observed in some cells indicating interconnections between the VTA and LPA-LH. In addition, horseradish peroxidase (HRP, Sigma Type VI, 25%) was applied microiontophoretically in the LPA-LH. Alternate brain sections were processed with DAB and BDH for the brown and blue reactions. Labeled neurons were identified in the VTA utilizing light and dark field microscopic examination of sequential sections. Therefore, shorter latency effects of electrical stimulation are monosynaptic and are probably not due to stimulation of fibers of passage in the VTA. These results, in terms of neurophysiological and neuroanatomical data, demonstrate unequivocally significant direct and indirect presynaptic inputs to neurons in the LPA-LH area mediated by neurons in the VTA. (Supported by NIH Grant NINCDS USPHS No. 13543)

24.4 MESENCEPHALIC CENTRAL GRAY PRESYNAPTIC INPUTS TO HYPOTHALAMIC NEURONS. W. H. Tsai, F. C. Barone, M. J. Wayner, S. L. Scharoun*, R. Guevara-Aguilar and H. U. Aguilar-Baturoni. Brain Research Lab, Syracuse Univ, 601 Univ Ave, Syracuse, NY 13210 and Depto de Fisiologia, Facultad de Medicina, U.N.A.M., Mexico.

Single unit electrical recordings were made from central neurons in urethane anesthetized male hooded rats. The central gray area in the mesencephalon (MCG) was stimulated by applying 0.5 msec single rectangular pulses of 0-500 μA through a concentric bipolar stimulating electrode and the effects on ipsilateral lateral preoptic and lateral hypothalamic (LPA-LH) neural activity were determined. Reliable current dependent responses were observed. An analysis of response latencies indicated that effects were mediated mono and polysynaptically. Significant responses occurred with relatively short latencies and at low current intensities. In addition, horseradish peroxidase (HRP, Sigma Type VI, 25%) was applied microiontophoretically in the LPA-LH. Alternate brain sections were processed with DAB and BDH for the brown and blue reactions. A light and dark field examination of sequential sections revealed positively labeled neurons in the MCG area. These results, in terms of neurophysiological and neuroanatomical data, unequivocally demonstrate a significant input from neurons in the MCG to neurons in the LPA-LH. The shorter latency LPA-LH neuronal effects of MCG electrical stimulation must be due to monosynaptic connections and apparently are not due to the stimulation of fibers of passage. (Supported by NIH Grant NINCDS USPHS No. 13543)

24.5 THE TECTORETICULOSPINAL PATHWAY IN THE GOLDEN HAMSTER. J. A. Muntz, R. C. Shults, and J. D. Rose. Dept. of Psychol., Univ. of Wyoming, Laramie, WY 82071.

The behavioral functions of the deep tectum have become of increasing interest recently. Previous work in this laboratory has demonstrated a role for this region of the dorsal midbrain in the lordosis response of female golden hamsters. The present investigation demonstrates the organization of descending projections from the deep tectum to pontomedullary reticulospinal neurons by means of neuroanatomical and electrophysiological methods. In the anatomical studies 50% horseradish peroxidase (HRP) and 2% dimethyl sulfoxide in normal saline was injected through a micropipette into caudal pontine and anterior medullary gigantocellular nuclear regions. The cerebellum was ablated prior to injection to prevent spread of the enzyme. After a 3-day survival period brains were processed using the technique of Mesulam (1978) employing tetramethyl benzidine as a chromogen. The injections resulted in retrograde labeling of neurons at all rostro-caudal levels of the deep superior colliculus. The labeled cells lay principally in a lateral position in the stratum profundum and almost exclusively contralateral to the injection site. Microelectrode recordings were performed in urethane-anesthetized hamsters. Tectoreticular neurons identified by antidromic response to stimulation of the contralateral ponto-medullary gigantocellular nuclei were found to lie in the same deep tectal region as cells retrogradely-labeled with HRP. Electrophysiologically identified tectospinal neurons were located in the anterior deep tectum exclusively, in accord with previous neuroanatomical findings (Shults and Rose, 1979). Single neurons comprising the reticular portion of the tectoreticulospinal system were identified in the medullary gigantocellular nucleus by orthodromic response to contralateral deep tectal stimulation and antidromic response to ipsilateral cervical spinal cord stimulation. The properties of tectoreticular, tectospinal and tectoreticulospinal neurons were relatively uniform in that these cells showed little or no spontaneous activity and were largely unresponsive to somatosensory stimulation. The inactivity and unresponsiveness of these caudally-projecting neurons stood in marked contrast to the presence of active, highly responsive neurons lying around them, and may reflect the close association between tectoreticulospinal and tectospinal neurons and motor function. The tectoreticular neuronal population was clearly larger and more spatially extensive than the tectospinal one in neuroanatomical as well as electrophysiological experiments.

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24.6 PROJECTIONS FROM THE PONTINE GIGANTOCELLULAR TEGMENTAL FIELD IN THE RAT. B.E. Jones, Neuroanatomy Lab., Montreal Neurological Inst., McGill University, Montreal, Quebec, Canada H3A 2B4.

The pontine gigantocellular tegmental field (FTG) has been shown to be important in the control of eye movements (Bender and Shanzer, 1964), head movements (Peterson et al, 1978), posture (Kuypers, 1964), locomotion (Mori et al, 1977) and paradoxical sleep (McCarley and Hobson, 1971; Jones, 1979). Early anatomical studies demonstrated diffuse projections from this area of the reticular formation ascending into the diencephalon and descending the full length of the spinal cord, whereas more recent experiments have emphasized local projections onto bulbar oculomotor neurons (Grayfield, 1977) and heavy spinal projections onto the ventral horn (Holstege et al, 1977; Martin et al, 1979).

To investigate the entire projection from the pontine FTG in the rat, small localized injections of radiolabelled amino acids were performed with a pressure micro-injection system. Amounts ranging from 10-250 nl of ^{3}H-leucine or proline (100nCi per nl) were delivered through glass micropipettes with 1-5 micron tips. Animals were sacrificed by perfusion 24-48 hours after injections for autoradiographic processing of the tissue that involved 14-90 day exposures (NTB-2 emulsion).

Large injections (∿250 nl) labelled the small celled fields in the more lateral reticular formation and the more rostral central tegmental field in addition to the giant cell field (FTG) of the pons and they resulted in a pattern of projections which could be described as diffuse, that is including local propriobulbar projections (onto 6th and 7th n. nuc., vestibular nuc., and brainstem RF), long ascending projections into the zona incerta and thalamic intralaminar nuc., and long descending projections along ipsi- and contralateral ventral funiculi to ventral horn and the contralateral dorsolateral funiculi to the dorsal horn.

In contrast very small and restricted injections (10-100 nl) into the pontine FTG produced a limited and fairly specific pattern of largely ipsilateral projections. Reticulo-reticular projections extended ipsi- and contralaterally into the paramedial regions through the brainstem, but were especially heavy into the bulbar giant cell field and magnocellular region (FTM). Cranial nerve nuc. were not directly innervated nor was the lateral reticular formation. Long projections were evident only down into the spinal cord passing through the ventral funiculus into the intermediate zone (lamina 7 and 8) on the ipsilateral side.

In summary the major projections of the pontine FTG are bilateral reticulo-reticular into the paramedial zone of the brainstem and unilateral reticulo-spinal into the intermediate zone of the ventral horn. This area probably functions in the control of postural mechanisms during waking and sleep.

24.7 LOCUS COERULEUS NEURONS HAVE AXONS THAT BRANCH TO BOTH THE CEREBRUM AND CEREBELLUM: AN HRP AND IRON DEXTRAN DOUBLE-LABELLING STUDY IN THE MOUSE. D.A. Steindler* (SPON: E. Eisenstein). Dept. of Anatomy, Michigan State University, East Lansing, Mi. 48824.

Particular monoaminergic neurons within the brainstem (i.e. nucleus locus coeruleus (LC) and raphe nuclei) have been described as giving rise to extremely divergent axonal projections to numerous CNS areas (Ungerstedt, Acta Physiol. Scand. Suppl., 367: 1971; Nakamura and Iwama, Brain Res., 99: 1975). Collateral axonal branching represents one way in which divergent projections may be achieved by meager numbers of neurons and at the same time allow these neurons to affect and possibly interrelate the ontogeny, maturation, and adult patterns of activity of various CNS neurons.

The present double-labelling study utilizes the retrograde axonal transport of horseradish peroxidase (HRP) and iron dextran (ID) in order to identify LC neurons that are involved in a branched innervation of the cerebrum and cerebellum. One group of adult white mice received HRP injections within the prefrontal, frontal, or somatosensory cerebral cortices as well as injections of an iron dextran complex (Anchor Laboratories, concentrated to approximately 200mg/ml in 0.9% NaCl containing 0.03% poly-L-ornithine or DDH_2O) within portions of the ipsilateral cerebellum (particularly the crus II and neighboring hemispheric lobules). In a second group these injections were reversed. After survival times of up to 8 days, and HRP histochemistry (using DAB/glucose oxidase) followed by the Prussian blue reaction on 50µm sections (Cesaro et.al., Neurosci. Lett., 15: 1979), retrograde labelling was assessed in the thalamus, locus coeruleus, and pre-cerebellar nuclei.

Populations of single-labelled neurons were found in the ventral tier, mediodorsal and intralaminar thalamic nuclei, pons, inferior olive, principal sensory, oralis and interpolaris divisions of the trigeminus, locus coeruleus, as well as other areas. Neurons within these nuclei displayed varied degrees of retrograde ID-labelling. In many situations, analysis was carried out at high magnification in order to discern weak labelling in neurons containing as little as 3-10 blue granules per cell. Other times, the ID-labelling can be quite pronounced with neurons containing several blue granules that appear to coalesce resulting in intensely labelled portions of somata and dendrites. The uptake and detection of both tracers can be manipulated in order to achieve distinct poplations of brown and blue granules within single branched neurons. In addition to HRP or ID-labelled cells, many double-labelled neurons have also been observed in LC, and it is possible that particular portions of the nucleus may be involved in interrelating portions of the fore- and hindbrain cortices.

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24.8 TERMINAL DENDRITIC STRUCTURE AND DENDRITIC APPENDAGES OF NECK MUSCLE MOTONEURONS IN THE ADULT CAT. P. K. Rose, S. Keirstead* and S. Vanner*. Dept. of Physiology, Queen's University, Kingston, Canada K7L 3N6.

Most descriptions of motoneuron morphology have been concerned with the size and distribution of their dendritic trees. However except for several reports, published in the late 19th century, little is known about dendritic appendages and terminal dendritic structure of motoneurons. Consequently we have re-examined the fine structural characteristics of motoneurons in the adult cat. All motoneurons examined were intracellularly stained with horseradish peroxidase and electrophysiologically identified as neck muscle motoneurons.

Distal dendrites often followed a tortuous course and frequently were varicose or appeared as a string of beads joined by narrow processes. This was in contrast to proximal dendrites which were smooth and cylindrical and followed a straight path. The structure of the dendritic terminal itself was variable. Some dendrites ended as a swelling whereas others had a single fine spike-like process originating from a pre-terminal swelling. Occasionally a distal dendrite divided into two or more short branches, forming a tufted or claw-like ending.

Spines were a common occurrence on all motoneurons examined. They were most frequently located on proximal dendrites. Most spines were either short (2-3 µm) and stubby or long (5-8 µm) and narrow. Another kind of dendritic appendage was also characteristic of all motoneurons. Unlike spines, these appendages were long (50-250 µm) and usually emerged from the apex of a cone-shaped protrusion. The origin of each appendage was narrow (less than 0.5 µm) and cylindrical in contrast to the central and distal parts which were 1-2 µm in diameter and varicose. Most of these processes were found on proximal dendrites but they were also common on the central part of the dendritic tree of biventer cervicis and complexus motoneurons.

The functional significance of the unusual terminal dendritic structure is not clear. However, the long dendritic appendages are strikingly similar to descriptions of presynaptic dendrites. Neck muscle motoneurons may consequently influence neighbouring neurons via two routes: 1) axon collaterals or 2) dendrodendritic contacts. The significance of this arrangement may be related to the fact that unlike the output mediated by axon collaterals, the output of presynaptic dendrites would be dictated by synaptic inputs distributed to only a fraction of the total dendritic tree.

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24.9 POSTERIOR TIBIAL-TO-PERONEAL NERVE CROSSOVER: AN ELECTROMYOGRAPHIC STUDY. R. R. Weisz, R. J. Cox*. Electromyographic Lab., *Department of Neurology, Indiana University Medical Center*, Indianapolis, Indiana 46202.

A Posterior Tibial-to-Peroneal nerve anastomosis was found as an incidental finding in a patient studied electromyographically. In contrast with the well described and recognized Peroneal-to-Posterior Tibial nerve crossover, the reverse anastomosis has hitherto not been documented. It is important to recognize the anastomosis because it can be a source of error when one evaluates the results of an electromyographic study. The main difficulty in identifying the anastomosis is differentiating it from an Accessory Deep Peroneal Nerve. The technique of proving the anastomosis will be presented, and methodology employed to avoid pitfalls will be discussed. The signficance of the anastomosis in terms of electrodiagnostic studies and functional human neuroanatomy will be discussed.

24.10 NEUROMUSCULAR ORGANIZATION IN THE HINDLIMB OF THE OPOSSUM, DIDELPHIS VIRGINIANA. D. A. Thomas* (SPON: J. A. Kugel). *Department of Anatomy, West Virginia University, Medical Center*, Morgantown, West Virginia 26506

Numerous studies of neural organization in the opossum, a metatherian mammal, have generally revealed systems which are qualitatively, typically mammalian in arrangement. Yet the total size of opossum central nervous system is extremely small when compared to body size or to the CNS in comparably sized placental mammals. This study is a preliminary examination of neuromuscular innervation in two antagonistic hindlimb muscles, the medial gastrocnemius (MG) and the tibialis anterior (TA). It is intended to quantitate directly the innervation, motor unit size and fiber types of these muscles in the opossum for comparison to other mammals. Detailed dissection of the lumbosacral plexus revealed that both of these muscles are supplied by branches of the sciatic nerve (origin from L5, L6, S1, S2). One or two descending sciatic branches reach the head of MG; multiple branches enter the head of TA just below the popliteal fossa. Nerves to these fibers were osmium stained, Epon embedded, sectioned, and fibers were counted and measured. Mean nerve fiber count for MG is 255 and for TA is 347. All sizes were charted on histograms. The muscles were also removed, frozen sectioned and reacted with alkaline ATPase. Paraffin embedded tissue from adjacent areas was used for total fiber counts. MG (from a small sample) has a range of 11,202 - 16,718 muscle fibers; the ratio of fast to slow muscle fibers is 1:1. TA has from 8,265 - 14,766 muscle fibers. The ratio of fast to slow muscle fibers in TA is 3:1. Cross sectional areas of muscle fiber types (slow or fast) were similar within each muscle. Until deafferentation studies are completed, estimation of motor unit size is tentative. Assuming that the percentage of afferent nerve fibers is the same as reported in the cat (40 - 55 %), motor unit size for these muscles would fall into the typical mammalian range.

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24.11 DISTRIBUTION OF FIBER TYPES IN MONKEY FOREARM MUSCLES. J.S. McIntosh, M. Ringqvist*, E.M. Schmidt. Lab of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Histochemical staining methods were applied to the following forearm muscles from one Macaca mulatta and two Macaca fascicularis monkeys: ext. carpi ulnaris, ext. carpi radialis longus, ext. carpi radialis brevis, ext. digitorum, ext. digiti 4th and 5th proprius, flex. carpi radialis, flex. carpi ulnaris (FCU) and brachioradialis (BR).

In all the muscles studied we found a mosaic of type I and type II fibers and in all but FCU, the histochemical mosaic was essentially the same throughout the muscle cross section. FCU is a bipennate muscle with distinctly different proportions of type I and II fibers on either side of a central tendon (see Table). BR in M. Mulatta had very low percentages of type I and IIA fibers as compared to the other monkeys studied (see Table).

Monkey forearm muscles except FCU appear to be similar to human in that they have a mosaic pattern of fiber types, fairly uniformly distributed throughout the muscle.

Mean values and S.D. for percentage distribution of fiber types in the forearm muscles of one Macaca mulatta and two Macaca fascicularis monkeys.

	Type I	Type II		
		IIA	IIB	IIA + IIB
	%	%	%	%
All muscles Studied Except FCU and BR	29.4 ± 5.7	27.0 ± 8.7	43.6 ± 11.7	70.6 ± 5.7
FCU Medial part	25.6 ± 1.8	25.1 ± 5.6	49.3 ± 6.6	74.4 ± 1.8
FCU Lateral part	64.2 ± 5.9	19.2 ± 5.2	16.6 ± 3.3	35.8 ± 5.9
BR M. fasc.	29.3 -	32.1 -	38.6 -	70.7 -
BR M. mulatta	7.1 -	11.7 -	81.2 -	92.9 -

24.12 ORIGINS OF CARDIAC PREGANGLIONIC NEURONS IN THE RAT; A COMPARISON OF ATRIAL AND VENTRICULAR INNERVATION. S. L. Stuesse. With the technical assistance of K. S. Stockton.* Neurobiology Program. N. E. Ohio Univ. Col. of Med., Rootstown, Ohio 44272

A retrograde cell labeling technique was used to compare origins of preganglionic neurons in the sinoatrial and mid-ventricular regions in adult rats. Animals were anesthesized with halothane, thoracotomized, and 2-5μl of 30% horseradish peroxidase (Sigma, type VI) was injected into the myocardial wall in either an atrial or mid-ventricular location. After a 24-48 hr. survival time, the animals were reanesthesized, perfused, and their brains removed. 40μm sections were made from the brainstems and upper spinal cords, and the sections were then reacted with tetramethyl benzidine (TMB). Sections were lightly counterstained with Neutral Red to visualized cellular anatomy. Whole hearts were also reacted with TMB to confirm injection sites.

The majority of labeled cells were found in the nucleus ambiguus (NA) about 1mm rostral to the level of the obex; fewer cells were found in the dorsal motor nucleus (DMN); no labeled cells were found in the nucleus tractus solitarius. In the NA, labeled cells were also found in the most caudal part of the medulla. At this level, a few periambigual cells were labeled. Slightly caudal to the obex, 2-4 labeled cells were found in some sections in an intermediate zone between the NA and DMN.

The same nuclei were labeled with atrial and ventricular injections but fewer cells were labeled with the latter. Labeling was bilateral in both cases. In rats in which either right or left vagotomies were performed several days prior to the HRP injections, the labeling was ipsilateral to the intact vagus.

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24.13 ORIGINS OF SUPRASPINAL PROJECTIONS TO THE SPINAL CORD IN THE TEGU LIZARD. W.L.R. Cruce, D.B. Newman*, and J.A. Finkelstein, With the technical assistance of D.F. Ayers.* Neurobiology Department N.E. Ohio University College of Medicine, Rootstown, OH 44272 and Anatomy Department, Uniformed Services University of the Health Sciences School of Medicine, Bethesda, MD 20014

Cells of origin for pathways from the brain to the spinal cord were localized in the Tegu lizard, Tupinambus nigropunctatus, by making spinal injections of horseradish peroxidase (HRP), 30%-40%, or Bisbenzimide (BIS), 10%. Small and large injections were made unilaterally and bilaterally at cervical, thoracic, and lumbar levels. Survival times ranged from 2 to 11 days. Frozen sections were cut at 40 micra thickness and reacted with tetramethyl benzidine (TMB) for HRP or immediately mounted for BIS. Alternate sections were counterstained with Neutral Red for cytoarchitectonic localization. Sections were viewed with brightfield or darkfield transmitted light for HRP or with incident fluorescent light methods for BIS.

Labelled cells were found on the ipsilateral side in the interstitial nucleus and the superior, middle and inferior reticular fields. The raphe nucleus was labeled in the midline. Contralateral labeling was found in nucleus ruber. Bilateral labeling was found in the nucleus of the solitary tract, the dorsal motor nucleus of the vagus, the ventrolateral vestibular nucleus (Deiter's), and locus coeruleus. Within the hypothalamus a few labeled cells were observed in the periventricular region. Our previously described subdivisions of the lateral portion of the superior reticular field were identified: the dorsolateral (MDL) and ventrolateral (MVL) metencephalic nuclei. Ipsilateral labeling was seen in MDL and bilateral labeling was found in MVL. Our previously described subdivisions of the inferior reticular field were also identified: the dorsal (RID) and ventral (RIV) medial parts and the ventral lateral (RVL) part. Labeling in these cells was ipsilateral except for RID which was bilateral. Further studies are under way to determine if any of the bilateral labeling is due to spread of the injected label across the midline.

Comparison of injections at different levels of the cord suggested that there may be a somatotopic orginazation within the ventrolateral vestibular nucleus such that thoracic and lumbar projecting neurons are located caudally and ventrally to cervical projecting cells.

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25.1 LAMINAR DIFFERENCES IN THE SIZE AND SHAPE OF RECEPTIVE FIELDS IN RAT SOMATOSENORY (SI) CORTEX. John K. Chapin, Chia-Sheng Lin, and Donald J. Woodward, U. of Tx. Hlth. Sci. Cntr., Dallas,Tx. 75235

This study was conducted to quantitatively describe the differences in the spatial configurations of somatic receptive fields (RF's) of cells in different layers of the primary somatosensory (SI) cortex. Post-stimulus time histograms of single unit responses in nembutal anesthetized rats were generated by delivering standardized punctate mechanical stimuli to each of 25 to 40 closely spaced points on the skin of the contralateral forepaw or hindpaw, or of 26 adjacent whiskers on the face. Computer techniques were used to calculate the magnitudes of the excitatory peaks in each histogram within such a series. These were used to plot a two-dimensional "response contour map (RCM)" showing the exact spatial configuration and point-to-point topography of the receptive fields of cells in various cortical laminae.

The RCM's of all cortical cells studied here exhibited central regions of high sensitivity surrounded by less responsive zones. In neurons encountered within single vertical penetrations through cortex such foci generally represented the same peripheral cutaneous location. No clear inhibitory surrounds were ever observed. The overall sizes of the RF's (defined here as the total area of the active zones seen within the response contour maps) were always larger than could be detected by aural monitoring. The RF's in layer IV and deeper layer III were quite small and exhibited steep slopes rising sharply to clearly defined central peaks. In the paw areas they were generally elongated in shape and were oriented proximo-distally along limb, paw or digit surfaces. In the vibrissa field they were more circular in shape and encompassed only 2-5 whiskers. The RF's were slightly larger in the more superficial parts of layer III and layer II. In the vibrissa areas they tended to be elongated rather than circular in shape and were oriented vertically across the rows of whiskers. By contrast, most cells in layer V exhibited very large RF's, often covering the whole paw surface or vibrissa field. Localized foci existed in the same location as in the overlying layer IV cells, but one or two additional foci were often clearly distinguishable in locations elsewhere within the RF.

In conclusion, the organization of the SI cortex appears to be somewhat similar to that of the visual cortex in that layer IV contained small, circular RF's while in deeper and more superficial layers they were larger and had more complex shapes. Despite these differences, the concept of a vertical "columnar" organization remains intact since RF's in all layers contained central foci corresponding to the same cutaneous area.

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25.2 CHRONIC CHANGES IN CAT SOMATIC SENSORY-MOTOR CORTEX (Sml) FOLLOWING SELECTIVE DORSAL-ROOT SECTION. Joel I. Franck*[1], Jacqueline Metzler, and Philip S. Marks* (SPON: Franklin C. Wagner, Jr.). Sect. of Neurosurg., Yale Univ. Sch. of Med., New Haven, CT 06510.

Several recent studies have reported both acute and chronic changes in the somatotopic map in spinal cord (Dostrovsky et al., 1976), DCN (Millar et al., 1976) and Sml (Metzler & Marks, 1979; Franck, 1980) following selective blocking of ascending spinal pathways. In order to examine further the long-term effects of selective deafferentation on the body surface projection to Sml, extracellular responses of single units were studied from 12-40 weeks following the sectioning of all dorsal roots caudal to L3 with the exception of L7.

Response and receptive-field (RF) properties of 451 units were studied in 3 awake, paralyzed, adult cats under N_2O analgesia and compared to both the control recordings and the recordings taken 8 to 55 days following deafferentation in the same animals, and the results obtained during acute blocking of the L4-L5 dorsal roots in another population. In all cats, the somatotopic map in Sml continued to change 12-20 weeks after deafferentation, with a progressive increase in the area representing the trunk and abdomen in the more medial, partially deafferented region which had responded to hind-limb stimulation in control preparations. While nearly 60% of the 212 cells examined during this period had highly specific response properties, the remaining 40% were classified as nonspecific, as compared to 33% in these same animals 8-55 days post-rhizotomy, and to 7% in control preparations. These cells had multiple, bilateral or uncharacteristically large RFs which often responded to multiple subclasses of stimulation. There was also an increase in the percentage of cells with higher thresholds of stimulation. During the 20-40 weeks post-rhizotomy, 239 units were examined and few additional changes in Sml somatotopy were observed. There was, however, a progressive decrease in the percentage of units with nonspecific response and RF characteristics such that, by 30 weeks after dorsal-root section, the percentage of nonspecific cells had decreased to nearly 18%. This proportion remained relatively constant through the 40 weeks of observation.

These findings support previous reports of the presence of widespread afferent connections throughout the somatosensory systems of intact animals which are usually ineffective. They suggest, further, that the increased effectiveness of afferents from the intact body segments in activating cells in the deafferented region may be essentially complete by 30 weeks following deafferentation.

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25.3 THE CORTICO-CORTICAL CONNECTIONS OF DIFFERENT BODY REPRESENTATIONS FROM SI IN THE RAT. C.S. Lin, S.E. Knowles, J.K. Chapin and D.J. Woodward. Department of Cell Biology, The Univ. Tx. Health Sci. Ctr., Dallas, Texas 75235

The aim of this study was to determine the cortical afferents converging upon electrophysiologically identified zones of body representation in SI cortex. Electrophysiological maps of body parts in SI were obtained prior to iontophoretic HRP injections (2-3 μ Amp for 15-20 min) which were made from electrodes (tip size 20-30 μM) containing a mixture of L-lysophosphatidyl choline in 20-30% HRP (Sigma VI). Brain sections were preheated with $CoCl_2$ and then reacted with DAB. These enhanced methods allowed visualization of dendritic morphology of cells projecting to the injection site as well as axonal arborizations of cell bodies whose axons left the injection site. Major findings were: a) The glabrous skin of the finger digits and the forepaw is represented twice in SI with the finger digits in between the rostral and caudo-medially located forepaw areas. A zone in which cells were unresponsive (UZ) to light cutaneous stimuli was found between the forepaw and vibrissae. Cytoarchitectonically, this zone lacked the thick layer IV, a characteristic of the other SI cortical areas. b) Afferents to forepaw, hindpaw and vibrissae areas of SI were found primarily from cells located ipsilaterally in layers III and V of both SII and MI. Anterogradely filled SI axons to the SII cortex arborized and formed terminals mainly in layers V and III. Only a few cells projecting to SI were found in layers III and V of the contralateral homotopic zones of SI. c) The callosal connections of the UZ were much denser than from any other part of SI. The labeled cells arose mainly from the contralateral homotopic UZ in layers II, III and V. A few retrogradely labeled cells were found in ipsilateral SII and MI in layers III and V. d) Reciprocal connections between SI and MI were identified by injecting HRP in MI and Fast green in SI after electrophysiological identification of similar body parts. e) Cortico-cortical connections between the representations of different body parts in the SI cortex originated from densely filled pyramidal cells located in the lower border of layer III and middle of layer V adjacent to the injection sites.

In summary, the present results indicate that SI predominantly communicates with other cortical areas (MI, SII, contralateral SI) via connections between areas of homotopic body representations. Corito-cortical connections between heterotopic body areas are most strong from adjacent regions within SI.

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25.4 COMPARISON OF THE RESPONSES OF NUCLEI RAPHE DORSALIS, MAGNUS, AND OBSCURUS TO SOMATOSENSORY STIMULI. S. Springfield* and G-M Moolenaar, Physiology Dept., Howard Univ. Med. Schl., Washington, DC 20059.

Experiments were done on chloralose-anesthetized cats to 1) discern the differences, if any, in the responses of cells in the rostral raphe (R) nucleus R. dorsalis and the more caudal nuclei, R. magnus and R. obscurus to somatosensory stimuli and 2) to compare their responses to those of reticular formation (RF) neurons. The responses of 230 cells were recorded extracellularly using glass micropipettes filled with 3M NaCl. Stimuli included brief tap, deep muscle pressure, an 80 db click, noxious pinch, cold (ethyl chloride) spray, and heat (test tubes of water heated to 47°C). Brief tap was the most effective stimulus in activating cells in all three nuclei. Like reticular neurons, raphe units had extensive receptive domains which often included both fore-and hindlimbs. Tap applied to the distal forelimb was particularly effective. Furthermore, the responses of R cells became attenuated with increasing stimulus frequency, a second feature in common with RF neurons. Among the nuclei, two major differences were observed. First, while cells which responded to noxious input could be located throughout the R, the majority were observed in R. magnus. These units were often multi-modal, responding to a variety of inputs. Preliminary data suggest a second difference which is related to the rate of spontaneous discharge and responsiveness to sensory input. Slowly firing neurons (<1 Hz), when responsive to stimuli, discharged with one or two spikes. Most of these slowly firing units were found in R. dorsalis. More rapidly firing cells (>10 Hz) were commonly inhibited by the natural stimuli. Duration of inhibition was related to the intensity of the stimulus. Such cells were found throughout R. However, the majority of cells which were excited by sensory input had spontaneous discharge rates between 1-10Hz. These cells responded with a burst whose duration was related to stimulus intensity. Excitation was followed by an intensity-related period of inhibition. Cyclic bursts of activity was seen primarily in R. obscurus. These cells fired in doublets or bursts of 4-6 spikes and could not be driven by somatosensory stimuli. These results suggest strong similarity to RF neurons and suggest, furthermore, a correlation between rate of R unit spontaneous discharge and characteristics of the response to somatosensory stimuli.

25.5 PROJECTIONS OF PERIPHERAL NERVES TO THE CUNEATE NUCLEI OF RATS. C. H. M. Beck, Psychology, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9.

Preliminary to electrophysiological single cell recording from the cuneate nuclei, anatomical maps were constructed of peripheral nerve projections to those nuclei in rats. Horseradish peroxidase (Sigma VI, 30% in Ringer's solution) was injected (.2µl) into the stumps of peripheral nerves distal to the brachial plexus. Survival times ranging from 40 to 48 hours were followed by sectioning of the brainstem, cord and nerves at 33µ and reacting chemically with tetramethylbenzidine.

Injections into median, radial and ulnar nerves cut 1 cm above the elbow revealed that each nerve had a distinct projection zone in the cuneate nuclei. The radial nerve projected quite heavily to the external cuneate and to a narrow band along the dorsal edge of the internal cuneate. The ulnar and median nerves projected mainly to the medial and lateral portions of the internal cuneate ventral to the projection of the radial nerve. Injection into the deep radial and the superficial radial nerves just below the elbow revealed that they contributed to the external and internal cuneate projections respectively of the radial nerve. Reacted ventral horn cells in the most dorso lateral portion of the ventral horn of the spinal cord were apparent after all injections except those of the superficial radial nerve. Reaction particles were visible in the nerve stumps and in the dorsal root ganglia cells of the affected nerves.

25.6 FUNCTIONAL PROPERTIES OF RACCOON CUNEATE NEURONS RESPONDING TO TACTILE STIMULATION OF GLABROUS SKIN. Mark J. Rowinski, John H. Haring*, Susan Warren*, Christine H. Maliniak*, and Benjamin H. Pubols Jr. Department of Anatomy, College of Medicine, Pennsylvania State University, Hershey, PA. 17033.

The functional properties and dorsal column projection of mechanoreceptive afferent fibers innervating the glabrous skin of the raccoon's forepaw have been extensively characterized (Pubols, Pubols & Munger, Exp. Neurol., 1971; Pubols & Pubols, J. Neurophysiol., 1973). The present investigation was undertaken to gain insight into transformations of tactile information occurring in the cuneate nucleus. Unit activity was recorded in 77 neurons in 17 methoxyflurane-anesthetized raccoons. Recording loci were between 1.5 mm rostral and 5.5 mm caudal to the obex, and peripheral receptive fields were located entirely on glabrous skin.

Units were classified according to their response to mechanical stimuli as rapidly adapting (RA), slowly adapting (SA), or Pacinian (Pc). Of those neurons antidromically activated by electrical stimulation of the forepaw region of the contralateral thalamic ventrobasal complex (47% of sample), 19 were RA, 7 were SA, and 10 were Pc. Of those not antidromically activated (53% of sample), 33 were RA, 3 were SA, and 5 were Pc. One unit classed as SA also gave an "off" response, suggesting convergence from primary SA and RA fibers. Still other cuneate units (not included in the above tabulations) had receptive fields spanning both hairy and glabrous skin, indicative of another type of modality convergence.

Receptive field (RF) areas are larger on palmar (range = 11.0-99.2 mm^2) than on digital (range = 1.2-62.2 mm^2) surfaces ($P < .0001$). The median digital RF area (= 7.4 mm^2) is > 40 X that for primary afferents ($P < .0001$), and the palmar median RF area (= 30.3 mm^2) is > 100 X that for primary afferents ($P < .0001$); however, primary afferent RF areas do not differ significantly for those located on digits versus palm ($P = .20$). There is no systematic relationship between RF area and anteroposterior recording locus within the cuneate nucleus ($r = .194$; $P > .20$).

Most cuneate neurons display a resting discharge, typically in the form of bursts of 2-5 spikes, and the major effect of mechanical stimulation is to decrease the interburst interval. The range of absolute displacement thresholds is ≈ 5-250 µm, similar to that for primary afferents. The range of power function exponents relating instantaneous burst frequency to skin displacement velocity = .50-.86 (encompassed by the range of exponents for primary afferent instantaneous spike frequency). Evidence was obtained that repeated mechanical stimulations lead to a decrease in post-stimulus inhibition of resting discharge. (Supported in part by research grant NS-13418, USPHS.)

25.7 CUNEATE NUCLEAR PROJECTIONS TO THE CEREBELLUM AND OTHER REGIONS IN THE RACCOON. Susan Warren*, Mark J. Rowinski, Christine H. Maliniak*, John H. Haring*, and Benjamin H. Pubols Jr. Department of Anatomy, College of Medicine, Pennsylvania State University, Hershey, PA 17033.

Experiments were designed to study the projection of cuneate nucleus neurons to the cerebellum in the raccoon, and to compare this projection with cuneate projections to other regions as previously described by others. The direct axonal trajectories and corresponding terminal fields of cuneate neurons were demonstrated by ^{3}H-leucine autoradiography in four raccoons (4.54-7.10 kg). The subjects were anesthetized with halothane, surgically prepared under sterile conditions, and injected with 0.05-0.20µl of ^{3}H-leucine (50µCi/µl)-saline solution in the rostral or middle region of the main cuneate nucleus. Injection was unilateral in one raccoon and bilateral but asymmetric in the other subjects. Survival periods (2 days or 1 week) and tissue processing followed conventional methods for tritiated amino acid autoradiography.

Neurons of the main cuneate nucleus project to the ipsilateral cerebellum by way of the restiform body. The axons of these neurons follow a trajectory which is predominantly anterior and lateral to the deep cerebellar nuclei, arching dorsomedially toward the medial regions of the anterior lobe. The projection apparently diverges over a wide extent of cortical volume. The primary target areas of the cuneocerebellar axons are lobules III, IV, and V of the anterior lobe. Both superficial and deep cerebellar folia exhibit cuneocerebellar terminations. Evidence was also obtained for termination of fibers in the ipsilateral interposed and lateral nuclei.

Cuneate neurons projecting to the cerebellum in the raccoon are intermingled with neurons which project to brain stem and diencephalic structures such as the inferior olivary complex, red nucleus, zona incerta, and thalamic ventrobasal, posterior, and magnocellular medial geniculate cellular aggregates. Injections which moderately label the cuneocerebellar projection result in a very strong projection to the contralateral thalamus, moderate projection to the ipsilateral inferior olive, and weak projection to the contralateral red nucleus. The cuneate nucleus rostral to the obex appears to be the most potent source of cuneocerebellar axons since minimal injections in this region produce labelling comparable to that seen in cases of maximal injection in the middle region (obex to 3 mm caudal). This finding is consistent with physiological studies of the incidence of antidromically activated cuneocerebellar neurons throughout the rostral and middle nuclear regions in the raccoon. (Supported in part by research grant NS 13418, USPHS.)

25.8 MECHANOSENSORY PROJECTIONS TO DORSAL COLUMN NUCLEI IN A TREE SQUIRREL (FOX SQUIRREL, SCIURUS NIGER). E. M. Ostapoff* and J. I. Johnson. Neuroscience Program and Psychology, Biophysics and Zoology Depts., Michigan State Univ., East Lansing, MI 48824.

Our purpose is to compare the distribution and organization of mechanosensory projections to three pairs of dorsal column nuclei (cuneate, gracile and external cuneate) in an arboreal jumping rodent, with those previously determined for an arboreal marsupial (American opossum) and an arboreal carnivore (raccoon). Micromapping procedures were used to record unit and unit cluster activity with tungsten microelectrodes in squirrels anesthetized by means of ketamine followed by equithesin. Electrode penetrations were spaced at intervals of 200-500 µm forming mediolateral rows traversing all three nuclei in transverse planes. Rows were placed to sample rostral, caudal, and central (near the obex) regions of the nuclei. The locations of rows and penetrations were verified in Nissl-stained sections of the experimental brains. These preliminary conclusions are based on 130 responsive loci in 63 penetrations in four wild-caught squirrels.

In fox squirrels the three nuclei appear to be about the same size, with the gracile and external cuneate slightly smaller than the cuneate nucleus in cross-sectional extent and total volume. Most responses in the external cuneate were evoked by stimulation of deep-lying tissues or moving joints in the hand or forelimb, although a few responses were found to cutaneous or hair stimulation. Most responses in the gracile nucleus were to hair and cutaneous stimulation of the tail, hind foot and leg, and trunk, in that order going dorsoventrally in the nucleus, but some responses were to deep or movement stimulation. A similar distribution of responses was found in the cuneate to stimulation of hand, forelimb and neck. The pattern of projections in fox squirrels differed most strikingly from that seen in raccoons and opossums, in the relatively small extent and number of projections from the glabrous skin on the volar and plantar surfaces of the hands and feet. These surfaces provide a majority of projections to these nuclear regions in opossums and raccoons, but their representation is small in our squirrels: 10 of the total of 25 activating loci on the hand from a total of 72 on the forelimb; and 3 from a total of 15 from the hindlimb. In squirrels projections from hairy receptive fields were especially frequent: 33 on the forelimb including 9 on the hand of a total of 72; 9 of 15 on the hindlimb, and 11 of 18 on the trunk. (Supported by NSF grant BNS-7903421).

25.9 QUANTITATIVE EM ANALYSIS OF THE NORMAL SYNAPTIC ORGANIZATION OF THE RACCOON CUNEATE NUCLEUS. B.C. Albright. Dept. of Anatomy, Med. Sch., Univ. of North Dakota, Grand Forks, N.D. 58202.

The synaptic organization of the cuneate nucleus was studied in the cell nest or column region in a 2 mm area immediately caudal to the obex. Tissue blocks were not oriented in any particular plane and sections were collected from a variety of levels throughout the blocks. A random sampling procedure was used in the selection of grids, sections and micrographs. Quantitative methods involved the computer assisted direct measurement of the area, perimetry and length of identified neuronal profiles from micrographs. Only those profiles that were observed to related synaptically to one another by the presence of a distinct synaptic site were studied. Eighty-five percent of all measured axon terminals were axodendritic, 13% were axoaxonic and 2% axosomatic. Sixty-five percent of the axodendritic terminals synapsed on a single dendritic profile. These terminals contained either spherical, flat or pleomorphic vesicles and averaged 1.64 μm^2 in area. The associated dendritic profiles averaged 3.1 μm^2 in area. A one to one relationship between axon and dendritic profiles existed in 34% of the cases. Another major category of axodendritic terminals included those which were presynaptic to two or more dendritic elements. These averaged 4.3 μm^2 in area and were often postsynaptic (48%) to a smaller axon profile. They were presynaptic to an average of 4 dendritic profiles which had a mean area of 2.57 μm^2. More than half of these dendritic profiles were also related synaptically to other axon terminals. The axoaxonic terminals involved in these complexes averaged .8 μm^2 in area and primarily contained either flat or pleomorphic vesicles. One-third of these axoaxonic profiles were also in an axodendritic position. Some of the dendritic profiles of these terminals received synaptic input from the larger axonal postsynaptic component of the axoaxonic complex. These terminals therefore possess both axonic and dendritic terminations and may modulate dendritic activity either directly or indirectly in a preinhibitory manner. Axon terminals on dendritic spines accounted for 2% of the axodendritic terminals and averaged .8 μm^2 in area. Associated dendritic spines averaged 2.3 μm in length. Axosomatic profiles accounted for 2% of all terminals studied. They averaged 1.27 μm^2 in area and contained either spherical, flat or pleomorphic vesicles. The results of this study will be discussed relative to other cytoarchitectural and degeneration studies in the raccoon dorsal column nuclei.

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25.10 PROJECTIONS FROM DENTAL STRUCTURES TO THE BRAIN STEM TRIGEMINAL COMPLEX: TRANSGANGLIONIC HRP TRANSPORT. L. E. Westrum, R. C. Canfield* and T.A. O'Connor*. Departments of Neurological Surgery and Biological Structure and Department of Restorative Dentistry, University of Washington, Seattle, WA 98195.

There has been a recent increased interest in the trigeminal primary afferents from teeth and periodontal structures to the brain stem. The precise CNS representation for these structures, however, continues to be controversial. We are using transganglionic neuronal transport methods in an attempt to delineate these various pathways and to test our previous observations using degeneration techniques following pulpectomies or tooth extractions. Horseradish peroxidase (HRP), both crystals and a 30% solution in 2% DMSO, was deposited in pulpal chambers of 10 adult cats, either as single or repeated applications. Thus far emphasis has been directed towards projections from single maxillary canines, but additional experiments are underway or planned for other teeth. Following survival times of 2-5 days the animals are reanesthetized, perfused and the tissues processed by the TMB method of Mesulam or with diamino benzidine. HRP-positive fibers enter the ipsilateral ganglion, where numerous cells are labeled, then fibers pass through the central root, enter the spinal tract and are distributed variably to each of the ipsilateral subnuclei. For maxillary canines the terminal distribution is greatest to the dorsal division of the main sensory nucleus, adjacent pars oralis, to pars interpolaris near obex and diminishes in pars caudalis. The pattern is variable but is rather extensive in the dorsoventral and mediolateral distributions. Fibers and terminal granules are distributed through broad regions of each nucleus, except for pars caudalis, with clusters of granules ventrally, medially and especially in the dorsal 1/3 of the nucleus. In pars caudalis, there is a distribution to the middorsal superficial layers, including substantia gelatinosa. A few fibers are also seen passing medially beyond the nucleus into the reticular formation, but no obvious crossing fibers could be identified. The results suggest a more extensive ipsilateral CNS representation for some dental structures than previously indicated corresponding well with certain physiological studies, but the reduced termination in pars caudalis and apparent absence of contralateral pathways are at variance with other studies. Supported by NIH grants DE-04942, NS-09678 and NS-04053. Dr. Westrum is an Affiliate of the CDMRC at the University of Washington.

25.11 CENTRAL PROJECTIONS OF PRIMARY AFFERENT BRANCHES OF THE OPHTHALMIC DIVISION OF TRIGEMINAL NERVE IN THE CAT. W.M. Panneton and H. Burton. Dept. Anat/Neurobiol., Wash. Univ. School of Med., St. Louis, MO 63110

The transganglionic transport of horseradish peroxidase (HRP) was utilized to investigate the central projections of branches of the ophthalmic division of the trigeminal nerve (V_1), including the innervation of the cornea. The frontal, lacrimal, and infratrochlear nerves were isolated, transected and soaked in a 50% HRP solution. Corneal experiments involved injection of the HRP solution into the corneal stroma. After 48-72 hours, the animals were sacrificed and serial sections through the brainstem were processed using tetramethylbenzidine as the chromagen.

Results show that all branches of V_1 distribute processes throughout the longitudinal extent of the ipsilateral trigeminal sensory complex, including the principle nucleus and subnuclei of the spinal nucleus. The centripetal transport of HRP within all branches of V_1, including those that innervate the cornea, was confined primarily to the most ventral parts of the principle nucleus and subnucleus oralis except at levels of the trigeminal motor nucleus where label extended along the lateral border of the principle nucleus. HRP also was transported to ventral parts of subnucleus interpolaris where a topography was apparent, e.g., peripheral fields innervated by the lacrimal, frontal and infratrochlear nerves were represented in lateral, intermediate, and medial positions, respectively, while the cornea was represented most ventrally. A topography of V_1 was especially evident within the intermediate laminae of subnucleus caudalis. In the rostral to caudal direction our results show that the nerve innervating the most anterior receptive fields, the infratrochlear, distributes to laminae II-IV approximately 1.5-3.2 mm caudal to the obex while progressively more posterior fields are represented more caudally (e.g., cornea, 2.2-4.2 mm; frontal, 2.4-6.3 mm; and lacrimal 3.7-8.0 mm). Where the respective distributions of the V_1 branches overlap in the rostro-caudal dimension, topographical segregation is maintained about the medial-lateral radius of the nucleus. In contrast, cases indicated considerable overlap in the distribution of HRP to laminae I and V and label could be found from levels near the obex through at least the first cervical segment.

The central processes of the nerves studied were distributed to all laminae of subnucleus caudalis. However, the infratrochlear had a denser projection to lamina I when compared to the frontal and lacrimal nerves. Corneal cases also resulted in intense staining of lamina I but showed a preferential distribution of label to lamina II. (Supported by NINCDS grants NS09809 and T32-NS07071.

25.12 CENTRAL CONNECTIONS OF THE INFERIOR ALVEOLAR NERVE IN THE CAT AS DETERMINED BY THE HORSERADISH PEROXIDASE (HRP) TECHNIQUE. Carl Marfurt* and Jan Arvidsson* (SPON: J. Way). Department of Anatomy, Hershey Medical Center, Hershey, PA 17033, and Department of Anatomy Karolinska Institutet, S-10401 Stockholm, Sweden.

Previous horseradish peroxidase and transganglionic degeneration studies in our laboratories have demonstrated a somatotopical projection of cutaneous primary trigeminal sensory neurons onto the trigeminal brain stem sensory complex in cats and rats. The current investigation was undertaken to extend these observations by identifying the pattern of central termination of primary trigeminal afferent neurons whose peripheral processes distribute through the inferior alveolar nerve.

In 6 adult cats, the inferior alveolar nerve was transected and anchored in microtubing filled with a 50% solution of HRP in saline. Forty-eight to ninety-six hours postoperatively, the brain stem and trigeminal ganglia were perfusion fixed, sectioned, and reacted with tetramethyl benzidine. HRP labeled neuronal cell bodies, 15 to 85 microns in diameter, were observed throughout the posterolateral region of the ipsilateral trigeminal ganglion. Labeled central processes of these neurons terminated in the dorsal 1/4 to 2/5 of the ipsilateral trigeminal brain stem sensory complex. Terminals were most numerous in the trigeminal main sensory nucleus (MSN) and appeared to decrease slightly in caudal direction through the spinal nucleus of V. Heavy labeling extended into the most rostral and dorsomedial pole of MSN. Occasional fibers approached, but did not enter the solitary nucleus. Labeling was heavy in all laminae of pars caudalis and extended well into the subjacent reticular formation (lamina V). Terminals were somatotopically localized in the dorsomedial region of caudalis, with the exception of a prominent plexus of lamina I fibers which arched laterally into maxillary and even ophthalmic division territory. Fibers which descended into the upper cervical spinal cord terminated only in laminae I and V. Occassional fibers passed through the dorsal commisure at the levels of C1 and C2 to terminate in the contralateral dorsal horn.

Numerous large-diameter fibers, presumably conducting proprioceptive information from the periodontal ligaments, entered the mesencephalic tract of V. Labeled cell bodies of origin were distributed throughout the rostrocaudal extent of the ipsilateral mesencephalic nucleus. Labeled central processes of these cells projected to the cerebellum by way of the superior cerebellar peduncle and to the inferior and superior colliculi. No fibers were seen to project to the thalamus or the motor nucleus of V.

25.13 AN HRP STUDY OF THE COURSE AND TERMINATION OF THE SPINAL TRIGEMINAL TRACT AND CONVERGENCE WITH CERVICAL PRIMARY AFFERENTS IN RAT. R. W. Pacholka*, R. M. Beecher*, C. H. Phelps, B. A. Fisher*, and J. C. Pearson* (SPON: G. Crampton). Dept. of Anatomy, Wright State Univ. Sch. of Med., Dayton, Ohio 45435.

Myofacial pain dysfunction, in humans, involves perception of pain at several points in the face and neck, although pain typically originates in the muscles overlying the temporomandibular joint (TMJ). The neuroanatomical basis for this referred pain has not been previously described. The purpose of this study was to trace primary afferents in rat from sites known to be involved in the pain syndrome; i.e., masseter superficial to the TMJ and the sternocleidomastoid. Projections from these areas were traced using horseradish peroxidase (Sigma VI, 16% solution) dissolved in a dimethylsulfoxide/physiological saline solution with tetramethylbenzidine utilized as a chromagen. Following a survival time of 24 hours the animals were perfused and the tissues prepared for light microscopy. Retrograde transport from the masseteric injection site resulted in densely labeled cell bodies in the ipsilateral Gasserian ganglion. Anterograde transport to the ipsilateral brain stem and spinal cord resulted in dense labeling of the spinal trigeminal tract (STT) which was shown to extend caudally to the C-3 level. Projections from the sternocleidomastoid were visualized entering the cord at the C-2 level, bifurcating into ascending and descending branches and becoming coincident with the STT. Fibers from this tract were observed to terminate in the nucleus caudalis and substantia gelatinosa at the C-3 level.

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25.14 GOLGI STUDIES OF INTERNEURONS AND INTERNUCLEAR PROJECTION NEURONS IN THE VENTROLATERAL SUBDIVISION OF TRIGEMINAL NUCLEUS ORALIS. W.M. Falls and J. King*. Dept. of Anatomy, Michigan State Univ., East Lansing, MI. 48824.

Trigeminal nucleus oralis (TNO) at the upper end of the spinal trigeminal nucleus (SVN) can be divided into dorsomedial, intermediate and ventrolateral (VL) subdivisions in the adult rat. These three subdivisions extend the entire length of TNO and are distinguished by morphological differences in the texture of their neuropil, as revealed in 1µm sections and electron micrographs and by the morphology and distribution of the axons and dendrites of their neurons as seen in Golgi preparations. The VL neuropil consists of multiple neuronal cell types squeezed between numerous tightly packed deep axon bundles which run longitudinally through the entire SVN. Among these cell types are Golgi type II interneurons, i.e. neurons whose unmyelinated axons arborize within or near their dendritic arbors and internuclear projection neurons whose axons via the deep bundles link up different levels of SVN.

Golgi type II interneurons are found in small clusters throughout VL. Their cell bodies (9 - 14µm in diameter) give rise to dendritic arbors which extend 300-500µm in the rostrocaudal axis and are generally confined to the subdivision. The unmyelinated axons generate highly branched axonal arbors which more or less overlap the dendritic arbors. Some axonal branches extend at least 150µm beyond the dendritic arbors in the rostrocaudal axis. These neurons are similar to islet cells in the substantia gelatinosa (Gobel, S., J. Comp. Neur., 162:397, 1974) and are considered to be inhibitory interneurons interposed between primary trigeminal axons which arborize in VL and second order projection neurons.

Small internuclear projection neurons with cell bodies ranging from 8-10µm in diameter, are found in large numbers throughout VL. Their dendritic trees occupy spherical or elliptical domains up to 300µm in diameter and are usually confined to VL. The unmyelinated axons of many of these cells arise either directly from the cell body or from a primary dendrite and give rise to a single collateral 15-30µm from their site of origin. This collateral generates a fine axonal plexus within the dendritic arbor of the parent cell while the parent axon, without branching further, travels a short distance in VL and enters a deep axon bundle. On the basis of axonal morphology these neurons are thought to function not only as internuclear projection neurons, modifying the output of neurons at other levels of SVN, but also as intranuclear interneurons involved in local circuits in VL. Supported by General Research Support Grant to the College of Osteopathic Medicine, M.S.U.

25.15 FURTHER OBSERVATIONS ON STIMULUS-RESPONSE RELATIONSHIPS OF VIBRISSA-ACTIVATED NEURONS IN NUCLEI ORALIS AND INTERPOLARIS IN THE RAT. John M. Gibson and Wally I. Welker, Department of Neurophysiology, University of Wisconsin, Madison, Wisconsin, 53706, USA.

We have demonstrated significant quantitative differences in stimulus-response relationships of neurons of the oral and interpolar divisions of the spinal trigeminal nucleus (Gibson, et al., Neurosci. Abstr. 5:707, 1979). In this report, we will present additional evidence for differential specialization of these trigeminal nuclei.

Single-unit action potentials were recorded extracellularly with tungsten microelectrodes in the trigeminal complex of barbiturate-anesthetized albino rats. Stimuli consisted of quantitatively-controlled deflections of single mystacial vibrissae in the unit's "preferred" (maximally activating) direction. Data were recorded digitally as spike discharge times with a resolution of 0.1 msec. These data were subjected to a variety of quantitative analytic procedures.

In both oralis and interpolaris, about half the more slowly adapting units were capable of discharging 10 seconds or more in response to a 7-degree step deflection. The regularity of discharge of these units was not significantly different for the two samples, nor was there evidence for two distinct subpopulations in either sample.

The "average" neuron of oralis responds to a given stimulus with significantly more spikes than the "average" neuron of interpolaris. This is true for static deflection (step function), constant angular velocity deflection (ramp) and for impulsive deflection (pulse).

Intensity functions (spikes per trial as a function of stimulus amplitude) for all first order neurons were monotonically increasing, and generally had a a "staircase" shape. In contrast, about one-fourth of the neurons recorded from oralis and interpolaris had nonmonotonic pulse intensity functions. In the extreme case, a few neurons of interpolaris were found to cease firing completely at some suprathreshold amplitude. These nonmonotonic units had an interesting cluster of properties, generally being more rapidly adapting, having low thresholds, and having multiple-vibrissa receptive fields. However, population intensity functions (total spikes in all units as a function of stimulus amplitude) were all smooth and monotonic. The curve for oralis lies above that for interpolaris, which lies, in turn, above that for the first-order units.

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25.16 Electrophysiological Studies on the Spinothalamic Tract of the Cat.
J. R. Maddox* and J. A. Holloway. Dept. of Physiol. Biophys., Coll. Med., Howard Univ., Wash., D. C. 20059

Microelectrode recordings were obtained from spinothalamic tract (STT) neurons in the lumbosacral spinal cord of adult cats anesthetized with chloralose, paralyzed with gallamine triethiodide, and artificially ventilated. Recording electrodes contained 3M KCl saturated with fast green dye. Units were identified as cells of origin of the STT if they could be activated antidromically by thalamic stimulation, responding with fixed latencies to paired stimuli (2 msec separation) and short trains (< 200 uA, 0.2 msec duration) of high frequency stimuli (200-500Hz). Collision of antidromic and orthodromic action potentials was used as final confirmation of STT recording. Recording sites were verified histologically in 30um frozen formalin-fixed cord sections. STT neurons were excited by mechanical stimulation of one or more hind limbs. Sixty percent were activated by light tactile stimuli; forty percent were activated by firm manipulation of deep tissue within the thigh region. The peripheral receptive fields (RF) of the units were fully analyzed. Forty percent of the units responding to light touch of the hind paw possessed contralateral RFs. Twenty percent of the units had ipsilateral cutaneous fields which were excited by light tactile stimuli of the entire hind limb. Also, twenty percent of units in this study possessed bilateral RFs. The vast majority of STT neurons recorded were located in Rexed's lamina VII of the spinal cord gray matter. The estimated mean conduction velocity was 12 m. sec^{-1} (range 5-20 m. sec^{-1}). None of the units were excited by noxious mechanical or noxious thermal stimulation of the skin. These preliminary results suggest that the majority of spinothalamic tract neurons respond primarily to mechanical stimuli, and that some of these neurons may project to the thalamus ipsilaterally.

Rexed, B. The cytoarchitectonic organization of the spinal cord in the cat. J. Comp. Neurol. 96: 415-496, 1952.

25.17 RESPONSE PROFILES OF INTERPOLARIS NEURONS PROJECTING TO THE CEREBELLUM AND THE VENTROBASAL THALAMUS IN THE RAT. D. C. Woolston☆, J. M. Gibson and W. I. Welker. Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Horseradish peroxidase studies have shown that the interpolaris (IP) region of the medullary trigeminal complex projects heavily to both the cerebellum (C) and the ventrobasal thalamus (VB) (Watson, C. and Switzer, R., Neurosci. Let., 10:77, 1978; Fukishima, T. and Kerr, F., J. comp. Neur. 183:169,1979). We are determining, using a battery of precisely-controlled quantitative tactile stimuli, the response profiles of vibrissae-activated single neurons in IP that are driven antidromically by electrical stimulation of either VB or the uvular (folium 9A) tactile area of the cerebellum. Female Holtzman rats anesthetized with sodium pentobarbital served as subjects.

Major results of our study to date include:

1) No neurons were activated antidromically from both C and VB.
2) Although half the sampled neurons had single vibrissa receptive fields (RF'S), as was the case in a previous sample of IP neurons (Gibson, J. et al., Neurosci. Abst. 5:707, 1979), none of these neurons with punctate receptive fields were found to project to C or VB. C- and VB-projecting neurons had larger RF's (5 vibrissae median).
3) Median amplitude thresholds were very low for both C-projecting (0.07°) and VB-projecting neurons (0.06°), compared to the median for Gibson's sample of all IP neurons (0.37°).
4) C-projecting neurons had a somewhat lower median velocity threshold (2.86°/sec) than VB-projecting neurons (10.02°/sec). The median velocity threshold for Gibson's sample of all IP neurons was higher (22.0°/sec).
5) Most C- and VB-projecting neurons had nonmonotonically increasing responses to increasing deflection amplitudes.
6) Nearly all C-projecting and VB-projecting neurons adapted rapidly (within 100 msec) to a sustained deflection of 7.2°.

In summary, little difference between C-projecting and VB-projecting neurons has been found to date. However, the sample of projection neurons differs in several respects from Gibson's sample of all IP neurons, which we assume included interneurons and neurons which projected to CNS loci other than C and VB. The projection neurons' large RF's and nonmonotonic responses to increasing amplitudes suggest that extensive intranuclear processing occurs in IP.

(Supported by NIH grants 5 T32 NS07026 and NS 14748.)

25.18 ORIGIN, COURSE, AND TERMINATION OF TRIGEMINOTHALAMIC PROJECTIONS IN THE OPOSSUM. Ross J. Kosinski* and James C. Hazlett (SPON: A. Castro). Dept. of Anatomy, Loyola Univ. Stritch Sch. of Med., Maywood, Il 60153.

Trigeminothalamic connections were studied utilizing orthograde and retrograde axon transport techniques. HRP injections centered in the ventral basal complex resulted in dense retrograde neuronal labelling in both subdivisions of the contralateral chief sensory nucleus with intermittent labelling throughout all levels of the corresponding spinal trigeminal nucleus. In the subnucleus caudalis, labelled cells were located predominately in the marginal and magnocellular layers and to a lesser extent in the substantia gelatinosa and paratrigeminal nucleus. These results are in essential agreement with a recent report by Stritzel et al.,'80. Interestingly, a few reactive neurons were observed throughout the trigeminal complex ipsilateral to the injection. With a few notable exceptions, similar results were obtained following HRP injections centered in the parafascicular complex. The major differences are:1.the apparent absence of retrograde labelling in the ventral subdivision of the contralateral chief sensory nucleus;2.a marked reduction of reactive neurons in the marginal layer of the subnucleus caudalis and 3.an overall decrease in the number of retrogradely labelled neurons in those areas giving rise to thalamic afferents. The course and termination of the trigeminothalamic projections emanating from both the chief sensory nucleus and the subnucleus caudalis were studied utilizing the orthograde transport of ^{3}H-leucine. In both series of injections, the main bundle of labelled axons coursed rostrally through the brainstem in association with the contralateral medial lemniscus. From this location, fibers were observed to distribute to various brainstem centers. The ultimate target of these ascending projections was identified as the dorsal portion of the contralateral ventral basal complex. As expected, the orthograde labelling was always more extensive in this nucleus after the chief sensory nucleus injections. Additional sites of termination included the contralateral parafascicular complex and zona incerta.

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25.19 TRIGEMINOTECTAL PROJECTIONS IN THE RAT. R.S. Erzurumlu* and H.P. Killackey. (SPON: J.E. Swett). Dept. of Psychobiology, Univ. of Calif., Irvine 92717.

The projections from the brainstem trigeminal complex to the superior colliculus were studied using anterograde degeneration and horseradish peroxidase (HRP) retrograde labeling techniques.

Tectal projections from the brainstem trigeminal complex arise from the contralateral principal sensory nucleus and the subnuclei oralis and interpolaris of the spinal trigeminal nucleus. Tectal fibers from all three nuclei collect in the trigeminal lemniscus after crossing the midline at successive levels of the brainstem. In the rostral pons, these fibers leave the trigeminal lemniscus and ascend towards the superior colliculus which they reach inferolaterally. The terminal fields of trigeminal projections, originating from different portions of the brainstem trigeminal complex overlap in the superior colliculus. In most cases the terminal fields are confined to the stratum griseum profundum and the stratum album intermediale. The exception is the interpolaris projections, which are the heaviest and often extend into the deep portions of the stratum griseum intermediale. In general, the trigeminotectal terminal fields occupy the entire lateral portion of the superior colliculus along its rostrocaudal extent.

Following an injection of HRP into the superior colliculus, cells of origin of the trigeminotectal projections can be identified. In the principal nucleus and the subnucleus oralis they are confined to the ventral portions of these cell groups. Labeled cells in the principal nucleus, while few in number, are the largest cells in this nucleus and are easily distinguished from the thalamic projection cells. The labeled cells in oralis are polygonal in shape and are the largest in the entire trigeminal complex. After superior colliculus injections of HRP, the majority of labeled cells are found in the subnucleus interpolaris. These cells are dispersed throughout the nucleus. At the caudal and rostral poles of the nucleus the labeled cells are small and medium in size, but the majority of labeled cells which lie between the two poles of interpolaris are large in size and polygonal in shape. Further, these labeled cells look similar to the thalamic projection cells of this nucleus.

Based on this similarity and the finding of anterograde label in the ventrobasal complex after collicular HRP injections, we conclude that the same cell of interpolaris projects to both thalamus and superior colliculus, whereas different cells project to these two targets from the principal nucleus.

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25.20 CORTICAL PROJECTIONS TO THE SPINAL TRIGEMINAL NUCLEUS IN THE ADULT CAT. Dunn, R.C., Jr., Tolbert, D.L. Departments of Neurosurgery and Anatomy, St. Louis University School of Medicine, St. Louis, MO 63104.

As background for a developmental study of the corticotrigeminal projection in kittens, a correlative light and electron microscopic analysis of this pathway has been undertaken in adult cats. In some animals unilateral cortical ablations of the coronal and/or proreate gyri were performed. After survival periods of 3-21 days, sections through the spinal trigeminal complex from 3 mm caudal to 6 mm rostral to the obex were examined using silver impregnation techniques and electron microscopy. In other animals combined ^{3}H-leucine and proline injections were made into the same cortical areas. Following survival periods of 5-7 days, tissue from the spinal trigeminal complex was processed for autoradiography.

Data from the light microscopic studies indicate that corticotrigeminal fibers exit dorsally from the ipsilateral pyramid, cross the midline and enter the spinal nucleus of V from its ventromedial aspect. The degeneration and orthograde labeling techniques demonstrate similar terminal fields which are mainly within the contralateral spinal trigeminal nucleus. In subnucleus caudalis these afferents are localized primarily in the ventral magnocellular layer and in the inner zone of the substantia gelatinosa. In subnuclei interpolaris and oralis these cortical projections occupy a lateral position immediately adjacent to the spinal trigeminal tract.

The electron microscopic data confirm the light microscopic impression that the corticotrigeminal fibers are small axons. These axons are thinly myelinated and measure 0.8-1.6 μm in diameter. Corticotrigeminal terminals appear widely dispersed within the spinal trigeminal nucleus. They are small boutons containing round vesicles that form asymmetric synaptic contacts onto small dendritic profiles. Both electron-dense and vesicle depleted electron-lucent terminals have been identified.

Previous studies by Wold and Brodal (Neurobiol. 3:353, 1973; Brain Res., 65:381, 1974) described substantial differences in the spinal trigeminal projections arising from the proreate and coronal gyri. In contrast to the coronal projection, that from the proreate was characterized as sparse, purely contralateral and entering the nucleus through the spinal trigeminal tract. Our data, however, indicate: (1) the proreate and coronal spinal trigeminal projections appear similar in density and distribution; (2) both projections gain access to the spinal nucleus from the contralateral pyramid; (3) both are predominantly contralateral.

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25.21 CORRESPONDENCE BETWEEN PATTERNS OF SOMATOSENSORY RECEPTIVE FIELD (RF) PROJECTIONS AND MUSCLE-MOVEMENT FIELDS (MMF's) EVOKED FROM WITHIN THE TRIGEMINAL (Tr) NUCLEAR COMPLEX. G.M. Shambes and Wally Welker. Department of Neurophysiology, University of Wisconsin, Madison, WI 53706.

The trigeminal nuclear complex (Tr) is a major mediator of exteroceptive reflexes associated with head, face and perioral structures. It is also a mediator of head, oral and facial movements initiated from central motor circuits. Peripheral tactile projections to Tr are organized somatotopically within all its subnuclei. This study was designed to determine whether (a) the motor projections (via motor V and VII) are also organized somatotopically and (b) the motor output patterns are topographically related to the tactile input patterns. Nine albino rats were anesthetized with either pentobarbital or ketamine hydrochloride. Patterns of projection of tactile RF's to Principalis (P), Oralis (O), Interpolaris (I), and Caudalis (C) subnuclei of Tr were determined by using systematic micromapping sampling methods and threshold natural stimulation of oral, facial and head structures. Tungsten ball microelectrodes were used to record single-unit or multiple-unit responses. After defining the RF for each recording location, the electrode was switched to stimulus mode to electrically stimulate the same locus, and the muscle-movement field (MMF - the muscle or body structure moved) was determined. This paired recording-stimulating procedure was carried out for every electrode locus. Electrical stimulus parameters: 100 μsec pulses (biphasic, balanced polarity, 2/sec). RF's, puncture depths, threshold stimulus voltages and MMF's were recorded for each puncture site. Results: (1) Threshold oral-facial tactile projections to P,O,I,C were somatotopically organized; (2)Threshold electrical stimulation (10-50 μA) of these same oral-facial projection sites evoked ipsilateral vibrissal movements, jaw opening and closing, ear protraction, eye opening and closing, dilation of the nares, and retraction of upper and lower lips; (3) There was partial or complete correspondence of RF's and MMF's for 74% of the 200 recording-stimulating loci studied, e.g. when a vibrissal RF projection site in P,O,I,C was stimulated, a movement of the same peripheral vibrissal region was elicited; (4) Although RF's and MMF's were different in 26% of the loci, they seem related in some adaptive way, e.g. stimulation of an upper incisor site in Tr elicited jaw opening movements. The sensorimotor correspondences and organizational specificity discovered in Tr suggests that it is a basic integrating component for simple local reflexes as well as for more complex specialized facial behaviors involving cerebral, brainstem and cerebellar circuits connected to it. (Supported by NSF Grant BNS-16230)

25.22 SOMATIC PROJECTIONS TO SUPERIOR AND INFERIOR COLLICULUS: AN HYPOTHESIS CONCERNING SENSORIMOTOR INTEGRATION. Martine RoBards, Mark Stritzel*, and Richard T. Robertson, Department of Anatomy, College of Medicine, University of California, Irvine, 92717.

Investigations of the somatic-recipient part of the superior colliculus have shown that the trigeminal representation in the deep layers is somatotopic and comprises a facial "hemisphere" which lies in dramatic correspondence with the hemisphere of visual receptive fields of cells in the overlying visual stratum. This laminar assembly of visual and somatic afferents serves to anchor the visual world to "head space."

In apparent contrast with the somatic projection to superior colliculus, the somatic-recipient inferior colliculus (external and intercollicular nuclei) shows a mosaic of spinal and dorsal column-mediated somatic afferents which defy description along traditional somatotopic lines. Acoustically sensitive cells in these nuclei have also been difficult to understand, since they show little resemblance to the frequency-specific, tightly tuned cells characteristic of the central nucleus.

Our analysis of the afferent and efferent organization of the somatic-recipient midbrain of opossum and rat, using HRP and fluorescent retrograde methods, and degeneration and autoradiographic anterograde techniques, has shown: (1) almost all hindbrain somatic cells projecting to superior colliculus arise from the trigeminal complex and the dorsal forepaw and preaxial forelimb portion of the cuneate nucleus--the frontal 180° of personal space for a four-legged animal--while dorsal column nuclei efferents representing the caudal 180° account for most somatic projections to the inferior colliculus; (2) some somatic cells projecting to midbrain also project to thalamus; (3) dorsal column nucleus cells projecting to midbrain both resemble and lie among muscle afferent-activated cells and cells projecting to cerebellum; (4) the rostral cuneo-trigeminal confluence projects widely to midbrain, as well as to thalamus and cerebellum, and may represent a special nucleus controlling neck movements; and (5) the external nucleus projects to somatic-recipient superior colliculus, providing auditory information and somatic information from spinal levels to the tectal motor cells (where other auditory and direct spinal projections also converge).

The apparent lack of somatotopy and frequency specificity in external nucleus cells may reflect an economical collapsing of sensory information--orthogonal to frequency, only roughly correlated with somatotopy--onto a map of personal space which makes most sense from the motor perspective. Thus, in turning to orient to an event behind him, an animal's initial trunk and neck movements require only crude information about the locus of the stimulus. Final orientation, in frontal body space, requires increasingly more precise sensory information and refined movements. NSF#BNS7825744

25.23 EVIDENCE FOR TWO TYPES OF THALAMOCORTICAL RELAY CELLS IN THE VENTRAL POSTERIOR LATERAL NUCLEUS (VPL) OF THE OPOSSUM (DIDELPHIS VIRGIANA). John C. Pearson* and Maria Regina Coccia* (SPON: H. Davis). Dept. of Anatomy, Wright State University, School of Medicine, Dayton, OH 45435.

Golgi analyses of the lateral geniculate nucleus (LGN) in rat, cat, and primate have described two kinds of geniculocortical relay cells (classes 1 and 2) distinguished primarily by the presence or absence of appendage clusters located at primary dendritic branch points. Primary branch points of class 1 cells are free of appendages; whereas, those of class 2 cells contain clusters of small excresences. EM and physiological studies indicate that the synaptology associated with the clusters of appendages may provide for a functional difference between these two relay cell types in cat LGN. Golgi analyses of the ventral posterior lateral nucleus (VPL) are mostly limited to rat, cat, mouse and rabbit, and describe only one class of thalamocortical relay neuron (the bush cell) which contains neurons similar in morphology to class 1 cells in LGN. However, a recent study of VPL in a prosimian primate (Galago) described two types of relay cells which are similar to the two classes described in LGN. In view of the functional importance recently associated with class 2 cell appendage clusters and evidence that relay cells containing these clusters are present in VPL of a primate, a reinvestigation of cell types in VPL of non-primate mammals was initiated. In the present study, the zinc chromate and Kopsch modifications of the Golgi technique were utilized to study the morphology of cells in the VPL of five adult opossoms. Two types of relay neurons are described (type 1 and type 2 cells). Type 1 neurons have multiangular somata and primary dendritic branch points which are free of appendage clusters of any kind; whereas, type 2 neurons have more rounded cell bodies and many appendages of various shapes and sizes located at or near the point at which the primary dendrites branch in a tufted fashion. The distal dendrites of both cell types contain numerous, small appendages which are either "bump-like" in nature, contain small spherical terminals, or appear as inverted drumsticks.

26.1 CHOLINERGIC SLOW POTENTIALS INFLUENCE PARASYMPATHETIC GANGLIONIC TRANSMISSION. William H. Griffith, III, Joel P. Gallagher and Patricia Shinnick-Gallagher. Dept. of Pharmacol. and Toxicol., Univ. of Texas Med. Br., Galveston, TX 77550.

We have previously reported the existence of slow synaptic potentials in mammalian parasympathetic neurons. These potentials occur after nicotinic receptor blockade (hexamethonium, $5x10^{-4}$M) and repetitive preganglionic nerve stimulation (30-40 Hz for 1 sec). We now demonstrate how these slow potentials can alter ganglionic transmission.

Cat vesical pelvic ganglia (VPG) and conventional intracellular techniques were used for in vitro recording.

The nerve evoked slow potentials consisted of only a slow inhibitory post synaptic potential (S-IPSP) in 48% of the cells (N=145). Both a S-IPSP and a slow excitatory post synaptic potential (S-EPSP) occurred in 31% of the neurons. Only a S-EPSP was recorded in 13% of the cells. No slow potentials were found in the remaining 8% of the cells. All of these slow potentials could be mimicked by either iontophoretic application of acetylcholine or superfusion of the ganglion with muscarinic agonists, bethanechol or methacholine. Iontophoretic responses occurred after synaptic transmission was blocked with a zero Ca^{++}/high Mg^{++} EGTA (1 mM) solution. All slow potentials were blocked with atropine (10^{-7}M).

Concentrations of muscarinic agonists that elicited membrane polarizations varied among cells. Most responses required between 1-100μM of agonist. The hyperpolarizing response was always seen at lower concentrations and always occurred before the depolarizing response.

Since the ionic mechanisms for the two responses appear to be different and the hyperpolarizing response is more sensitive to muscarinic agonists, a differential sensitivity may exist for the muscarinic receptors on these ganglion cells.

During the S-IPSP a decrease (15±2%, N=12) in membrane resistance was observed; whereas an increase (5±1%, N=9) in resistance occurred during the S-EPSP. The reversal potential for the S-IPSP, determined with double barrel electrodes, was 104.1±4.6 mV (N=6). This value is close to the equilibrium potential of potassium. During the increase in membrane conductance induced by the S-IPSP, ganglionic transmission was depressed in three ways: 1) spontaneous action potentials were inhibited, 2) direct action potentials were depressed, and 3) iontophoretically induced ACh potentials were depressed. Frequently, spikes were generated during the S-EPSP. These data suggest that in parasympathetic neurons slow synaptic potentials can alter ganglionic transmission. (Supported in part by USPHS Grant #NS 16228.)

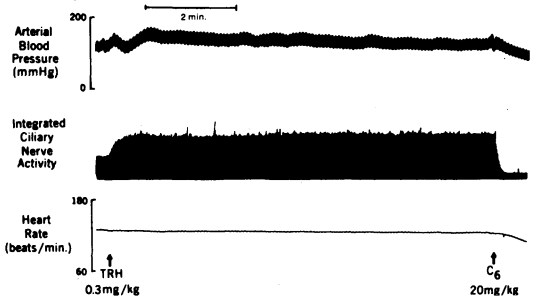
26.2 **THYROTROPIN-RELEASING HORMONE ACTIVATES CNS PARASYMPATHETIC OUTFLOW TO THE EYE.** Michael C. Koss. Dept. of Pharmacology, Univ. of Oklahoma Health Sciences Center and Dean A. McGee Eye Institute, Oklahoma City, Oklahoma 73190.

Thyrotropin-releasing hormone (TRH) is found in many central nervous system (CNS) regions other than the hypothalamus and exerts potent effects on the CNS which are independent of its release of thyrotropin and prolactin from the pituitary. TRH has been shown to act on the spinal cord to stimulate efferent motor activity to the skeletal musculature and to activate the extrinsic neural input to the gastrointestinal tract. The present series of experiments were undertaken in order to determine if TRH would produce a similar activation of the parasympathetic motor input to the eye. Toward this end, the effect of TRH on ciliary nerve activity was monitored in anesthetized cats.

Intravenous administration of TRH (0.03-5.0 mg/kg) caused a dose-dependent stimulation of tonic ciliary nerve activity which appeared to be independent of the excitatory light reflex. As shown by the example below, TRH produced a rapid increase in integrated nerve activity which was sustained for the period of observation. TRH (1 mg/kg) was administered in preparations in which the nerve fibers were crushed proximal to the ciliary ganglion. In these cases there was no activation of firing in the short ciliary nerve in response to TRH. TRH also produced a dose-dependent pressor effect which was not associated with a consistent alteration of heart rate.

These results demonstrate that TRH acts in the CNS to stimulate parasympathetic outflow to the eye, and suggest that it may play a neurotransmitter or neuromodulator role with regard to this autonomic system.

(Supported by USPHS Grant NS 14039)



26.3 A CENTRALLY MEDIATED MECHANISM FOR CONTROL OF OCULAR BLOOD FLOW. C. R. Auker, L. M. Parver* and D. O. Carpenter. Armed Forces Radiobiology Research Institute, Bethesda, MD 20014 and Georgetown University, Washington, D.C.

In a previous report (Soc. Neurosci. Abstr. 5:802, 1979) we showed that choroidal blood flow in the cynomolgus monkey eye serves an apparently homeostatic function in maintaining macular temperature at a relatively constant level.

We now report the existence of a centrally mediated reflex that elevates choroidal blood flow in response to light stimulation. We believe this is a protective mechanism that aids in removing excess heat generated by focusing of light on the macula.

Cynomolgus monkeys were anesthetized with Nembutal (30 mg/kg, i.v.) and placed in a stereotaxic head holder. A thermistor probe (YSI 524) was inserted through the pars plana and the probe tip lodged in the macular region. Intraocular pressure was maintained at a constant 18-20 mmHg by a lactated Ringer's reservoir connected to a needle inserted into the anterior chamber. In other experiments, scleral surface temperature was measured by a disc-shaped thermistor (YSI 409 or 427) inserted behind the eye through a slit in the conjunctiva.

When a 7.5-V light was shone into the contralateral eye, the macular and scleral temperature in the instrumented eye (which was shielded from the light) rose 0.10-0.17°C. Femoral arterial pressure and rectal temperature were constant throughout this maneuver. Closing the lid over the light-stimulated eye eliminated the response. Application of topical Proparacaine anesthetic to the cornea of the stimulated eye did not block the response.

These results suggest that blood flow was reflexly elevated in the instrumented eye when light was shone into the fellow eye. It is not yet clear whether the adequate stimulus is light or heat, but the receptors are apparently not located in the skin surrounding the eye nor in the cornea. The fact that blood pressure and rectal temperature were not changed suggests that this was not a generalized response to a noxious stimulus.

Additional work is under way in our lab to further characterize this reflex.

26.4 EFFECTS OF CHEMICAL SYMPATHECTOMY UPON THYMUS DEPENDENT IMMUNE RESPONSES. Nicholas R. Hall, John E. McClure*, Shu-Kuang Hu*, Nadine T. Tick*, Cary M. Seals*, and Allan L. Goldstein*. Department of Biochemistry, George Washington University School of Medicine, Washington, D.C. 20037.

Several immunologic parameters were measured in C57Bl/6J male mice following treatment with 100 mg/kg ip of 6-hydroxydopamine (6-OHDA). Twenty-four hours after drug treatment, a single ip injection of sheep red blood cells (SRBC) was given to the primary (1°) immune response subjects (n=8). Secondary (2°) immune response animals (n=8) received a second SRBC injection 21 days after the first sensitization. The immune system was evaluated 5 days after the final antigen injection. A single cell suspension of spleen cells was dispensed in triplicate onto agarose-coated slides for the plaque forming cell assay (PFC). An enzymatic assay was used to measure thymocyte levels of terminal deoxynucleotidyl transferase (TdT) and a radioimmunoassay was used to measure serum levels of thymosin α_1.

No significant differences between body, thymus and spleen weights were measured between the 6-OHDA versus the saline treated control group in either the 1° or 2° immune response. Antibody production as measured by PFC and hemagglutination assays was significantly reduced in the drug treated animals after 1° and 2° exposure to the SRBCs. The response to LPS was also reduced, but 6-OHDA increased the spleen cell response to Con-A, PHA-P and PWM. These increased measures were observed only in the 1° immune response group. Thymocyte TdT levels were significantly elevated by 6-OHDA treatment in the 1° response group but were not different from control values in the 2° response mice. Preliminary measurement of thymosin α_1 revealed a significant increase in levels of this peptide hormone in the serum of drug treated animals in both the 1° and 2° response groups.

These data suggest involvement of the autonomic nervous system in modulating the immune response to thymus dependent antigens. The possibility that the endocrine thymus performs an important function in a central nervous system-immune system axis is currently being investigated.

26.5 THE DEVELOPMENT AND CHARACTERIZATION OF AUTONOMIC INNERVATION OF THE THYMUS IN STAGGERER AND NORMAL MICE: AN INDICATION OF CNS REGULATION OF THE NEUROENDOCRINE-IMMUNE NETWORK. K. Bulloch and R. Loy. Dept. Neurosciences, UC San Diego, La Jolla, CA 92093

Recently the hypothesis has been put forth that many human as well as murine autosomal recessive diseases expressing multiple defects in the nervous, endocrine and immune systems have their etiology in a disruption of the neuroendocrine-immune network (Bulloch and Moore, 1980). It is now apparent that the functional integrity of the thymus gland during the perinatal period is crucial to the formation of this network. To determine if innervation is an integral part of the regulation of the neuroendocrine-immune network, we have undertaken a developmental study of autonomic nervous system mapping to the thymus of one such mutant, the staggerer and its normal counterpart. Parasympathetic innervation was studied using horseradish peroxidase (HRP) histochemistry with benzidine dihydrochloride as the chromagen. Acetylcholinesterase (AChE) staining was employed as an approximate determination of the localization of the parasympathetic terminals in the thymus. Sympathetic innervation was characterized utilizing a cryostat glyoxylic acid fluorescent histochemical technique for the demonstration of catecholamines. Our findings indicate that parasympathetic projections to the thymus are intact in the adult staggerer. There are no outstanding differences between the staggerer and normal animals in the topographical distribution of HRP-labeled cells in either the nucleus ambiguus or cervical spinal cord. Terminal distribution or acetylcholinesterase activity may be aberrant in the staggerer's thymus. Normal animals displayed light, consistent staining around medullary areas and little to no staining in the cortex and capsule. Staggerer, on the other hand, demonstrated very heavy AChE activity in the capsule and cortex and irregular, sporadic staining around the medulla. Sympathetic innervation also appears to be altered in staggerer mice. Catecholamine fibers did not follow the normal developmental or topographical patterns of normal mice. Some fibers were observed associated with the vasculature and capsule, but generally demonstrated erratic patterns in their cortical and medullary distribution. In addition, a delicate system of cortical thymic cells normally associated with catecholamine fibers were found irregular in both their organization and autofluorescent granule content. In view of the crucial role the thymus plays in the development of the neuroendocrine-immune network, such irregularities in thymic cell populations and innervation patterns may well be responsible in part for the phenotypes expressed in the staggerer mouse.

26.6 RECEPTIVE RELAXATION AND INTRAGASTRIC PRESSURE IN THE INTACT RAT AND IN THE ISOLATED STOMACH. C. Takata*, W.G. Young and J.A. Deutsch*. (SPON: E. R. Marchand) Depts. of Psychology and Neurosciences, UC San Diego, La Jolla, CA 92093.

3 rats, Sprague-Dawley males (400-500 g), were infused with a liquid diet (Carnation Evaporated Milk, diluted 1:1 with water) at a rate of 2 ml/min into the lumen of their stomachs via an indwelling catheter (Dow Corning Silastic tubing #602-305)(Deutsch, J. A. and H. S. Koopsman. Science, 179:1242, 1973). Egress from the stomach was prevented with an inflatable cuff that occluded the pylorus (Deutsch, J. A. Prog. in Neurobiol. 10:135, 1978). A total of 20 ml was infused. The intragastric pressure (GP) rose linearly from the outset of infusion to 27 cm $\pm$ 4 cm (SEM) of water pressure. This is normally not the case with rats voluntarily drinking. 4 rats tested drank an average of 9.4 ml $\pm$ 0.9 ml (SEM) and GP rose only to 4.3 cm $\pm$ 2.3 cm (SEM) of water at the end of intake. However, pentobarbital-anesthetized rats (n = 2) displayed linear rises in GP to 52 cm $\pm$ 2 cm (SEM) of water. GP measured in isolated stomachs (n = 4) rose only to 12 cm $\pm$ 8 cm (SEM) of water pressure.

It appears that in the intact rat, receptive relaxation (RR) is necessary to cause gastric muscular relaxation so that increasing gastric volumes may be accomodated without substantial rises in GP. Such is the case in rats indulging in deglutition. Rats, both awake and anesthetized, that are not indulging in deglutition demonstrate very high GP. There may be an intrinsic reflex present within the gastric musculature allowing for accomodation. Since RR is dependent on external vagal innervation (Martinson, J. Acta. Physiol. Scand. 64:453, 1965;(Jansson, G. Acta. Physiol. Scand. 75:245, 1969), it appears that the nature of such signals may be inhibitory (i.e., vagal inhibition of an intrinsic accomodation reflex) when the rat is not swallowing. However, when it is swallowing, these vagal inhibitory signals are lifted and this permits gastric relaxation (i.e., normal drinking pressures).

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26.7 AN ELECTROPHYSIOLOGICAL STUDY OF THE MICTURITION REFLEX PATHWAY IN NEONATAL KITTENS. G. Mawe* and W. C. de Groat, Dept. of Pharmacology, Medical School, Univ. of Pittsburgh, Pittsburgh, PA 15261

Previous studies showed that micturition in neonatal kittens could be induced by tactile stimulation of the perigenital region. This somatovesical reflex which is mediated by a spinal pathway disappeared in most animals by the age of 7-8 weeks but reappeared in chronic spinal adult cats and older kittens 3-12 days after transection of the spinal cord. The present study was undertaken to determine whether micturition reflexes induced by stimulation of bladder afferents undergo similar changes during development and after spinal injury.

Experiments were performed on kittens 5-67 days of age anesthetized with chloralose. Reflexes were recorded from postganglionic fibers on the surface of the bladder in response to distension of the bladder or electrical stimulation of afferents in the pelvic nerve. Bladder activity was recorded via a urethral cannula. In kittens 5-24 days of age reflexes occurred at very long latencies (mean 360 msec; range, 325-430 msec) in comparison to reflex latencies in older kittens (28-34 days, 150-275 msec; 56-67 days, 125-150 msec) and adult cats (95-140 msec). Recordings of axonal volleys on dorsal and ventral roots indicated that the peripheral afferent and efferent limbs of the reflex pathway in 5-24 day kittens were composed of slow conducting fibers (0.6-2 m/sec). The estimated central delay for the reflex ranged from 225 to 325 msec. Transection of the cervical spinal cord initially abolished spontaneous bladder contractions and pelvic nerve evoked reflexes but did not block responses evoked by perigenital stimulation. However, in chronic spinal kittens (8-21 days of age) examined 3 to 14 days after spinal transection, spontaneous bladder contractions had returned and pelvic nerve-evoked reflexes occurred with a latency (mean 135 msec; range, 90-200 msec) that was considerably shorter than the latency in intact animals.

We conclude that in neonatal kittens micturition induced by stimulation of bladder afferents is normally mediated by a spinobulbospinal pathway (SBS), whereas micturition induced by perigenital stimulation is mediated by spinal reflex mechanisms. The decrease in the latency of the SBS reflex during postnatal development is attributed to progressive myelinization of the peripheral and central axons. The maturation of the SBS pathway seems to correlate with the disappearance of the perigenital-bladder reflex. Recovery of bladder function in chronic spinal neonatal animals is associated with the emergence of a bladder to bladder spinal reflex that was undetectable in kittens with an intact neuraxis. (Supported by NSF Grant 79-06093).

26.8 REFLEX MICTURITION INDUCED IN CHRONIC SPINAL DOGS WITH "VOLUME CONDUCTION" STIMULATION OF SACRAL NERVE ROOTS. Pei Chin Tang. Chicago Medical School, Chicago, Il. 60612.

As reported previously, the "volume conduction" method consists of inserting 3 platinum electrodes near the sacral nerve roots through holes drilled in the sacrum. Stimulation via these electrodes with 60 Hz AC resulted in vigorous contraction of the bladder with pressures ranging from 50 to 100 mmHg in anesthetized spinal dogs. By studying the distribution of fatty tissues and locations of the sacral roots, we designed a 6-pronged stimulating electrode with which we routinely generated bladder pressures of 80 mmHg or higher. As the method stimulates all sacral roots, it caused contraction of bladder as well as its sphincter. In chronic spinal dogs we applied alternate 1/2 sec. on and 3½ sec. off stimulation to induce post-stimulation micturition. Urine flow occurred only after cessation of stimulation when the skeletal muscles of the external sphincter had relaxed while the smooth muscles of the bladder were still contracting. We were able to maintain spinal dogs, both male and female, in healthy condition by emptying their bladders twice daily with this method. Here the 6-pronged electrode was also superior to the 3-pronged one. Immediately following the spinal transection, each stimulation induced a small amount (around 10 ml.) of urine output, presumably due to stimulation of the bladder efferent fibers in the sacral roots. Soon afterward, the output following each stimulation became larger, sometimes over 100 ml. Duration of urine flow often lasted over 10 sec. This prolonged urine flow could be due to reflex micturition induced by simultaneous stimulation of the bladder afferents and efferents. Thus, the "volume conduction" method can induce brief micturition during spinal shock by stimulating the bladder efferents and reflex micturition after the shock by stimulating both bladder afferents and efferents in the sacral roots.

In several dogs, the soldered joint between the lead wire and the electrode was broken within the electrode holder which was anchored to the sacrum. We had no difficulty in replacing the entire electrode assembly under local anesthesia as the holder was on the surface of the sacrum and the solid mass of fibrous tissue encapsulating the holder could be cut off without touching the contents of the sacral canal. Re-inserting the electrodes into the existing holes in the sacrum and re-anchoring the holder could be done in a few minutes. We have replaced the electrode assembly in 5 dogs and every one was successful. The ease of electrode replacement extends further the longevity of this method. (Supported by NINCDS Grant #NS15353-02).

26.9 LESIONS OF THE SUBSTANTIA NIGRA PRODUCE ALTERATIONS OF PANCREATIC ISLET SIZE. B.J. Davis*, R.J. Carey, J.R. Sladek, Jr. and P.H. Smith* (SPON: N.R. West). Dept. of Anatomy, SUNY Upstate Med. Ctr Dept. of Psychiatry, Vet. Admin. Hosp., Syracuse, NY; and Dept. of Anatomy, Univ. of Rochester School of Medicine, Rochester, NY. Lesions of the ventrolateral hypothalamus (VLH) have been shown to reduce food intake (FI) and body weight (BWT) and also to alter pancreatic islet function (Smith, et al. 1979). Recently, it has been reported that lesions of the substantia nigra (SN) produce a syndrome of aphagia and adipsia similar to that observed following VLH lesions. Thus it seems likely that interruption of dopaminergic fibers from the SN that project through the hypothalamus in the medial forebrain bundle may contribute to the reductions of FI and BWT that follow VLH lesions. The focus of the present investigation was to determine whether alterations of the endocrine pancreas occur after lesions of the SN. Adult male rats were given bilateral injections of 6-hydroxydopamine (3.0µl in 1.5µl ascorbic acid soln.) into the SN following pretreatment with des-methylimipramine (25mg/kg). Sham and unoperated rats served as controls. After lesions, rats were given high fat diets and FI and BWT were recorded throughout the experiment. Ten weeks after lesion placement animals were killed by decapitation. Lesion sites were verified using Falck-Hillarp fluorescence histochemistry. Samples of the head, body and tail of the pancreas from each animal were fixed in Bouin's fluid, dehydrated and embedded in a single paraffin block. Serial sections (4µm) were processed for the immunocytochemical localization of insulin-, glucagon-, and somatostatin-containing cells. Islet sizes were measured by a point-counting technique and size-class distributions were constructed as follows: small=625-7500µm^2; medium=7625-17500µm^2; large=18125-37100µm^2. The results of this study show that when the endocrine pancreas of SN lesioned rats is compared to that of weight-matched controls there is a significant reduction of total islet area. Moreover, in the pancreas of lesioned rats, there is a shift of islet size distribution with a marked increase of total islet area represented by the smaller islets, and a reduction of total area contributed by the larger islets.

	Islet Area	% of Total Area		
		Small	Medium	Large
Controls (n=3)	$10.7 \times 10^5 \mu m^2$	18	26	56
Lesioned (n=3)	$6.9 \times 10^5 \mu m^2$*	36	27	37

*p<0.001

These findings indicate that alterations of endocrine pancreatic function known to occur following VLH lesions may be related to destruction of dopaminergic fibers originating within the SN. (Supported in part by NIH Grant AM-25325.)

26.10 EFFECTS OF LESIONS IN LOWER BRAIN STEM AND CEREBELLAR VERMIS ON MOTION SICKNESS-INDUCED EMESIS IN THE SQUIRREL MONKEY. K. R. Brizzee, J. M. Ordy and W. R. Mehler, Delta Regional Primate Research Center, Covington, LA 70433 and NASA-Ames Research Center, Moffett Field, CA 94035.

Twenty-one young adult female squirrel monkeys of the Bolivian subspecies were subjected to continuous, counter-clockwise horizontal rotary motion at 25 rpm, together with a sinusoidal vertical excursion of 6 inches every 2 seconds (0.5 Hz). Each animal was exposed to this motion regimen for a period of 60 minutes once each week for 3 consecutive weeks, and each exhibited from 1 to 5 emetic responses within the test period. Following the third weekly motion test bilateral electrolytic lesions were made in the lower portion of the nucleus of the tractus solitarius (NTS) in 3 animals, and in the nodule and uvula of the cerebellar vermis in 4 animals. In 8 animals bilateral ablation of area postrema (AP) was performed by thermal cautery. Three control animals were sham-operated after the third motion test while 3 additional controls were given the motion tests as noted above but were not operated. After a recovery period of 30 to 40 days in all operated animals and at a comparable interval in the nonoperated controls, each animal was again tested for motion sensitivity for 3 consecutive weeks. The brains of all of the animals were then fixed by left ventricular cardiac perfusion with Bouin's fluid and processed for histological evaluation of the lesions. One of the animals with NTS lesions was refractory to the motion test postoperatively while two exhibited about the same number of emetic responses postoperatively as preoperatively. One of the animals with cerebellar lesions failed to respond to the motion regimen while two others exhibited about the same number of emetic responses postoperatively as preoperatively. The fourth animal in the cerebellar group exhibited two emetic responses on the first weekly postoperative test as compared with 4 responses preoperatively. On the subsequent weekly postoperative tests, however, no further emetic responses were observed. Five of the AP-ablated animals were completely refractory to the motion regimen postoperatively, 2 exhibited a decreased number of emetic responses, and one exhibited the same number of responses before and after the AP lesions. The AP-operated animals which exhibited emetic responses to motion in the postoperative tests were found to have an appreciable amount of AP tissue remaining while those which were refractory had little or no AP tissue remaining. The controls exhibited no significant difference in emetic sensitivity on the second series of three weekly tests than on the first series. (Supported by NASA-Ames Grant NSG-2139 and NIH Grant RR00164-18.)

26.11 THE EFFECT OF PASSIVE LIMB MOVEMENT ON HYPOTHALAMIC-EVOKED THERMOREGULATORY RESPONSES IN RABBITS. I.M. Scott,* M.M. Toner* and J.A. Boulant. Department of Physiology, Ohio State Univ. Sch. of Med., Columbus, Ohio 43210.

A controversy exists over the role of proprioceptive limb afferents that may modify the hypothalamic control of thermoregulation during exercise. While some studies indicate no "exercise factor"; other studies suggest that exercise may lower the hypothalamic set-point temperature for heat-loss responses. This apparent set-point lowering was observed for salivation in the exercising dog (Sharp and Hammel, 1972) and for sweating in exercising man (Tam, Darling, Cheh and Downey, 1978). Two important questions still remain:

1) Since body temperature rises during exercise, is this modification of hypothalamic responses due to: a) extrahypothalamic, deep-body thermoreceptor afferents or b) proprioceptive afferents from the moving limbs? The proprioceptive role was tested in the present study by comparing hypothalamic-evoked thermoregulatory responses in the rabbit during rest and during passive limb movement (which did not increase core temperature).
2) In addition to heat-loss responses, does passive movement modify the hypothalamic control of heat-retention and heat-production responses? This was tested in the present study by determining the effect of limb movement on the hypothalamic control of panting or breathing frequency (Bf); relative skin blood flow (SBF)(or ear thermal conductance); and metabolic heat production (MR) due primarily to shivering.

In unanesthetized rabbits, preoptic-anterior hypothalamic temperature (T_{po}) was manipulated and measured using previously-implanted hypothalamic thermodes. The rabbits were supported in a sling so that their legs could be attached to movable rails. A motor connected to the rails produced the passive limb movements. Characteristically, decreasing T_{po} produced a proportional increase in MR, while increasing T_{po} produced proportional increases in SBF and Bf. During passive limb movement, MR was significantly reduced at T_{po}'s below 35°C; while Bf was higher at T_{po}'s between 37° and 39°C. Also the hypothalamic thermosensitivity for both responses was reduced. Finally, during movement, SBF was significantly increased at T_{po}'s above 40°C. The results indicate that proprioceptive afferents may reduce the hypothalamic thermoregulatory set-point by decreasing heat-production and increasing heat-loss responses during changes in T_{po}.

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26.12 FEVER IN THE DEVELOPING KITTEN. Ch.E. Olmstead and J.R. Villablanca. Depts. of Psychiat. and Anat. and the Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

In normal kittens thermoregulation as measured by the ability to maintain an adult rectal temperature (T_B) of 38.2±2°C, either in a normal ambient (T_A) of 23 to 25°C or in response to hot or cold challenges, develops over the first 45 days of life (Physiol. Behav. 23: 489, 1979). As part of our continuing investigation of the behavioral and physiological development of normal and brain-damaged kittens we now report the ontogeny of the febrile response to intravenous injections of an exogenous pyrogen, salmonella typhosa, in intact kittens (107 observations in 63 kittens from 16 litters).

The results are shown in Table I where a Friedman 2-way ANOVA showed significant group effects (X^2=121.8, df = 7, p< .001).

TABLE I

Age	N	Pre T_B°C	Peak ΔT_B°C	Hrs to Peak
0-5	12	37.6	.5	2.0
11-15	11	37.5	.6	1.5
21-30	14	37.5	.5	2.0
31-40	10	37.7	.6	0.5
41-50	7	38.3	.8	2.5
50-90	9	38.6	.9	1.0
Adults	5	38.6	1.4 - 1.5	2.0 - 4.0

In general there was an increase in both the magnitude and the duration of fever as a function of age, with the greatest change occurring between 41 and 50 days of age. There was an obvious relationship between the ability of the kitten to maintain its T_B at an ambient of 23-25°C as indicated by the baseline T_B and the magnitude of the fever. The dose necessary to elicit a fever in the very young kitten (0-20 days of age) was considerably higher (5.0-7.0 ml/kg standard dose) than that in the adult (0.5-1.0 ml/kg standard dose). Normal adult cats show a distinctive, bimodal response to the IV injection of exogenous pyrogen (Am. J. Physiol. 208:703, 1965) which, in the present study, was characterized by peak fevers of 1.4°C and 1.5°C at 2.0 and 4.0 hours, respectively, post-injection. No such bimodal response was seen in any of the kitten groups. Even in the 50-90 day animals the fever was transient and there was rapid return to the baseline. In summary, the febrile response to exogenous pyrogen develops over at least the first 7 weeks of life. The data will be discussed in relation to other physical parameters of development, to the animals behavior, and to the immunological status of the mother. (Supported by USPHS HD 05958 and HD 94612).

26.13 THERMOREGULATORY RESPONSES OF CHRONIC DECEREBRATE KITTENS. J.R. Villablanca and Ch.E. Olmstead. Mental Retardation Research Center and Depts. of Psychiat. and Anat., UCLA, Los Angeles CA 90024

We report here preliminary results showing that kittens (N=4) transected above the midcollicular level in the first month of life and followed chronically (survival 13-135 days) do not develop normal thermoregulation. Due to this impairment they must be maintained in an incubator. However, their body weight develops in parallel with that of intact littermates until about 90 days of age.

In intact kittens the ability to regulate rectal temperature (T_B) matures over the first 45 days of life (Physiol. Behav. 23: 489, 1979). This maturational sequence goes from a mean T_B of 37.5°C at 5 days of age to adult levels (38.2±2°C) at about 7 weeks of age. Up until 14 days of age the predominant response to being removed from the nesting box to an ambient temperature (T_A) of 23 to 25°C is a decrease of the T_B of about .02°C/min. For the chronically decerebrate kittens removal from the incubator to a similar T_A resulted in a drop of 0.07-0.10°C/min.

In response to stronger thermal challenges (T_A's of -15°C or +60°C) all of the intact kittens showed, from the first day of life, the ability to detect and move along a thermal gradient as reflected in the approach to warmth and the avoidance of coolth. Nothing resembling behavioral thermoregulation has been seen in the decerebrate. At -15°C normal kittens showed a decrease in the T_B of about .2°C/min at 10 days decreasing to about .1°/min at 40 days of age accompanied by shivering and piloerection which appeared immediately with cold onset. Although there appeared to be some non-specific motor activity, we saw no shivering or piloerection in the decerebrate kitten and it has been possible to regularly and easily decrease the T_B to 30°C at a rate of about .2°/min, regardless of age. In response to heating the intact kitten shows an increase of 0.05°/min at 10 days and 0.02°/min at 40 days of age. Panting was of very short latency as were other behavioral responses. In no case has it been possible to elevate the temperature of an intact kitten above 42°C in the T_A of +60°C. In contrast, the increase in the T_B of the decerebrate animal ranges from 0.08 to 0.20°/min and extreme care was necessary to avoid elevating the temperature into the lethal zone above 42°C, a condition which resulted in the death of our 13 day animal. We have not seen decerebrate kittens pant while being heated.

Contrary to the report (Exp. Neurol. 42: 519, 1974) that kittens decerebrated above the midcollicular level develop near normal thermoregulation, the present data demonstrate that body temperature regulation, which in intact kittens develops over the first seven weeks of life, is dramatically and permanently disrupted by an early high decerebration. (Supported by USPHS HD 05958 and HD 094612.

26.14 MEDULLARY THERMOSENSITIVE UNITS--THE EFFECT OF Δ^9-TETRAHYDROCANNABINAL. W.T. Schmeling* and M.J. Hosko (SPON: A.S. Bloom). Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

Recent studies have demonstrated that many sites in the CNS may play a role in thermoregulation. Previous evidence from our laboratory has demonstrated that thermosensitive neurons (TS) in both the hypothalamic preoptic region (POR) and mesencephalic areas may function in mediating the degree of hypothermia produced by administration of the primary psychoactive component of marihuana, Δ^9-tetrahydrocannabinol (THC). This laboratory has also demonstrated a functional role for the spinal cord (SC) in THC induced hypothermia. In order to further identify those CNS sites which play a role in the modulation of THC induced hypothermia, as well as to investigate the physiological role of such thermoregulatory sites, medullary (MED) TS neurons were studied using extracellular electrodes. Cats were anesthetized with urethane. Double-wall stainless steel thermodes were implanted in POR and MED. Epidural flexible thermodes were inserted into the spinal canal to provide SC thermal challenge. Thermocouples or thermistors were utilized to monitor POR, SC, MED and deep rectal temperature. Tungsten microelectrodes were used to isolate and record single unit activity. A high percentage (70%) of the units we have isolated in the medulla exhibited some TS to local thermal challenge. Of the medullary units exhibiting some TS, 47% demonstrated thermosensitive convergence to heating and/or cooling of SC and/or POR. The firing rate response to such convergent driving varied from neuron to neuron. Selected, stable TS units were challenged by systemic administration of small doses (0.5-2.0 mg/kg) of THC. Typically, THC decreased the spontaneous firing rate while the TS was differentially altered. Non-TS neurons (controls), exhibited little or no change in firing patterns after THC. These studies document the presence of TS neurons in the medulla. Many of these TS units receive convergent information from other known TS sites. The action of THC on these neurons may contribute to the hypothermic action of this agent. (Supported by PHS Grant #DA-00124).

26.15 SYMPATHETIC RHYTHMS IN SPINAL CATS. P.M. Gootman and M.I. Cohen. Depts. of Physiol. Downstate Med. Ctr., Bklyn., 11203 and Albert Einstein Col. Med., Bx., N.Y., 10461.

Simultaneous recordings from preganglionic sympathetic nerves at different levels, cervical sympathetic (CS) and greater splanchnic (SPL), reveal the presence of common periodicities as shown in crosscorrelation histograms (CCH). The major types of periodicities were cardiac, respiratory and 10/sec (Cohen & Gootman, Am. J. Physiol. 218:1092, 1970). The CCHs revealed that peak SPL activity lagged peak CS activity.

These common periodicities can be explained in two ways: (1) there are common periodic inputs to the two types of preganglionic neurons; (2) there are feedback connections in the spinal cord between the two groups of neurons. To distinguish between these two possibilities, spinal cord transections (at C-1) were performed on decerebrate cats with neuromuscular blockade (gallamine or decamethonium), vagotomy, pneumothorax, and artificial ventilation with 100% O_2. SPL and left and/or right CS discharge were monophasically recorded. The central respiratory cycle was monitored by recording phrenic (PHR) discharge. The EKG and systemic arterial pressure were also simultaneously recorded.

After spinal cord section, recordings were taken at hourly intervals for up to 12 hours. Throughout this period activity gradually increased but still remained small compared to presection levels. This low level activity showed no sign of periodicity. Asphyxia of sufficient duration produced increased activity in sympathetic nerves. SPL activity during asphyxia had 2-3/sec oscillations; but the CCHs of CS and SPL activity were almost flat.

Strychnine stimulated neurons in the spinal cord more effectively than asphyxia or doxapram: it produced markedly increased activity with bursting patterns in CS, SPL and PHR. Power spectra of the recordings showed a dispersed range of frequencies (2-10 Hz) without markedly distinct peaks such as were present in the intact animal. The CCHs showed locking of activity in PHR, CS and SPL nerves on a slow time scale (1-5 sec) but no appreciable locking of CS and SPL activity on a faster time scale (2-10 Hz) such as occurs in the intact animal.

These results indicate that, while there can be oscillation of activity at the spinal cord level, the normally occurring synchrony of sympathetic discharges at different segmental levels is dependent on inputs from the brain stem. (Supported by NIH Grants HL-20864 and HL-20800.)

26.16 PREDICTABILITY: A PSYCHOLOGIC/BEHAVIORAL VARIABLE AFFECTING STRESS-INDUCED MYOCARDIAL PATHOLOGY IN THE RAT. W. H. Bailey, D. R. Alonso*, J. M. Weiss*, and S. Chin*. Rockefeller Univ. and Dept. of Pathology, Cornell Univ. Med. Coll.,New York, NY 10021.

In studies with humans there is increasing evidence that psychological and behavioral aspects of stressful situations affect the functioning of the heart and the likelihood of later cardiovascular disease. The evaluation of such factors and their physiological consequences in animals is difficult, however, because of the confounding of psychological factors with potent physical characteristics of stressor stimuli. To avoid this problem, we exposed rats in pairs to the same stressor (3.5 mA inescapable shocks of 2 sec duration on a VI 1 min schedule through tail electrodes wired in series in which one of the rats received a tone 4 sec before each shock (predictable shock condition) . The other rat received a tone bearing no relationship to the shock (unpredictable shock). Since exactly the same physical stimuli (shock, tones) were given to both rats, differences between these two animals would have to relate to their ability to predict the occurrence of shock. To assess the effect of the shock stressor as a separate variable, the experimental design also included a third animal which was presented tones but not shock. Hearts were removed from 23 such triplets 48 hr after a single 24-hr stress session, and multiple sections at four different levels were prepared and stained with hematoxylin-eosin. Focal areas of myocardial necrosis were identified by the degeneration of myocardial cells and their replacement by leucocytes, macrophages, and fibroblasts in a majority of rats given unpredictable shock. Significantly fewer such lesions were found in the hearts of rats given predictable shocks. Indeed, the incidence of myocardial damage observed in rats given predictable shock was hardly greater than that found in a few control rats. Such a result indicates that a psychological variable, predictability, can almost completely counteract the adverse effects of stressful physical stimulation per se. The size and prominent distribution of these focal lesions in subendocardial regions is consistent with the hypothesis that the release of endogenous catecholamines may be directly or indirectly responsible. Histochemical procedures based on fuchsinophilia or fuchsinorrhagia were found to be completely unreliable indicators of stress-induced myocardial pathology in rats. (Supported by NIH grant HL 19974.)

26.17 CARDIOVASCULAR AND CATECHOLAMINE RESPONSES IN INDIVIDUALS DIFFERING IN REACTIVITY. R.H. Cox,* J.E. Lawler,* K.A. Lawler,* and E.T. Howley* (Spon: K. Kant). Depts. of Psychology and Physical Education, Univ. of Tenn., Knoxville, TN 37916.

The difficulty encountered in attempts to induce a permanent hypertension in animals with psychologically stressful paradigms has contributed to doubts about the importance of this factor among some investigators. However, recent evidence suggests that selective breeding can produce an animal with a predisposition to develop hypertension. This fact has contributed to a renewed interest in individual differences which might produce a vulnerability to hypertension. A cardiovascular (CV) system which is reactive to stress may be one manifestation of such a vulnerability. However, for such a characteristic to have significance it must be demonstrable in a variety of situations. This study was undertaken in an effort to demonstrate the generality of CV reactivity and to define its relationship to the sympathetic nervous system and to obtain basic information about the CV and oxygen uptake capacities of individuals who differ in reactivity to stress.

Thirty-four male undergraduate volunteers were exposed to three forms of psychological challenge: 1) a reaction time-shock avoidance (RT-AV); 2) a reaction time-money reward (appetitive or RT-AP); and 3) a mental arithmetic stress (MA). Heart rate, systolic and diastolic blood pressure and urinary excretion of epinephrine and norepinephrine were measured. The primary focus of the study was on those 14 individuals showing extremes in CV reactivity. Eight subjects showing a heart rate increase of over 30 bpm were compared to six subjects who responded with less than 10 bpm increase to the RT-AV task. The reactive group displayed a greater change in heart rate to the RT-AP task but not the MA condition. Their systolic blood pressures, while no different at rest, were higher during the RT-AV, RT-AP and MA conditions. No difference in urinary excretion of catecholamines between the two groups was detected but significant positive correlations between baseline epinephrine values and resting heart rate and mean heart rate change in all psychological conditions were found. A significant relationship between CV reactivity and familial hypertension was also found.

The reactive and non-reactive groups did not differ in aerobic capacity or maximum heart rate and both met the metabolic demands of exercise with similar rises in CV parameters. There was evidence however that as a group the reactive subjects show higher heart rates and systolic blood pressures than the non-reactive group to the same relative and absolute workloads.

26.18 AUTONOMIC RESPONSES AS MEASURES OF LEARNING IN PREWEANLING RATS. Louise T. Martin and Jeffrey R. Alberts*, Dept. Psychology, Indiana Univ., Bloomington IN 47405

The purpose of the present study was to investigate ontogenetic differences in the effectiveness of various autonomic and behavioral response systems as measures of learning. Preweanling rat pups were trained in 1 of 2 classical conditioning paradigms: odor-illness and odor-temperature conditioning. During a 24-hr retention test, both autonomic (heart rate, respiration) and behavioral (ultrasounds, gross motor activity) responses were simultaneously monitored at 4 different ages (5, 10, 15, and 20 days). Pups were restrained in a wire mesh cage, which was placed in a wind tunnel that received the effluent airstream of an olfactometer. Test trials consisted of 30-sec presentations of the conditioned odor and a novel odor; responses were monitored for 1 min prior to and following each odor presentation.

Only the cardiac measure proved to be a sensitive index of learned associations throughout the preweanling period. However, the direction of the cardiac response appears to undergo a major developmental shift around Day 20. At 5, 10, or 15 days of age, aversive associations lead to large and sustained accelerations in heart rate; by Day 20 the cardiac response is primarily deceleration. Younger Ss that have acquired specific olfactory aversions decelerate in response to novel odors, whereas 20-day-olds show cardiac acceleration. Unpaired aversive experiences are also reflected in the cardiac response patterns. Younger Ss show acceleration following offset of a novel odor and sustained deceleration to a familiar-safe odor. Again, by Day 20 these directional differences in heart rate are reversed.

These results demonstrate that in a restrained testing situation, cardiac responses can provide an effective measure of learning at young ages. Changes in heart rate do not appear to be coupled with changes in respiratory rate or motor activity until around Day 20, the age at which tonic parasympathetic restraint on the heart becomes established. We have interpreted these distinctive cardiac response patterns as orienting and defensive reflexes to sensory stimulation. The observed shift in the organization of these responses at Day 20 may correspond to a shift in the organization of behavior -- from stimulus-bound, reflexive responses to behaviors that are more instrumental and voluntary in nature.

We are currently monitoring these same response systems during a spatial orientation test. Under this less restrained condition we hope to determine the age at which behavioral measures surpass a cardiac measure as indices of learning in preweanling rat pups.

26.19 INTERACTION BETWEEN BRAIN DEGENERATION AND PSYCHOSOCIAL STRESS. J.N. Naranjo*. (SPON: C.A. Marotta). Department of Anatomy, Harvard Medical School, Boston, MA 02115 and McLean Hospital, Belmont, MA 02178.

A considerable body of data indicates that mice that are psychosocially stressed in free wheeler and Reimer-Petras colonies consistently exhibit a chronic elevation in blood pressure. The present study examines the effects of psychosocial stress on the central nervous system. The C57/USC mice that were placed in the stress colonies had a significant increase in blood pressure ($p < .001$) in relation to the age matched controls. The brains of both groups were analyzed using a reduced silver stain (Naranjo and Greene, 1977) and electron microscopy. Using coded slides, the light microscopy was analyzed by four trained observers; a consensus indicated that there was a significant amount of widespread neural atrophy ($p < .004$) in all stressed animals and a virtual lack of degeneration in the same age controls.

An analysis of the tissue not only confirmed that there were differences in the amount of neuronal degeneration between the stress colonies (probably due to the level of stress in each colony) but also there were individual differences in the amount of degeneration among the mice in each stress colony. Axonal degeneration was observed in the optic tract, corpus callosum, fimbria, fornix, internal capsule, neocortex, hypothalamus and hippocampus. Neural damage in fiber tracts was noted as black coarse fragmented fibers, fiber debris, numerous small impregnated spheroidal or slightly irregular structures, argyrophilic material and a proliferation of oligodendroglia. Damage to cellular areas consisted of small circumscribed regions of retrograde dust, fragmented dendritic processes and silver impregnated soma. The silver staining results were confirmed by electron microscopy, and the ultrastructural changes in neurons and glia will be presented.

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26.20 NERVE GROWTH FACTOR-LIKE ACTIVITY IN SERUM SAMPLES FROM PATIENTS WITH PERIPHERAL AND DIABETIC NEUROPATHY. D. J. Wells* and B. L. Strehlo* (SPON: J. Krier). Section of Cardiovascular Research, Mayo Clinic and Foundation, Rochester, MN 55901.

Nerve growth factor-like proteins (nerve growth stimulating activity, NGSA) are likely to be involved in the normal and pathological development of the human sympathetic and sensory nervous systems. Development of a radioligand assay for measuring serum and tissue levels of NGSA has been hampered by the lack of proven cross-reactivity of antibodies developed against mouse nerve growth factor (NGF) with a purified sample of human protein and by the presence of NGF binding proteins in serum which compete for labeled NGF.

The well-established chick embryo dorsal root ganglion biological assay for NGSA has been utilized in this laboratory to measure the levels of NGF-like material in male and female subjects of varying age. Serum samples were obtained from laboratory normal value study subjects or from pediatric patients undergoing routine physical examinations. Samples were diluted with isotonic saline and incubated with 8 day-old chick embryo dorsal root ganglia at 37°C for 24 hours. NGF activity was determined by comparing the amount of neurite outgrowth obtained with the patient sample dilutions with the outgrowth produced by a series of dilutions of purified mouse NGF protein. Subjects ranging in age from 6 months to 75 years gave no significant sex or age difference (females: $N = 37$, $\bar{x} = 11.3 \pm 10.5$ B.U./ml; males: $N = 33$, $\bar{x} = 15.5 \pm 15.4$ B.U./ml).

Patients diagnosed with peripheral neuropathies did not show significant differences in levels of NGF-like activity from controls although individual exceptions were observed (females: $N = 9$, $\bar{x} = 18.8 \pm 22.3$ B.U./ml; males: $N = 6$, $\bar{x} = 9.5 \pm 9.7$ B.U./ml). Samples of cerebral spinal fluid from these patients showed no NGF-like activity. In contrast to the results obtained with the control subjects, serum samples from patients with either diabetes mellitus ($N = 6$) or diabetic neuropathy ($N = 6$) gave no detectable NGF-like activity. The amount of mouse NGF protein which gave maximal biological activity did not vary when the assay dilutions were done with isotonic saline or pooled serum samples, indicating that serum did not exert an inhibitory effect on the assay. Continued efforts will be made to assess the relevance of these results to development of diabetic and peripheral neuropathies.

All experiments reported here were approved by the Human Studies Committee, Mayo Clinic. This research was supported by the Mayo Foundation.

26.21 RESPONSES OF DENTAL-PHOBIC AND NON-DENTAL-PHOBIC CHILDREN TO DENTAL TREATMENT: COMPARISON OF FOUR AUTONOMIC MEASURES. G. A. West and K. H. Reid. Dept. Physiology & Biophysics, Health Sci. Ctr., Univ. of Louisville, Louisville, Ky. 40292.

Seventeen children, ages 6-12, were monitored during routine dental restorations in the Pedodontic Clinic at the U of L Dental School. Continuous recordings were made of red light transmission through the distal phalanx of the left little finger, and of the voltage developed between two 1 cm^2 electrodes placed on the palm and dorsum of the left hand during passage of a 8 microampere constant current between them. Pulse rate (PR) and finger pulse volume (FPV) values were derived from the light transmission signal, while absolute skin conductance (ASC) and skin conductance response (SCR) values were derived from the voltage signal. Both signals were recorded on chart paper for monitoring and on FM magnetic tape for later processing.

PR and FPV values were obtained by graphic averaging over 3 second epochs, followed by averaging these numberical values over the time devoted to each dental procedure. In 7 children PR and FPV records were incomplete due to movement artifact. SCR values were obtained by summing SC amplitude changes over the time of each dental procedure. ASC values were obtained by calculating a mean baseline value over the time devoted to each procedure. The separation of children into phobic and non-dental-phobic groups was done by asking mothers to fill out a questionnaire which indicated how they felt their children would react to dental treatment. This assessment was supplemented by observation of each child's reaction to the restoration procedure itself.

Each of the 4 autonomic measures was evaluated using analysis of variance, repeated measures, with procedures and subjects as main effects. The group of 4 measures was tested by discriminant analysis for ability to separate groups. Salient results were:

1. ASC best separated dental-phobic from non-dental-phobic children ($N=10$, 5/group, $p < .05$)
2. SCR most consistently separated dental procedures ($F=8.35$, $p < .01$)
3. PR showed the largest inter-subject variability.
4. Using SCR, PR, or FPV as measures, we found no significant difference between dental-phobic and non-dental-phobic children.
5. For all 4 measures, the largest effect was seen during the injection of the local anesthetic. We interpret this as indicating that this was the most stressful of the routine dental procedures.

27.1 [^{3}H]-2-DEOXYGLUCOSE UPTAKE IN THE MOLLUSCAN CENTRAL NERVOUS SYSTEM. Stephen C. Reingold & Terrence J. Sejnowski+. Department of Biology, Princeton University, Princeton, New Jersey 08544.

We have used [^{3}H]-2-Deoxyglucose ([^{3}H]-2-DG) to localize metabolically active neurons within the buccal ganglion of the mollusk Limax maximus. Autoradiographic analysis gives a resolution of 0.5 um, with label concentrated in cell somata and neuropil processes. Within somata, nuclei appear less heavily labelled than cytoplasm. Labelling of neurons may be correlated with intracellular stimulation and spontaneous activity within the ganglion.

Individual buccal ganglia were dissected and incubated in high Mg^{++}, low Ca^{++} saline to reduce transmission at chemical synapses. Peripheral nerve roots were recorded with suction electrodes. Glass microelectrodes were used to impale individual neurons within the ganglion. Upon impaling a neuron, the bath solution was replaced by 100-200 uCi [^{3}H]-2-DG/ml high Mg^{++} low Ca^{++} saline. The cell was depolarized with intracellular current pulses to fire at a rate of 1-2 spikes/second, while monitoring peripheral nervous system activity. After 30-60 minutes of intracellular stimulation, the microelectrode was removed and the ganglion washed in 5 changes of non-radioactive saline over 30 minutes. Individual ganglia were freeze-substituted in acetone at -70°C or dehydrated in dry acetone at 4°C. Ganglia were embedded in Araldite or Spurr's Medium and serially sectioned at 3-5 um. Sections on slides were dipped in Kodak Nuclear Emulsion (NTB-2), exposed for 2-4 weeks, and subsequently developed and counterstained.

In control experiments in which a neuron is impaled but not driven and peripheral activity is absent, several somata are often labelled. Rarely, a single cell was activated with current injection in the absence of spontaneous activity, and a single cell soma was labelled with [^{3}H]-2-DG. In most experiments, several cells were labelled, even though only a single cell was experimentally activated. Such multiple cell labelling may result from spontaneous firing of normal or autoactive neurons; from activation of follower cells by the impaled neuron through electrical or still-functioning chemical synapses; or from subthreshold activity or non-electrical metabolic activity.

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27.2 ORTHOPTERAN JUMPING MOTOR NEURONS: A COMPARISON. John A. Wilson, Christine E. Phillips, DeForest Mellon, Jr., and M. E. Adams*. Dept. of Biol., Univ. of Virginia, Charlottesville, VA 22901 and Max Planck Inst. V., Abt. Huber, Seewiesen D-8131, Fed. Rep. Germany.

The extensor tibiae muscle provides the propulsive force for jumping in locusts, grasshoppers, and crickets. In the locust Schistocerca gregaria and grasshopper Gomphocerippus rufus the hind legs are much larger than the front and middle legs, but in the cricket Gryllus bimaculatus the difference is not nearly as pronounced. This difference is highlighted by the roles of the hind legs in walking. Locusts and grasshoppers use them occasionally, but the cricket uses them in every step. We wanted to know if these behavioral and structural differences would be reflected in the anatomy of the fast extensor tibiae motor neurons.

Fast extensor tibiae motor neurons from the 3 species were physiologically identified and iontophoretically injected with cobalt. Neurons were then fixed for examination in the light and electron microscope. To date, we have found no significant differences in the morphology of the locust and grasshopper fast extensors. The cricket fast extensor is significantly different at the level of the light microscope. Whereas the locust and grasshopper fast extensors have primarily small diameter (less than 2μM) processes leaving their primary neurites, more than 5 processes with 3μM or greater diameter leave the cricket fast extensor primary neurite. Also, only very few small branches are seen to leave the primary neurite of locust and grasshopper between the cell body and the "turn" but the cricket has at least 2 branches of greater than 3μM diameter leaving the neurite in this region. When the structure of these neurons are compared with the serial homologues of the locust fast extensor, the slow extensor of the front and middle legs, the greatest similarity is seen between the cricket fast extensor and the locust slow extensors. The changes in structure from the front and middle leg's slow extensors to the cricket fast extensor and finally to the locust and grasshopper fast extensors closely parallels the presumptive evolution of jumping abilities.

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27.3 SEGMENTAL VARIATIONS IN MGF-MEDIATED ESCAPE BEHAVIOR IN INTACT EARTHWORMS. C. D. Drewes* and S. L. Pallas* (Spon: D. E. Emery) Department of Zoology, Iowa State University, Ames, Iowa 50011.

Non-invasive electrophysiological recording methods were used to study segmental variations in the functional properties of MGF (medial giant fiber) efferent pathways in the earthworm, Lumbricus terrestris. In each segment of the animal MGF spikes are followed in one-to-one fashion by spikes in the serially arranged giant motor neurons, termed GMN1. A single GMN1 spike evokes a small longitudinal muscle potential but no visible shortening. When two GMN1 spikes occur (due to firing of two MGF spikes) there is a marked facilitation of the second GMN1-mediated longitudinal muscle potential. Such facilitation is more pronounced in anterior than in middle or posterior segments. This marked anterior-to-posterior gradient in facilitation of muscle potentials correlates well with the observations that GMN1-mediated shortening in anterior segments is greater, occurs earlier, and requires fewer antecedent spikes than does shortening in middle segments. In the most posterior segments of the animal the MGF mediates a dorsoventral flattening of the tail, rather than a longitudinal shortening. Such tail flattening responses usually precede shortening in anterior segments by several milliseconds, even though the MGF activity is initiated in anterior segments.

The observed segmental gradient in longitudinal shortening appears to represent a mechanism for focussing rapid escape withdrawal into anterior segments which are most vulnerable to attack when the animal is extended from its burrow. The early onset of MGF-mediated tail flattening would ensure that the animal's tail is securely anchored within the burrow before shortening begins.

27.4 EFFECTS OF CINGULATE AND FORNIX LESIONS ON CONTACT DEFENSIVE IMMOBILITY IN THE RABBIT. M. L. Woodruff and R. H. Baisden. Department of Anatomy, College of Medicine, East Tennessee State University, Johnson City, Tn. 37601.

Contact defensive immobility (CDI) is a name suggested by Woodruff (Psychol. Rec., 27: 161, 1977) for a behavior also called animal hypnosis, death feigning, tonic immobility, and the immobility reflex. CDI is an ethologically significant response emitted by a prey after other behaviors have failed to allow escape from a predator. For example, Sargeant and Eberhardt (Am. Midl. Nat., 94: 108, 1975) observed that assumption of CDI by ducks significantly enhanced the chances of survival of an encounter with foxes. Evidence reviewed by Gallup (Psychol. Bull., 81: 836, 1974) also supports the contention that CDI has survival value for prey animals and, additionally, the hypothesis that fear is an important variable influencing CDI. Because CDI is a form of species typical behavior and is influenced by the emotional state of the animal, it is reasonable to expect that lesions of limbic structures would influence it. Therefore, the cingulate cortex and the fornix were chosen for destruction. Two separate behavioral experiments were conducted. Each group contained 12 rabbits. Postoperative recovery time was 35 days for both experiments. The procedure for inducing CDI has been described in detail (Woodruff & Lippincott, Brain Behav. Evol., 13: 22, 1976). In the first experiment rabbits with fornix lesions remained in CDI significantly longer than sham-operated or unoperated controls [$F(2,33)=5.99, p<.01$]. In the second experiment lesions of the cingulate cortex in the region approximately overlying the fornix produced significantly shorter durations of CDI than the durations of the control groups [$F(2,33)=8.38, p<.01$]. Other rabbits were given either unilateral fornix or cingulate lesions and their brains were processed using the Fink-Heimer technique. In addition to projections unique to each lesion, degenerating terminals after either lesion were observed in the anterior thalamic nuclear complex, the midline thalamic nuclei, the midbrain tegmentum, and the presubicular region. The observation of opposite effects on CDI duration produced by these lesions is congruent with previous experiments in which such lesions produced opposite effects on avoidance behaviors (McCleary, Prog. Physiol. Psychol., V.1, 1966). The presence of overlapping terminals in thalamus and mesencephalon suggests possible anatomical sites where efforts might first be made to determine how the cingulate cortex and hippocampus exert reciprocal effects on behavior. CDI is a good behavior to use for such an endeavor because it is relatively simple. The sensory systems which initiate it and the areas in the brainstem involved in its production have been delineated (Klemm, Psychol. Rec., 27: 145, 1977). (Supported by Biomedical Research Development Grant 1-S08-RR 09171.)

27.5 NEUROETHOLOGY OF PREY CAPTURE BY LARVAL FIREFLIES: INHIBITORY EFFECTS OF FIREFLY EXTRACTS ON CARDIAC FUNCTION IN TERRESTRIAL MOLLUSKS. Jonathan Copeland. Department of Zoology, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201.

The larval form of Photuris lucicrescens, a North American firefly of the beetle family Lampyridae, is predatory upon soft-bodied terrestrial annelids and mollusks, including the slugs Limax maximus and Agriolimax reticulatus. Larval Photuris lucicrescens attacked and consumed small (less than 1.2 g) Limax and Agriolimax when confined overnight in a test chamber. Slugs, when observed after attack, were flaccid and areflexive.

Because European Lampyridae have been reported to inject their prey with a paralyzing toxin, I examined the mandibles of Photuris lucicrescens for morphological evidence of toxin injection capabilities. Using SEM I have found a rectangular (40 µM long x 10 µM wide) pit less than 100 µM from the tip of each fang-like mandible. The pit is the external opening of a duct which runs through each mandible. The duct opening is ideally suited for the injection of a toxin into a prey organism.

The relative toxicity of a number of body compartments of the firefly was tested by utilizing the in vitro cardiac preparation of Limax maximus (MacKay and Gelperin, Comp. Biochem. Physiol. 43A, 877-896, 1972). Slug heartbeat was recorded with extracellular electrodes from either auricle or ventricle. Extracts of larval firefly mandibles, head, thorax-abdomen, or hemolymph were prepared and added at full or reduced concentration for 30 - 180 seconds. Extracts prepared from larval Tenebrio molitor served as a control. Larval firefly extract produced either a complete cessation of heartbeat or a reduction in beat frequency. Inhibition was usually complete, although occasionally cardiac escape occurred. Cardiac inhibition was sometimes preceeded by a transient increase in beat frequency and a decrease in beat amplitude. All effects were completely reversible. The order of potency was hemolymph>thorax-abdomen>head>mandibles.

Future experiments will test the hypothesis that the hemolymph, or some derivative of the hemolymph, contains an active agent or agents which are injected into prey during capture by firefly larvae.

27.6 VISUAL PREY ACQUISITION BEHAVIOR IN THE FROG: EFFECTS OF VARIOUS UNILATERAL LESIONS. S.K. Kostyk and P. Grobstein. Dept. of Pharmacol. Physiol. Sci., Univ. of Chicago, Chicago, IL, 60637.

We compared the effects on visual prey acquisition behavior in Rana pipiens of three lesions: a) unilateral optic nerve section, b) unilateral tectal ablation, c) hemisection at a level between tectum and cerebellum. Tectal ablation and midbrain hemisection were done by aspiration and subsequently verified histologically. Visual behavior was tested by observing responses to live mealworms presented at a series of positions at eye level around the frog.

Frogs with one optic nerve sectioned oriented with reasonable accuracy to stimuli in a region of visual space on the side of the intact eye and extending about 45° across the midline in front; they failed to respond to stimuli outside this region. Thus either eye alone is sufficient for orientation toward stimuli within the central 90° of visual field which is normally seen by both eyes. Frogs with unilateral tectal ablation oriented with comparable accuracy in a region on the side ipsilateral to the lesion and again extending about 45° across the sagittal plane; they failed to respond to stimuli outside this region. This indicates that the effects of unilateral tectal removal are laregely equivalent to the effects of unilateral optic nerve section. Either tectal lobe alone is sufficient to produce orienting toward stimuli within the central 90° of visual field.

Frogs with hemisections responded to stimuli at any position around the animal. The nature of the response, however, depended on the stimulus position with respect to the sagittal plane. For positions contralateral to the lesion, frogs oriented with accuracy comparable to that of normals. For any position ipsilateral to the lesion, frogs responded as if the stimuli were directly in front of them. The results indicate that at the level of the lesion there are no tracts or structures essential either for detection of stimuli at any position or for production of accurate turns toward stimuli located contralaterally. They suggest that tracts or structures on one side of the brain may be essential for turns toward stimuli ipsilateral of the sagittal plane.

The behavioral deficits of hemisected frogs differed from those of the first two groups in that: 1) there was no stimulus position at which frogs failed to respond, and 2) the region of normal orienting was bordered by the sagittal plane instead of extending 45° across the midline. The former indicates that lesions at this level dissociate turn production from other aspects of prey acquisition behavior. The latter suggests that the bilaterally redundant representation of spatial information evident at retinal and tectal levels may be lost at subsequent levels of the neuronal circuitry underlying this behavior.

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27.7 MOTOR CONTROL OF VOCAL BEHAVIOR IN RING DOVES (Streptopelia risoria). Jeffrey Cohen and Mei-Fang Cheng. Inst. Anim. Behav., Rutgers Univ., Newark, NJ 07102.

In recent years data have been gathered on the neural control of vocalizations of songbirds. One of the most intriguing findings is the phenomenon of left hypoglossal dominance (Nottebohm, J. Exp. Zool. 177: 229, 1971). Except for the chicken, few comparable data exist for birds with relatively simple vocal repetoires, such as the ring dove. For this reason, and because the ring dove has been the subject of extensive brain and behavior analysis, we decided to investigate the motor control of its vocalizations.

In the first study, the question of whether the ring dove exhibits left hypoglossal dominance was examined. Recordings of the male's bow-coos and nest-coos were made on a Uher Report L tape recorder (7 1/2 cps) and analyzed on a Kay sonagraph model 7029A. Following preoperative recordings males received either left or right hypoglossal nerve sections (HNS), bilateral HNS or a sham operation. Only minor changes occurred following left HNS or sham operation. Structural changes in coos were more noticeable after right HNS, but the most dramatic changes in coo structure followed bilateral HNS.

Because of the greater changes in cooing after right HNS, we wondered whether this would be reflected anatomically by an increase in size of the syringeal musculature on that side (as is true for left hypoglossal dominance and the left tracheolateralis muscle (TLM) in songbirds). In the second experiment, syrinxes from intact doves were processed using paraffin sectioning and the sections were traced. The relative volumes of the TLM's were calculated with the aid of a polar planimeter. In 75% of the animals, the right TLM had a larger volume than did the left TLM (mean % increase = 33%). Thus the relatively greater deficits induced by right HNS are reflected by a greater volume of the ipsilateral TLM.

In the third study we sought to determine the site of origin of the hypoglossal nerves. Unilateral HNS was performed, and after varying survival times, birds were sacrificed. The brains were cut and stained with cresyl violet, and examined for retrograde degeneration. Following unilateral HNS, there was a reduction in the number of cells in the n. hypoglossus tracheosyringealis (formerly called n. intermedius) of the ipsilateral side, but no changes in other nuclei on that level. It thus appears, that although the ring dove does not have left hypoglossal dominance, its hypoglossal nerves have the same CNS site of origin as do songbirds.

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27.8 NEURAL CONTROL OF HATCHING IN THE CHICK. A. Bekoff and J. Kauer*. Dept. EPO Biology, Univ. of Colorado, Boulder, CO 80309.

Hatching normally occurs only once in the chicken, during an interval of about 45 to 90 minutes, at the end of the 21-day incubation period (Hamburger and Oppenheim, J. Exp. Zool., 166: 171, 1967). In the present study the leg motor output typical of normal hatching was first characterized using electromyographic (EMG) and videotape recordings. Then the following questions were examined: 1)is the pattern generator for the leg hatching movements retained in the post-hatching chick? and 2)under what conditions can it be turned on?

We found that the leg motor output typical of hatching could be re-elicited in post-hatching chicks at least up to 4 weeks of age by folding them into the hatching position and placing them into artificial eggs of the appropriate sizes. Therefore the pattern generator for hatching leg movements is present and remains functional long after the occurrence of normal hatching. By selective manipulation of the position of various body parts we determined that only bending the neck to one side was both necessary and sufficient to turn on leg motor output with characteristics typical of hatching.

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27.9 FACILITATION OF SPONTANEOUS MOUSE-KILLING IN THE RAT BY CHOLINERGIC STIMULATION OF THE LATERAL HYPOTHALAMUS. B.C. Yoburn* and M. Glusman (SPON. M. Potegal). Dept. of Psychiatry, Columbia Univ., and N.Y. State Psychiatric Institute, New York, NY 10032.

Lateral hypothalamic cholinergic systems have been implicated in the mediation of interspecific aggression in the rat (Bandler, R.J., Brain Research, 20:409, 1970; Bandler, R.J., Nature, 224:1035, 1969). In these studies the application of the cholinergic agonist carbachol reduced the latency to kill prey in spontaneously aggressive rats. However, the actual dose of the drug delivered to the brain could vary between 3 and 10µg and only one dose level was presented. Therefore, the present study examined the effects of three precisely controlled doses of carbachol injected into the lateral hypothalamus on spontaneous mouse-killing in rats.

Eight male rats which spontaneously killed mice were implanted with a 22ga guide cannula aimed at the right lateral hypothalamus. Following postoperative recovery, each animal was injected using a 28ga internal cannula connected to a microsyringe mounted on a microdrive with .5µl of buffer, and .5µl of 5, 12.5 and 20µg of carbachol dissolved in buffer. The order of presentation was counterbalanced and a minimum of 48 hrs elapsed between injections. Ten minutes following the injection, subjects were exposed to five aggression tests spaced ten minutes apart in which a white mouse was introduced into the homecage and the latency to killing was recorded. If the rat failed to kill in two successive tests or if seizure activity was apparent, the session was terminated. Sessions with seizures were excluded from the data analysis.

The latency to kill was decreased in seven of the eight animals by at least one dose of carbachol and in four animals by at least two different doses relative to the buffer injection. In general, aggression was facilitated by the lowest dose and this facilitation was decreased as the dose was increased. For five animals the 5µg dose was the most effective in facilitating aggression, while the 12.5µg dose was maximally effective for only two animals, and the 20µg dose was not maximally effective for any animals and actually suppressed aggression for three animals. The 5µg dose was not observed to induce seizures in any animals, although the 12.5 and 20µg doses did so for one and two animals, respectively.

These results are consistent with suggestions that interspecific aggression in the rat is controlled by cholinergic systems in the lateral hypothalamus. However, the specific effectiveness of carbachol in this study varied with dose and the individual animal studied. The interaction of these two variables indicates the importance of accurately controlling drug dose and evaluating several doses in each subject. (Supported by NIH Grant MH 15174.)

28.1 BEHAVIORAL CHANGES IN AGING APLYSIA. C. Bailey,* V. Castellucci, J. Koester, M. Chen, and U. T. Koch.* Div. of Neurobiol. and Behavior. Depts. of Anatomy, Physiology and Psychiatry, Columbia Univ., P & S, New York, N.Y. 10032, and The NYS Psychiatric Institute.

We have used Aplysia californica to explore the effect of age on two simple forms of learning and memory; sensitization and the retention of habituation in the siphon-withdrawal reflex. Changes in arousal as reflected by changes in heart rate were also examined. Since weight is correlated with age we have, in initial experiments, used this parameter to separate animals. The mean weights of the animals used in these studies were 1291g ± 41 (S.E.M.), N=60 for large, presumably old animals; and 136g ± 6, N=53 for small, presumably young animals.

For the sensitization studies all animals received five blocks of habituation training sessions (Carew et al., 1973). Each block consisted of ten tactile stimuli to the siphon and was separated by 1.5 hrs. Experimental animals received an electrical shock (200 mA, 60 Hz for 2 sec) as a sensitizing stimulus between block 4 and 5. The shocked young animals (N=14) had a significant increase in their response (duration of siphon withdrawal) when block 5 was compared to block 4 (Wilcoxon, $p < 0.01$, 2-tailed). By contrast, experimental old animals (N=19) showed no significant increase. The sensitization scores of young animals were significantly higher than the scores in old animals (Mann-Whitney U-Test $p < 0.002$). These results suggest that the ability to undergo sensitization in old animals is impaired.

To examine the retention of habituation, animals received 4 blocks of training on the first day. Retention was tested by giving a single block 24 hours and 1 week later. Young animals (N=12) exhibited significant retention of habituation at 24 hrs (Wilcoxon, $p < 0.01$) and 1 week ($p < 0.05$). In old animals (N=15) significant habituation of the response was present at 24 hrs (Wilcoxon, $p < 0.01$) but not 1 week after training. Therefore the retention of habituation in older animals is of shorter duration.

We have also examined the effects of age on food arousal. Presentation of seaweed to Aplysia produces a state of arousal that results in an increase in biting rate with a concomitant increase in heart rate. This change in behavioral state was compared in young (N=5) and in old (N=5) animals. The change in biting rate during food arousal was the same in both groups but the change in heart rate was significantly greater (5-fold) in young animals than in older ones.

Our results suggest that aging in Aplysia may lead to specific learning and memory impairments as well as altered states of arousal. We are currently investigating the cellular basis of these age-related changes in behavior. (Supported by NSF grant BNS 7824476, NIH grant NS 14385 and an Irma T. Hirschl Career Scientist Award.)

28.2 AGE-IMPAIRED FUNCTION OF CENTRAL NEURON L_7 IN APLYSIA. B. Peretz, G. Ringham and R. Wilson. Depts. of Physiology and Biophysics and Pharmacy, Univ. of Ky. Med. Ctr., Lexington, KY 40536.

Three functions have been ascribed to L_7, a central neuron in the abdominal ganglion: i) motorneuronal, in gill pinnules (Kupfermann and Kandel, 1969; Peretz, 1969; Kupfermann et al, 1971; Jacklet and Rine, 1977); ii) dishabituatory of the gill withdrawal reflex (Peretz and Lukowiak, 1975; Lukowiak and Peretz, 1977); iii) modulatory of central rhythmicity mediating periodic respiratory movements of the gill (GPM, Sinback, 1975). These functions were observed in young, ca. 60 days old, and in sexually mature animals, ca. 120 days old. Recently, it was reported that in old animals, 200 days and older, L_7 lost the capability of dishabituating the gill withdrawal reflex (Peretz, et al, 1979). We report here a comparison of L_7's motor neuronal function in mature and old Aplysia.

In the preparation used the innervation between the abdominal ganglion and the gill was intact. Intracellular stimulation of L_7 at low spike rates, 1-2/sec, reliably resulted in one-for-one evoked potentials in the ctenidio-genital nerve and in the pinnules, as recorded with suction electrodes, in both mature and old animals. With the force of gill contraction generated during periodic GPM's being approximately 50 mg/g of gill wt for the two age groups, the maximum force elicited by L_7 stimulation in mature animals was ca 45% of the GPM, whereas that elicited in old animals was only 12% of the GPM, at the same spike rates. The latency of onset of pinnule contractions was longer in old than in mature animals, 660 ms vs 325 ms. The difference in latencies was longer than can be accounted for by the difference in conduction time between L_7 and its terminations in the pinnule, 250 ms in old vs 100 ms in mature animals.

Possible age-dependent changes of contractile properties of gill musculature do not explain the differences between L_7 elicited pinnule contractions in the two age groups since the force generated during GPM's was comparable. In old animals impaired function of L_7 does not appear to be the result of conduction block between L_7 and the pinnule as demonstrated by one-for-one following in the nerve and in the pinnule. L_7's age-impaired motor neuronal function may result from changes in L_7's terminations in the gill pinnule. (This research supported by a grant from the National Institute of Health, MH18611.)

28.3 RETENTION DIFFERENCES IN YOUNG, MID-AGED AND SENESCENT MICE. S.M. Fraley* and A.D. Springer, (Spon: R. Browner). Dept. of Anatomy, New York Medical College, Valhalla, N.Y. 10595

Distinguishing between performance and learning deficits in old animals is often problematic since sensory systems are impaired during aging. Therefore, we chose to study age-related retention changes using a simple situation in which learning did not depend on the use of specified sensory systems. Young (2 month), mid-aged (12 month) and senescent (24 month) male C57/B16 mice were given nine 3 minute exposures to a novel environment in which activity was measured. In this paradigm decrements in activity reflected adaptation resulting from learning the stimulus characteristics of the environment. Conversely, between-session increments in activity reflected forgetting. Within the first 4 days, all age groups showed a progressive decline in activity, and baseline levels were maintained for the duration of testing. However, throughout the entire testing period, the activity levels of the mid-aged and senescent animals were higher than that of the young mice. This age difference was related to the older animals showing appreciable forgetting during the 24 hr interval between sessions.

Attempts were made to attenuate the between-session forgetting in the older animals by providing them with longer daily exposures to the environment. Eighteen days after the end of the 3 minute sessions, the same animals were given daily sessions of 12 minutes in duration. Results indicated that prolonged exposure to the environment improved retention in the mid-aged and senescent mice. Aged animals, therefore, appear to require a longer time to process information concerning stimuli in their environment.

In other experiments, 2, 12 and 24 month old mice were given a 3 minute exposure to the novel environment and retested 4 and 8 days later. At both time points, total activity of the mid-aged and senescent mice was higher than that of young animals. These results corroborated those of the previous experiment in showing that aged animals demonstrate greater forgetting of a simple learning situation. Other groups of 2, 12 and 24 month old mice were tested only 8 days after a 3 minute exposure to the novel environment. In this instance, forgetting was evident in all age groups. Other experiments attempted to determine the effect of neuropeptides on retention in aging mice.

(Supported by Grant AG02150 from the National Institute of Aging).

28.4 EFFECTS OF DRUGS ON MEMORY PERFORMANCE IN AGED NON-HUMAN PRIMATES AND RODENTS. R.L. Dean and R.T. Bartus, Dept. CNS Research, Med. Res. Div. of American Cyanamid, Pearl River, NY.

Serious impairments on memory and related cognitive tasks occur with age, in both humans and other animal species. Because it is commonly assumed that functional changes in the CNS contribute to these behavioral impairments, many clinical trials have attempted to alleviate the neurobehavioral disturbances by giving elderly humans various drugs. However, no single drug has yet achieved universal acceptance as being efficacious. For this reason, a clear need exists to develop animal test procedures to measure these deficits in animals and assess the ability of drugs to reduce them.

Two such procedures have been used in our laboratory for these purposes. One, a variation of the delayed alternation procedure, uses aged F344 rats. The other, a variation of the delayed response task, uses aged Cebus monkeys. Both procedures allow extensive drug testing to be performed on individual subjects, allowing each subject to serve as its own control, facilitating direct comparisons between doses and drugs.

A number of drugs currently on the international market for geriatric indications (piracetam, naftidrofuryl, centrophenoxine, and dihydrergotoxine) were tested in each procedure. Further, various cholinergic agents (choline, physostigmine and arecoline) were also evaluated, due to the current interest in the cholinergic hypothesis of impaired geriatric memory.

The results of these tests revealed that many of these drugs can, in fact, reduce the memory impairments observed in aged animals. However, in certain cases, substantial differences in individual responses to particular doses of drug minimize the group effects. Further, although the test procedures were not able to differentiate between aged subjects within each species, some animals failed to exhibit facilitative effects at any dose, whereas, others exhibited marked improvement. These results demonstrate that: 1) animal procedures can be developed to evaluate the effects of drugs intended to improve geriatric cognition; 2) statistical proof of efficacy is possible with many agents available in Europe, even within the strict confines of a laboratory setting; and, 3) unless individual differences in response to dose and drug are taken into account, subtle facilitative effects may be obscured.

28.5 CHANGES IN ^{3}H-NALOXONE BINDING IN THE BRAINS OF SENESCENT RATS. J. N. McDougal*, N. W. Pedigo, P. R. Marques*, H. I. Yamamura and T. F. Burks. Department of Pharmacology, Arizona Health Sciences Center, Tucson, AZ 85724.

Investigations of the changes in pharmacologic response due to the aging processes are becoming increasingly important because of the growing size of the elderly population. Aged laboratory animals respond differently than young animals to morphine. Thermic effects of morphine in senescent rats are more pronounced at low doses and are attenuated at high doses in comparison with young rats (McDougal, Marques and Burks, Proc. West. Pharmacol. Soc. 23: in press). In addition, senescent rats acquire tolerance to the thermic effects of morphine less readily than young rats. When a low dose of morphine (5 mg/kg s.c.) was readministered three days after the same initial dose, 27 month old rats responded the same to both doses, but the 3 month old rats responded with increased hyperthermia to the second dose. When a higher dose of morphine (25 mg/kg s.c.) was injected and then readministered three days later, 3 month old rats had a decreased hypothermic response, but the 27 month old rats responded the same as with the initial dose. However, upon morphine pellet implantation, all groups showed a decreased hypothermic response to morphine (25 mg/kg s.c.). These age-related changes in hypothermic response were not present with the administration of two doses of ethanol (1.5 and 3 mg/kg i.p.).

To determine if changes in opiate receptor binding are responsible for these altered pharmacological responses to morphine, we assayed opiate receptors in the corpus striatum, frontal cortex, hippocampus, nucleus accumbens and olfactory tubercle, hypothalamus, and brain stem of 3, 10 and 27 month old Fischer 344 rats from the colony supported by the National Institute on Aging. Specific ^{3}H-naloxone binding (1 nM in Tris buffer) was defined as the difference between binding in the presence and absence of 1 μM naltrexone. Preliminary results show a significant decrease (33%) in the specific ^{3}H-naloxone binding in the hypothalamus of senescent rats when compared with 3 month old rats. Binding in the 10 month old animals was intermediate between 3 and 27 month old rats. No significant age-related changes were noted in the other brain regions examined. The decrease in hypothalamic opiate receptors with aging may explain the observed age-related differences in the thermic effects of acutely administered morphine as well as the differences in acquisition of tolerance. (Supported by USPHS Grants AG01289, NS15420, NS06217 and RSDA to H.I.Y. MH00095.)

28.6 MORPHINE EFFECTS ON PAIN THRESHOLDS AND OPEN FIELD ACTIVITY IN YOUNG AND OLD RATS. B.J. Vasquez, R.B. Messing, R.A. Jensen, V.R. Spiehler, T.J. Hannan*, J.L. Martinez, Jr. and J.L. McGaugh. Department of Psychobiology, University of California at Irvine, Irvine, CA 92717.

Previously, we have reported alterations in forebrain dihydromorphine binding in old (24-26 month) as compared to young (3-5 month) male F344 rats.

We now report that morphine effects on nociception and locomotor activity are altered in these animals.

Flinch and jump thresholds to electric shock were unchanged in young rats when measured immediately following i.p. administration of 2.5 or 5.0 mg/kg of morphine. However, jump thresholds of old rats were significantly elevated immediately after injection of these doses of morphine. Pain thresholds of old and young rats given saline injections were not different from each other.

In contrast to these data, open field activity was higher in old as compared to young rats when rats were exposed to an open field for the first time. In this experiment rats were placed in an open field with a floor divided into 16 squares (25 x 25 cm). Activity was measured for 5 min by counting the number of squares crossed. When rats were injected i.p. with saline immediately following the initial 5-min testing period, and placed in the open field one hr later, activity levels of young rats were unchanged, but there was a decline in the open field activity of old rats. Morphine (1.0 mg/kg immediately following initial testing) increased the locomotor activity of young rats one hr later. In old rats given morphine (1.0 or 3.0 mg/kg) however, activity levels did not change upon subsequent exposure to the open field.

Taken together, these results indicate that differential effects of morphine in young and old rats probably depend on age-related changes in the neurochemical substrates on which the drug is acting, as well as on alterations in drug metabolism in old animals.

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28.7 FUNCTIONAL ASPECTS OF THE NIGRO-STRIATAL DOPAMINERGIC SYSTEM IN SENESCENT RATS. M. Roffman, F. Cordasco* and A. Kling. CMDNJ-Rutgers Medical School, Piscataway, New Jersey 08854.

Chronic administration of neuroleptics to mature rats have been shown behaviorally to produce changes in the dopaminergic receptors of the striatum indicative of hypersensitivity. However, there is a paucity of data on the effects of such treatment in senescent rats. The present experiments sought to determine the effects of chronic haloperidol treatment on the functional integrity of the nigro-striatal dopaminergic system as determined by apomorphine-induced stereotypy. Stereotypy was determined according to Costall and Naylor (EUR. J. Pharmacol. 21:350, 1973). With no prior treatment the senescent rats were less sensitive to the effects of apomorphine (0.25 mg/kg) with respect to the number of subjects exhibiting stereotypy (0.08%) and intensity of the behavior (1.1) than mature rats (67% and 0.083, respectively).

After chronic treatment with haloperidol both the senescent rats and the mature rats exhibited significantly ($P<.01$) more stereotypy than corresponding rats that had been chronically treated with saline. Seven days after cessation of chronic haloperidol treatment, a threshold dose (0.25 mg/kg) of apomorphine caused the senescent rats to emit a degree of stereotypy (1.25) that was similar to that elicited by the mature rats (1.86). However, by 14 and 21 days after chronic treatment no such behavior was detected in the senescent rats whereas the mature rats continued to emit the stereotypy (1.5 at both 14 and 21 days). On the other hand, after administration of 0.75 mg/kg of apomorphine the senescent rats exhibited similar stereotypic scores (1.5) at 7, 14 and 21 days after cessation of chronic drug treatment whereas the behavior of the mature rats was more intense than that of the senescent rats at 7 and 14 days (3.0 and 2.9 respectively) but not at 21 days (1.8) after chronic haloperidol treatment. These data indicate that the number of dopaminergic receptors in the nigro-striatal pathway are decreased in the untreated senescent rat. However, after chronic antipsychotic treatment these receptors appear to remain capable of becoming supersensitive and that this sensitivity may be more persistent than that occurring in mature rats.

Changes in the functional capabilities of the dopaminergic receptors within the nigro-striatal system of senescent rats may explain the clinical observation that geriatric patients are, in general, more prone to some of the neurological side effects, especially extrapyramidal symptoms, of the antipsychotic medication than are younger patients.

28.8 SODIUM PENTOBARBITAL IMPAIRS DELAYED-RESPONSE PERFORMANCE IN AGED RHESUS MONKEYS. J. S. Abelson and J. G. Marriott. Pharmacology Dept., Warner-Lambert Research Labs, Ann Arbor, MI 48105.

Barbiturates are commonly prescribed to geriatric patients for anxiety, pain, and insomnia. However, these drugs are often contraindicated in the elderly, for they may produce motor and cognitive side effects such as ataxia, forgetfulness, and confusion. The nature of the cognitive impairments produced in the elderly by these drugs has not been studied in laboratory models. The bulk of research on the effects of barbiturates upon cognition in young animals has attributed poor performance on cognitive tasks to depressed sensory and motor activity and not to specific memory impairments. We examined the effects of sodium pentobarbital (SP) on delayed-response (DR) performance of aged rhesus monkeys to determine the activity of barbiturates in an animal model of cognition in old subjects.

Ten test- and drug-sophisticated aged rhesus monkeys (18 + years) were tested on an indirect DR task. Subjects were presented with a visual stimulus on one of nine panels in a 3 x 3 matrix. Recall of the spatial position of the stimulus was tested following retention intervals of various durations. Both 0 sec (control) and non-zero (memory) intervals were tested. Duration of the non-zero retention intervals was established by a titration procedure, allowing the interval length to be adjusted dynamically by each subject's performance. SP (1.0, 2.0, 4.0, 8.0, and 16.0 mg/kg) was administered PO 30 min prior to behavioral testing twice a week. In general, higher doses of the drug led to increased variability in DR performance. The highest dose, 16.0 mg/kg, impaired performance in all animals. Statistical analysis revealed that overall DR performance with 1.0-8.0 mg/kg SP was not different from non-drug control, whereas 16.0 mg/kg significantly impaired both 0 sec and non-zero performance of the task ($p<0.5$). Further, there was a significant interaction of dose and retention interval; the difference between 0 sec control and non-zero performance increased as SP dose increased ($p<.01$), yielding greater effects of non-zero delay intervals with increasing dose.

These results clearly indicate that barbiturates have specific effects on short-term memory (STM) in aged subjects. The debilitating effects of SP are not simply due to impairments in motor activity, for DR performance at non-zero delays was more severely disrupted than 0 sec control responding. In this respect the present results in aged subjects are different from the young animal studies which have generally not noted specific STM impairments with barbiturates, demonstrating that aged subjects are more sensitive to these drugs than are young ones. Thus, the present results substantiate the view that barbiturates are contraindicated in geriatric patients.

28.9 BRAIN DAMAGE, STRESS, AND LONGEVITY: AN EXPERIMENTAL STUDY.
D. Wozniak*, S. Finger, H. Blumenthal*, and R. Poland*. Dep't of Psychology, Washington Univ., St. Louis, Mo., 63130, and U.C.L.A., Dep't of Psychiatry, Harbor General Hospital, Torrance, Ca. 90509.

Our principal objectives were to investigate whether (a) brain damage, (b) environmental stressors, or (c) the interaction of these factors would affect lifespan or susceptability to disease. To examine these questions, over 300 C57 BL/6J mice underwent bilateral frontal lobotomies or sham operations at sexual maturity. Approximately every 4 months, half of the animals were subjected to stress sessions involving cold-restraint or cold alone while other animals remained in their home cages. Following each stress session a small number of animals was sacrificed from each experimental condition to assess gastric lesions and serum corticosteroid levels (immediate effects of the stressors), and to provide well-preserved animals for pathological study. The remaining mice were allowed to die from "natural causes", and attempts were made to retrieve and autopsy each body.

The mean life span of the mice was about 28 months, although certain animals lived beyond 36 months. However, neither the lesion, nor the stress, nor the interaction of these variables was found to affect longevity.

The stress procedures were observed to elevate circulating corticosteroid levels and on occasion to produce a significant incidence of gastric lesions. The lesion variable, by itself or interacting with stress, was never found to produce significant effects with regard to gastric erosions or corticosteroid levels. Prevalent causes of death included leukemia, lymphosarcoma, metastatic pulmonary tumors, pulmonary adenomas, and pneumonias. Analyses assessing the potential relationships between the lesion and stress variables and the cause of death are currently being conducted.

It may be concluded that brain damage need not necessarily affect life span or alter an animal's resistance to stress-related pathophysiological changes. Conclusions as to whether brain lesions and/or stress will affect susceptability to different diseases will also be presented.

29.1 ALTERATIONS IN REGIONAL CEREBRAL BLOOD FLOW IN THE BRAIN-INJURED CAT WITH MORPHOLOGICAL CORRELATES. E. L. Auen, A. B. Malik, L. R. Nelson, K. D. Barron, and R. S. Bourke. Div. of Neurological Surgery and Depts. of Neurology and Physiology, Albany Medical College, Albany, N.Y. 12208.

Using the radioactive microsphere (15micron) reference blood flow technique, we injected 3 differently labelled microspheres (^{141}Ce, ^{113}Sn, & ^{46}Sc) at 3 different times into the left heart of brain injured and control cats to determine regional cerebral blood flow (rCBF). The brain injury model utilizes repetetive translation plus rotation acceleration impulses applied to the skull encased brain of the anesthetized cat (Nelson, L.R., et. al. Neurosci. Abs. 5:516 1979). Microsphere injections were made before, during, and 40 min. after the injury. Parallel experiments were done in control cats. In another set of experiments, control and brain injured cats were perfused for electron microscopy at times corresponding to the microsphere injections. Mean arterial blood pressure, intracranial pressure, EKG, EEG, and arterial blood gases were monitored throughout the experiment. The rCBF data for 19 different brain regions, and cervical and lumbar spinal cord were normalized based on tissue dry weight determinations.

There was a decrease in blood flow of 27.8%±1.9% in the cerebral hemispheres of the brain injured cats at 40 min. after the brain injury compared to controls. There was a decrease in blood flow in the cerebellar hemispheres of 14.3%±0.8% at the same time but blood flow in the brain stem, cervical, and lumbar spinal cord did not change significantly. The reduction in blood flow to the supratentorial regions was not associated with significant alterations in mean arterial blood pressure, intracranial pressure, electroencephalogram, or blood gases. Electron microscopy of supratentorial sites 40 minutes post injury demonstrated scattered focal perivascular astroglial swelling. These electron lucent perivascular foot plates often completely surrounded the capillary endothelium. The capillary endothelium of the brain injured animals often displayed luminal villous "finger like" projections. The relatively greater flow reduction observed in supratentorial regions 40 min. after trauma may be due in part to the perivascular astroglial process edema and endothelial alterations observed electron microscopically at that time.
Supported by NIH grants NS 13042, GM15426, and RCDA HL00363 to A.B.M.

29.2 EVALUATION OF TREATMENT MODALITIES USING AN ANIMAL MODEL OF SEVERE HEAD INJURY. L. R. Nelson, E. L. Auen, & R. S. Bourke. Div. of Neurological Surgery, Albany Medical College, Albany, NY 12208.

We previously have reported the development of an animal model of severe head injury (Nelson, L.R. etal Neural Trauma, pg.297-311, ed. Popp, A.J., Raven Press N.Y. 1979). The model utilizes a repetetive acceleration injury to the skull encased brain of the anesthetized cat followed by a 1 hour period of hypoxia induced by mechanically ventilating the paralyzed cat with 6% O_2 in N_2. This paradigm results in a 51% delayed mortality rate (control group) with an impaired rate of neurological recovery in survivors.

We have used this model to evaluate the relative effectiveness of 6 drugs in reducing both mortality and morbidity in head injury. Animals were randomly assigned to treat or control groups. Following the trauma each treat animal received a single dose of either Edecrin, DCPI, DCPHI, DCPIB, Acetazolamide (1to5mg/kg IV) or Methylprednisolone (30mg/kg IV q6h).

RESULTS

GROUP	MORTALITY RATE	P VALUE
Control	38/75=51%	
Edecrin*	14/39=36%	NS
DCPI*	11/42=26%	< .01
DCPHI*	8/22=36%	NS
DCPIB*	9/32=26%	< .025
Acetazolamide	8/16=50%	NS
Methylprednisolone	9/18=50%	NS

When all acylaryloxyacetic compounds (*) are grouped together the mortality rate is 42/135=31% P<.005. With a translational only mode of injury, the Edecrin and DCPI groups also showed a significantly improved rate of neurological recovery as measured by our numerically quantifiable neurologic examination. The other groups have not been tested with this mode of injury. With a translational plus rotational mode of injury none of the groups showed a significantly improved rate of neurological recovery. This data suggests that the translational only mode of injury is a superior model for drug testing since with this mode of injury one can assess the effectiveness of a drug both in terms of reducing mortality and morbidity.
Supported by NIH grant NS13042.

29.3 VASCULAR ENDOTHELIAL RESPONSES TO ACUTE HYPERTENSIVE INSULT: A SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDY. D.S. DeWitt*, J.T. Povlishock, R.L. Hayes, D.P. Becker. Departments of Neurosurgery and Anatomy, Medical College of Virginia, Richmond, Virginia 23298

Hypertension has been reported to cause balloon- or crater-like lesions in vascular endothelium. The temporal sequence of the genesis and resolution of these lesions has not been investigated and, to this end, the present investigation was undertaken.

Cats were anesthetized and infused with norepinephrine bitartrate (Levophed) to produce a rapid elevation in arterial pressure ($\bar{x}$=173 mm Hg). In two animals, perfusion was initiated two minutes after the onset of the hypertensive episode, while in all other animals, the hypertensive insult was sustained for approximately eight minutes. The animals were perfused with 2% paraformaldehyde and 2.5% glutaraldehyde at two minute, ten minute, thirty minute, and four and eight hour intervals following the onset of the hypertensive insult. The brains were removed and samples were taken from the cerebral cortex, diencephalon, the brain stem and pial arterioles. The brain parenchymal samples were then sectioned on a vibratome and together with the bisected pial vessels they were osmicated and processed for scanning electron microscopy. Selected samples were also processed and embedded in Epon 812 for transmission electron microscopy. The luminal surfaces of intraparenchymal vessels contained within these tissue samples as well as the luminal surfaces of the pial arterioles were viewed with a Hitachi S-500 scanning electron microscope. On the basis of randomized selection micrographs were taken at a constant magnification (1500X) of the luminal surfaces of the pial and intraparenchymal vessels. Endothelial lesions were characterized and quantified from these micrographs.

Through such a regimen, endothelial balloons and craters were recognized within two minutes of the onset of the hypertensive episode. Lesions were observed in the vessels of the brain parenchyma as well as in pial arterioles, and in both cases,they occurred predominantly along the marginal lines. With increasing survival times, more lesions were recognized with the maximum number occurring at thirty minutes. By the fourth hour after the insult, the number of endothelial lesions was significantly decreased. At eight hours, although there was no significant decrease in the number of endothelial lesions, the lesions appeared to be resolving as they were less conspicuous.

The results of the present investigation suggest that an acute hypertensive insult may initiate immediate endothelial damage which, with time, progresses and ultimately begins to resolve.
Supported by NIH Grant NS-12587.

29.4 PROLIFERATION AND IMMUNOFLUORESCENT STAINING OF GLIAL CELLS WITH MULTIPLE SCLEROSIS SERUM. V.K. Singh, D.A. Thomas* and R.D. Mashal*. Div. of Immunology, Dept. of Pathology, University of British Columbia, Vancouver, Canada, V6T 1W5.

The proliferation of rat brain glial cells in tissue culture supplemented with normal human or multiple sclerosis (MS) patient serum was studied. The proliferative response (PR) was measured by the incorporation of ^{3}H-thymidine into DNA. The PR with MS patient sera as compared to normal human sera was found to be very diffused. Only 2 patient sera caused about 50% inhibition; 4 patient sera had no effect, and the remaining 9 patient sera enhanced the PR by 30-90% over the control values. Increased PR corroborated with morphological differentiation whereas the suppressed PR was recorded with the degeneration of glial cells in cultures. Simultaneous detection, by means of indirect immunofluorescent (IF) technique,of glial cell antibodies in MS patient serum revealed that 5 out of 15 MS sera were strongly positive. Two sera were equivocal whereas the remaining 8 sera were completely negative. None of the 7 control sera exhibited any positive staining. The antibody staining with positive sera was localized with the cell surface membranes and the antibodies appear to be of IgG class. Those sera which caused inhibition of PR also displayed positive IF-staining whereas the sera which had no effect on PR exhibited equivocal IF-staining. The remaining patient sera with enhancing effect on PR were generally IF-negative. These observations indicate that glia proliferation in tissue culture with MS patient's serum correlates significantly with the antibody staining of glial cells by MS serum.
(supported by grants from BCMRF, BCHCRF and Vancouver Foundation

29.5 FURTHER ULTRASTRUCTURAL EVIDENCE TO SUPPORT THE PRESENCE OF A PROGRESSIVE AXOPLASMIC DEGENERATIVE PROCESS IN CASES OF JAKOB-CREUTZFELDT DISEASE. J.P. MACHADO-SALAS. Laboratorio de Neuromorfología Experimental y Aplicada.Depto.Anatomía.Facultad de Medicina, UNAM. México, 20.D.F. MEXICO.

Jakob-Creutzfeldt disease (J-C D) and Kuru are fatal neurological diseases in which a long-latency viral process appears to trigger some unknown mechanisms that end up with a severe destruction of neuropiles. For a long time it has been recognized that these alterations are characterized by the presence of vacuoles throughout the cortical gray matter.

Most of the ultrastructural studies that have been done in cases of J-C D., have localised these vacuoles at the level of nerve- or glial-cells bodies or their shorter branches.Few reports have mentioned that this alteration can also be observed in axons. Whatever the final localisation of these vacuoles, there still it remains to uncover the etiology and/or mechanisms that are involved in their production.

We have studied the ultrastructural features shown by the cerebral biopsy obtained from a young subject, who had shown a dementia of rapid evolution and myoclonic jerks.A sequence of changes can be identified as the putative degenerative process that ends up with axonal vacuolization, which, by the way, it was rather outstanding in the present case.

First, it appears to occur a darkening of the axoplasm and/or the development of osmiophilic bodies, and distortion of mitochondria. In a later stage, a centrifugal displacement of the axoplasm, progressively takes place, until it becomes attached to the myelin sheath.Finally, a distended, flattened and empty myelin sheath stands out as the remaining structure.Ocassionally, some distortion of myelin may parallel these changes.

Some probable mechanisms involved shall be discussed.

29.6 INDUCTION OF MYOTONIA IN UTERO BY 20, 25-DIAZOCHOLESTEROL TREATMENT. S. K. Mishra, M. Hobson*, S. H. Subramony and D. Desaiah. Neurology and Research Service, VA Medical Center, Department of Neurology, University Mississippi Center, Jackson, Mississippi 39216.

Myotonia is characterized by the continuous active contractions of muscles which persists after the cessation of voluntary efforts or stimulation (a slowness in the relaxation of muscles). The purpose of this study was to develop an animal model which mimics the genetically determined human myotonic muscular dystrophy. Myotonia was produced in offsprings of 20, 25-diazocholesterol (DAC) induced myotonic female rats which were mated either with normal or DAC-treated males. The rats were administered with DAC by gastric intubation of 80 mg/kg b.w. at weekly intervals. Myotonia was confirmed in adults by electromyography (EMG) preceeded by breeding (5 successive treatments). DAC treatment was continued in adult females thru 21 days post partum. After weaning offsprings were treated as adults. Myotonia was confirmed in the offsprings at 28 days of age (3^+ on a scale of 1-5) by routine EMG examination. At the age of six weeks biopsies of plantaris quadriceps and soleus muscles were taken for histopathological analysis. Frozen sections were cut and stained with various histological and histochemical stains and the following changes were noted: 1. Variability of fiber size, 2. internal nuclei, 3. decreased PAS positive fibers, 4. moth eaten fibers, 5. dark angulated fibers, 6. decrease and absence of type 2-B fibers (most effect seen in quadriceps muscle), 7. fiber splitting and 8. spindle abnormalities. The onset of myotonia in the offsprings was seen at 28 days as compared to 42 days in adults. Similarly, early histological and histochemical changes were seen in the myotonic offsprings (after 6 weeks) as compared to adults (after 16 weeks). The present data demonstrate for the first time that the myotonia is produced in utero by DAC treatment in rats. These results also suggests that the observed abnormalities are compatible with the genetically determined disorders seen in human myotonic muscular dystrophy. (Supported by Medical Research Service of Veterans Administration.

29.7 CELL LOSS IN DIENCEPHALIC NUCLEI OF PURKINJE CELL DEGENERATION (pcd) MUTANT MICE. S.V. O'Gorman and R.L. Sidman*. Dept. of Neuropathology, Harvard Medical School & Dept. of Neuroscience, Children's Hospital Medical Center, Boston, MA 02115.

Previous studies of mice homozygous for this autosomal recessive mutation have demonstrated a rapid degeneration of nearly all cerebellar Purkinje cells during the second and third postnatal weeks, and more slowly developing, progressive losses of retinal photoreceptors and olfactory bulb mitral cells (Mullen et al., 1975, PNAS 73: 208). We have found that additional cell losses occur between 50 and 60 days of age (P50-P60), when discrete neuronal subpopulations of the mediodorsal, submedial, ventrolateral, reticular and posterior thalamic nuclei, and all neurons of the ventral medial geniculate nucleus (vMG) degenerate. The ventrobasal, ventromedial, intralaminar, lateral geniculate, and anterior thalamic nuclei suffer no obvious neuronal loss between P24 and P180. The timing and morphological features of degeneration appear to be similar in each affected thalamic nucleus. vMG neurons, for example, are morphologically normal at P40 when examined in one micron sections of Epon embedded material stained with toluidine blue. By P50, many somatic profiles contain large areas of homogeneous, lightly-stained material, and a few frankly pyknotic neurons are present. Very few vMG neurons survive at P60. Examination of P50 material by electron microscopy shows that lysosomal number and size are increased in most vMG neurons, and many cells contain one or a few small, spherical (0.2-0.6μm diameter) aggregates of fine (8nm) particles, commonly apposed to the convexity of cresentic or cup-shaped cisternae of endoplasmic reticulum. Cytoplasmic organelles are reduced in number and curiously distributed in more severely affected P50 vMG neurons. Large areas of perinuclear cytoplasm are occupied exclusively by polyribosomes, while the remaining organelles are often clustered at the base of a primary dendrite. Collicular and cortical afferent terminals form morphologically normal synapses on vMG dendrites at P40, and intact boutons are often apposed to degenerating dendrites at P50. At P60, but not at P50, vMG axons in the thalamic radiations and their terminals in auditory cortex are intensely argyrophilic in Fink-Heimer preparations. The pcd locus affects an apparently disparate assortment of neuronal classes at different chronological ages, with the presumed common denominator yet to be established. We have no evidence to suggest that thalamic cell loss is a consequence of synaptic agenesis or dysgenesis, and doubt that it is related by transneuronal mechanisms to previously described cerebellar, retinal or olfactory bulb pathology.

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29.8 AXONAL NEUROFILAMENTS AND NEUROFILAMENT AGGREGATES EXPERIMENTALLY INDUCED IN THE RABBIT BY ALUMINUM AND MITOTIC SPINDLE INHIBITORS ARE IMMUNOHISTOCHEMICALLY DIFFERENT. A. Bignami, D. Dahl, N. Chi* AND N. Bich*. Spinal Cord Injury Research Laboratory, Harvard Medical School and West Roxbury VA Medical Center, Boston, MA. 02132.

Based on indirect evidence it is now believed that the mammalian neurofilament is formed by a triplet of polypeptides at approximately 200K, 150K and 70K daltons (Lazarides, E., Nature 283:249, 1980). We have previously shown that the 70K and 150K proteins are biochemically and immunologically not related (Dahl, D., FEBS Lett. 103:144, 1979; ibid. 111:152, 1980). We now report that the localization of the two proteins are different. Aggregates of neurofilaments in the spinal cord neurons of the rabbit were experimentally induced by the intrathecal injection of aluminum and mitotic spindle inhibitors (colchicine, vinblastine and vincristine). As previously reported (Dahl, D. and Bignami, A., Exp. Neurol. 58:74, 1978; Dahl, D. et al., Acta Neuropath., in press), neurofibrillary tangles stained intensely by immunofluorescence and the PAP procedure with neurofilament antisera raised to degraded chicken antigen (Dahl, D. and Bignami, A., J. Comp. Neurol. 176:645, 1977). These antisera strongly reacted with the 70K polypeptide in brain filament preparations as indicated by immunoaffinity chromatography (Dahl, D., BBA 622:9, 1980). Neurofibrillary tangles were not stained with antisera to antigen extracted from the 150K band in SDS gel electrophoretograms of brain filament preparations. Both antisera (anti-150K and anti-chicken antigen) demonstrated the same structures in cryostat sections of cerebellum, that is, Purkinje cell baskets, a fine mesh of fibers in the lower half of the molecular layer and granular layer, and myelinated axons in white matter. Large motor axons adjacent to neurofibrillary tangles in the anterior horns were equally well stained with both antisera. The findings may suggest that neurons contain a distinct class of filaments sharing with other classes of intermediate filaments the property of colchicine-induced aggregation. An alternative explanation is that the 150K polypeptide is not a "core" neurofilament protein but a neurofilament associated protein involved in axonal transport. Supported by grants from the NIH (NS 13034), the NSF (BNS-7912962) and by the Veterans Administration.

30.1 EVIDENCE FOR SORTING OF PROTEINS DURING THE INITIATION OF RAPID AXONAL TRANSPORT. G.C. Stone and R. Hammerschlag. Div. of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

During the initiation of fast axonal transport, newly synthesized proteins destined for transport may be routed through neuronal somata in a manner similar to that of secretory proteins and integral membrane proteins in non-neuronal cells (Droz, in The Nervous System 1:111, 1975; Hammerschlag, & Lavoie, Neurosci., 4: 1195, 1979). This analogy predicts that fast transported proteins (FTPs) are synthesized in the endoplasmic reticulum and pass through the Golgi apparatus prior to reaching the transport system proper. To further investigate this possibility we have examined the synthesis and turnover of microsomal proteins in bullfrog dorsal root ganglia (DRG) and compared these membrane proteins to identified FTP species. In vitro fast axonal transport was performed as described previously (Stone et al., Brain Res. 144:287, 1978) in which ^{35}S-met FTPs accumulate proximal to ligatures of spinal nerves. In separate preparations DRG were pulse-labeled with ^{35}S-met and fractionated. Proteins present in crude microsomal fractions (100,000 x g pellet) as well as FTPs in 4 mm nerve segments proximal to ligatures were separated via two-dimensional gel electrophoresis (m.w. ~20 K - 200 K daltons; pI ~ 4.5 - 8.0). Gel fluorographic patterns of microsomal and FTPs were compared using comigration analyses. Qualitative results demonstrate that nearly all of 47 FTP gel spots could be identified in the microsomal fraction. However, of the 12 most abundant FTPs, 10 were present at barely detectable levels in the DRG sub-fraction. Despite varying the pulse period from 15 min to 4 h and allowing up to 10 h of chase, this population of FTPs still did not appear in the microsomal fraction in appreciable amount. Thus, it is unlikely that differential rates of incorporation could explain our basic observation. Since most of the population of proteins that were not readily detected in the DRG fraction are of lower m.w. (~20 K - 35 K daltons) it is possible that their more abundant appearance at the ligature is due to proteolytic processing of higher m.w. precursors during translocation. This explanation is weakened since no higher m.w. species show obvious decreases at the ligature relative to the DRG. Another possibility is that proteins of this sub-group do not accumulate in the cell bodies because they experience a markedly shorter transit time from soma to axon. Added evidence to support this explanation is that cobalt, an agent known to interfere with somal events of fast transport, depresses the amount of the low m.w. FTPs arriving at the ligature to a lesser extent than the higher m.w. species (Stone & Hammerschlag, Trans Am. Soc. Neurochem., 11:143, 1980). (Supported by a MDA fellowship and by NSF grant BNS 79-24125.)

30.2 MILD CNS ISCHEMIA ACCELERATES FAST AXONAL TRANSPORT. M. Jacewicz* and D.E. Levy. Lab. of Cerebral Metabolism, Dept. of Neurology, Cornell Univ. Med. College, New York, NY 10021.

We recently reported (Neurology, 30:376, 1980) the effects of transient central nervous system (CNS) ischemia on fast axonal transport. Employing a radiotracer technique in a rat model of unilateral optic nerve ischemia, we found that ischemia blocked transport. With reperfusion, however, some proteins remained blocked, but others showed accelerated movement down the axon. We now report that a similar surge of radioactive material may occur with persistent, but relatively mild ischemia.

In a double-label experiment, we first injected both eyes of awake rats with 30 μCi of ^{3}H-leucine, allowed 1 hour for protein synthesis and transport, and then induced unilateral eye and optic nerve ischemia. Ischemia was achieved by occluding the right external carotid and pterygopalatine arteries with clasps placed the previous day. The left side served as a control. Following 1 hour of ischemia, animals were injected intravenously with the blood flow marker, ^{14}C-iodoantipyrine, and were decapitated 6 seconds later. Optic nerves and tracts were rapidly removed and frozen; 1 mm segments were prepared for double-label liquid scintillation counting.

Radioactivity of both labels was measured for each optic nerve and tract and was expressed as a function of distance from the eye. Most animals showed marked blanching of the right eye; ipsilateral blood flow was reduced to 30% of control in the proximal optic nerve and rose to 80% of control near the chiasm. All such animals demonstrated blocked fast axonal transport during ischemia. A few animals, however, showed minimal or no blanching, and blood flow in this group fell to only 60% of control proximally and was 100% of control distally. This group showed increased levels of incorporated leucine (TCA-precipitable) along the entire length of optic nerve and tract (3-4 times the control side).

We conclude that fast axonal transport in the CNS is very sensitive to the level of blood flow. Severe ischemia blocks the appearance of radioactive protein down the axon, but mild ischemia accelerates this process. Prolonged, mild ischemia and brief, severe ischemia followed by reperfusion may have similar effects on fast axonal transport.

30.3 BIOCHEMICAL AND RADIOAUTOGRAPHIC STUDY OF THE CALCIUM REQUIREMENT FOR FAST AXONAL TRANSPORT OF PROTEINS. P.-A. Lavoie and G. Bennett*. Dépt. de pharmacologie, Université de Montréal, and Dept. of Anatomy, McGill University, Montreal, Canada.

Many fast transported proteins are glycoproteins. Dorsal root ganglia (DRG) from bullfrogs were exposed to [^{3}H]fucose in vitro, and the fate of the [^{3}H]glycoproteins was followed during a subsequent incubation period where the ganglia and spinal nerves from one side of the animal served as control preparations and those from the other side served as experimental preparations. The DRG of the experimental preparations were incubated in a medium which either lacked Ca^{2+} or was supplemented with Co^{2+} (a Ca^{2+} antagonist). Two parallel studies were done: in one, the effect of the modified medium on fast axonal transport of [^{3}H]glycoproteins was quantitated by liquid scintillation counting of the TCA-insoluble radioactivity in nerve segments; in the other, the effect of the modified medium on the distribution of label in the DRG was assessed by light microscopic radioautography.

With either calcium-free medium + 1 mM EGTA or normal medium + 0.18 mM $CoCl_2$, the biochemical analysis showed that the quantity of [^{3}H]glycoproteins carried by the fast transport system was greatly reduced as compared to the contralateral control. However, the incorporation of [^{3}H]fucose into glycoproteins, which occurs mostly during the incubation period (Hammerschlag & Lavoie; Neuroscience 4: 1195, 1979), was normal. This would indicate that proteins destined for transport had migrated normally to the Golgi apparatus. In radioautographs of control ganglia, reaction in most neurons was diffusely scattered over the cytoplasm of the cell body; in instances where the beginning (unmyelinated) portions of axons were seen in continuity with neuronal cell bodies, the axoplasm exhibited a diffuse reaction of the same intensity as that of the cell body. After longer exposure times, diffuse reaction also appeared over the axoplasm of myelinated axons. In experimental ganglia, reaction in most neuronal cell bodies was concentrated in the paranuclear Golgi region and less diffuse reaction was present; in instances where the beginning portions of axons were observed, the axoplasm was usually unlabeled. Similarly, even after long exposure times, the axoplasm of myelinated axons remained relatively free of label. These results provide further support for the concept that glycoproteins destined for fast axonal transport pass through the Golgi apparatus, and suggest that Ca^{2+} is necessary for the glycoproteins to leave the Golgi apparatus.
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30.4 SEQUENTIAL DAMMING ANALYSIS OF GLYCOPROTEIN AXOPLASMIC TRANSPORT. Johnson* J.L. & J.-H. Kim, Div. of Biochem., Physiol. & Pharmacol., USD School of Medicine, Vermillion, S.D. 57069

The precise nature of the distribution of most highly mobile glycoproteins in the axonal advancing wave has not yet been precisely defined. The purpose of this study therefore is to use a sequential damming technique in order to define this distribution. Following dorsal root ganglionic injection of ^{3}H-fucose an *in vivo* flow time of 4 hrs, 10 hrs or 24 hrs was allowed after which the cat was sacrificed and the sciatic nerves separated from the ganglion, cleared of excess material and crushed at 30mm, 60mm, 90mm and 120mm distances from the most proximal site. The nerves were then placed in an oxygenated Ringer's solution (37°C) for 4 hrs of *in vitro* damming (4+4, 10+4 and 24+4 hrs). At 4+4 hrs, damming was only seen at 30+60mm sites, while at 10+4 hrs damming was seen at all sites (transport rate = 360mm per day). In all cases, the damming level decreased exponentially when passing towards the ganglion side from the 120mm site. For the 10+4 hr, the equation for this was $\ln Y = 0.0125X + 4.556$ (Y=% damming, X=distance in mm, correlation coefficient = 0.998, N=4). This may reflect an exponentially decreasing output of the pulse injected fucose label, and predicts, when extrapolated back to the ganglion, that at 10 hrs there was no more output of this labeled fraction. Consistent with this was the observation that by 24+4 hrs the damming amount was much less than that seen at 4+4 or 10+4 hrs. The slope of this trailing edge was 26% steeper at 10 hrs than at 4 hrs. Consistent with this was the observation that the distal damming was greater at 10 hrs than at 4 hrs. This suggests an enrichment of the fast transported material with passage away from the ganglion. Assuming a constant output of these fast transported proteins, it was calculated that over the entire distribution at 10 hrs, 40% was labeled protein and 60% was newly formed and unlabeled. Analysis of the damming site using SDS-acrylamide gel electrophoresis showed that although several molecular weight ranges were involved in this damming, glycoprotein in the range of 36-58,000 are the most enriched. This is consistent with data (Brain Res., 121, 1977, 215) suggesting that glycoproteins in this range contain components that are rapidly conveyed to the synapse as well as deposited in the axon.
SUPPORTED BY PARSONS TRUST FUND

30.5 BATRACHOTOXIN BLOCKS SALTATORY ORGANELLE MOVEMENTS IN ELECTRICALLY EXCITABLE NEUROBLASTOMA CELLS. D. S. Forman and W. G. Shain, Jr. Naval Medical Research Institute and Armed Forces Radiobiology Research Institute,NNMC,Bethesda,MD 20014.

The fast axonal transport of intra-axonal organelles that can be visualized by light microscopy proceeds by a series of saltatory movements, similar to those seen in other types of cells (Forman et al., Brain Res. 136:197, 1977). Saltatory organelle movement in neurites of neuroblastoma cells is a convenient model for studying this process in a cell with neural properties. Batrachotoxin inhibits fast axonal transport of labeled materials, and there is evidence suggesting that this effect may be independent of the well-established action of batrachotoxin in opening action potential Na^+ channels (Ochs and Worth, Science 187:1087, 1975; Kumara-Siri, J. Neurobiol. 10:509, 1979). It was therefore of interest to determine whether batrachotoxin would inhibit saltatory organelle movements. N115 neuroblastoma cells were grown on glass coverslips, and were observed in a continuously perfused chamber using phase optics and time-lapse Video Intensification Microscopy (Willingham and Pastan, Cell 13:501, 1978). Batrachotoxin inhibited saltatory movement in a time- and concentration-dependent manner. In 0.5 µM batrachotoxin saltatory movement stopped in most cells within 1 hr. In 1.0 µM batrachotoxin this time was reduced to 30 minutes, while in 0.1-0.2 µM more than an hour was required, and little or no effect was seen after 3 hours in 0.01 µM. This dose-response curve is consistent with that for the activation of action potential Na^+ channels in neuroblastoma cells by batrachotoxin (Catterall, J. Biol. Chem. 250:4053, 1975). The inhibition of saltatory movements by 0.5 µM batrachotoxin was reversible. Batrachotoxin also produced other changes, including neurite retraction and organelle clumping, but membrane ruffling movements were not inhibited. 1 µM tetrodotoxin, which blocks the Na^+ channels opened by batrachotoxin, completely prevented the effects of 0.5 µM batrachotoxin. Batrachotoxin (1.0 µM) had no effect on a number of cells that are not electrically excitable: 3T3 fibroblasts, and N103 and LB4 neuroblastoma cells. Replacing Na^+ in the medium with choline partially prevented the effects of 0.5 µM batrachotoxin, although morphological changes such as neurite retraction occurred in many cells. This evidence is all consistent with the hypothesis that the primary action of batrachotoxin is the activation of the action potential Na^+ channels, and that the inhibition of saltatory organelle movements (and by inference, fast axonal transport) may be a consequence of the disruption of normal internal ionic concentrations following depolarization of the cells.

30.6 4S RNA IS AXONALLY TRANSPORTED IN INTACT AND REGENERATING SCIATIC NERVES OF RATS. T.D. Lindquist*, N.A. Ingoglia, and R.M. Gould. (SPONS: F.P.J. Diecke) CMDNJ-New Jersey Med. Sch., Newark, NJ 07103 and New York State Inst. for Basic Res. Ment. Retard., Staten Island, NY 10314.

Experiments were designed: (1) to determine if following application of ^{3}H-uridine to ventral horn cells of the rat spinal cord ^{3}H-RNA could be demonstrated within intact and/or regenerating axons of the sciatic nerve, and (2) to test the hypothesis that only 4S RNA is transported axonally in this system.

In the first set of experiments the left sciatic nerve of rats was crushed. Two days later 170 µCi of ^{3}H-uridine was injected into the vicinity of the ventral horn cells giving rise to the sciatic nerve, thus labeling the nerve by axonal transport. Ten days after injection, rats were sacrificed and sciatic nerves were prepared for light and EM autoradiography. Photomicrographs were taken of randomly labeled areas of intact and regenerating nerves and grains were counted over Schwann cells, myelin, axons and other unspecified areas. Approx. 22% of the grains were associated with axons, 43% with Schwann cell cytoplasm, with the remainder either over myelin or other non-neural structures. Thus >20% of the ^{3}H-RNA in the nerve is present in axons.

In the second series of experiments the left sciatic nerve was crushed and 10 days later (^{3}H)-uridine was bilaterally injected intraspinally into 6 rats. Four control rats were sacrificed at 14 or 20 ds after injection. In the remaining 2 rats the sciatic nerve was cut 14 ds after injection and the distal part of the nerve was allowed to degenerate for 6 ds before sacrificing the rat. Thus, the distal portion of the nerve contained Schwann cells labeled by axonal transport but lacked intact axons. RNA was isolated from experimental and control nerve segments by hot phenol extraction and ethanol precipitation. RNA species (28S,18S and 4S) were separated by polyacrylamide gel electrophoresis and radioactivity was measured in a liquid scintillation counter. Control groups had RNA profiles similar to those already described, with approx. 40% of the radioactivity present as 4S RNA. The proximal portions of nerve taken from the group in which nerves were cut, also had approx. 40% of the radioactivity present as 4S RNA. However, in the distal segments of these nerves (in which the axons had degenerated thus creating an "axon-less" nerve) the amount of radioactivity in the 4S peak decreased to approx. 15% of the total RNA, suggesting that 4S RNA is the predominant if not the only RNA present in these axons. These results strongly indicate that both intact and regenerating axons in the sciatic nerves of rats selectively transport 4S RNA along their axons.

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30.7 POSTNATAL CHANGES IN AXONALLY TRANSPORTED ^{3}H-FUCOSYL MEMBRANE PROTEINS AND GLYCOLIPIDS OF HAMSTER OPTIC NERVE SYNAPTOSOMES. Susan Corey Specht and Teresa Candelas*. Dept. of Pharmacology, Univ. Puerto Rico Sch. Med., San Juan, PR 00936.

The glycoconjugates axonally transported to optic nerve endings of neonatal and adult hamsters were studied following intraocular injection of ^{3}H-fucose. Subsynaptosomal fractions were isolated by the method of Cohen et al. (J. Cell Biol., 74, 181, 1977), and precipitated with 5% TCA for analysis of incorporated ^{3}H-fucose. One day post-injection labeling was predominately in the external membrane fraction of adults with only 9% in the "vesicle" fraction, whereas approximately 35% was in the "vesicle" fraction of 12 and 16 day neonates. Nonetheless, in adults sacrificed 3 h post-injection, 35% of the ^{3}H-fucose was present in the "vesicle" fraction, suggesting rapid turnover in adults.

A Folch-Suzuki procedure was used to extract and partition glycolipids. Although the "vesicle" fraction contained only 8-14% of total synaptosomal protein, at least 80% of the label was in CM-insoluble or CM-extracted glycoprotein; a maximum of 15% was found in the ganglioside phase of both adults and neonates. Hence, "vesicle" fraction glycoprotein has the highest specific activity (dpm/mg protein) of subsynaptosomal components.

Synaptosomal membranes isolated by the flotation gradient method of Jones and Matus (Biochim. Biophys. Acta, 356, 276, 1974) were subjected to SDS-polyacrylamide slab gel electrophoresis 24 h following intraocular injection of ^{3}H-fucose. Protein bands comigrating with α-tubulin, β-tubulin, actin and at 55K were noted at all ages in addition to 48-52K bands. The most heavily labeled band in adults and 16 day hamster optic nerve membrane had an apparent molecular weight of 48-52K. This band did not contain detectable ^{3}H-fucose at 5 days postnatal (major labeling was in bands of more than 100K), and was a minor labeled band at 12 days postnatal. Hence, fucosylation of a major synaptic membrane protein demonstrates a developmental pattern. (Supported in part by USPHS grant EY 02334.)

30.8 SLOW AXONAL TRANSPORT IN GOLDFISH OPTIC AXONS. Roberta M. Alpert*, Bernice Grafstein, and D. Louise Edwards. (SPON: T.J.Cunningham). Dept. Physiology, Cornell Univ. Med. Coll., New York, NY 10021.

Axonal transport of proteins in the optic axons of goldfish can be studied by injecting a radioactively labeled amino acid into the eye and then determining the rate of displacement of radioactively labeled protein along the optic nerve. Slow axonal transport manifests itself in the appearance of a gradient of radioactivity extending along the nerve for several mm behind the eye. A semi-logarithmic plot of the distribution of radioactivity gives a straight line, the slope of which changes with time after the injection as the slow transport advances (B. Grafstein and M. Murray, Exp. Neurol. 25, 494, 1969).

In the present study, measurements of the change in slope were made to determine whether the rate of advance of slow transport was affected by various experimental conditions. The experiments were carried out on fish in which the optic tract had been cut 6-8 days earlier, in order to accelerate the rate of change of slope. After the injection of 4 µCi of ^{3}H-leucine into the eye, 2 days were allowed to elapse to permit the slowly transported protein to emerge from the eye into the nerve, and then one of the following procedures was carried out on the experimental group of animals: a) block of retinal protein synthesis by intraocular injection of cycloheximide or actinomycin-D; b) removal of the retina; c) block of physiological activity in the retina by intraocular injection of tetrodotoxin; d) lowering the temperature. In each case the experimental conditions were maintained for 24 or 48 hours, while the control animals remained under normal conditions for the same period of time. The optic nerves were then fixed in Bouin's solution and divided into 250 µ segments, which were assayed by liquid scintillation counting.

These experiments have led to the following conclusions: slow transport is immediately arrested when retinal protein synthesis is blocked; the transport continues for 24 hours in axons that have been separated from their cell bodies; abolition of action potentials by tetrodotoxin does not affect the transport velocity; the transport velocity does not change with lowering of the temperature, but transport is blocked at a temperature of about 10° C. The arrest of slow transport upon inhibition of cell body protein synthesis and the insensitivity of the transport velocity to low temperature are features that distinguish this mode of transport from fast axonal transport.

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30.9 DIRECT VISUALIZATION OF THE CYTOSKELETAL NATURE OF AXOPLASM. Thomas J. Deerinck* and Mark H. Ellisman Dept. of Neuroscience, U. Calif. at San Diego, La Jolla, CA 92093

It has been suggested that the electron scattering properties of epoxy resins hinder the full visualization of the finer components within the matrix of axoplasm. To circumvent this potential problem, we have adapted a technique that allows the direct visualization of axoplasm in thin sections with transmission EM (TEM) or with scanning EM (SEM) without an embedding matrix.

The technique involves fixing rat spinal roots in glutaraldehyde, paraformaldehyde, and tannic acid, post-fixing in osmium tetroxide and infiltrating with a water soluble polyethylene glycol (PEG) compound. Sections are cut on a special glass knife into water and mounted underwater on polybutene-coated grids. The PEG is further washed out with water and the sections are dehydrated and critical point dried.

Earlier, in standard epoxy preparations we noted that the so-called wispy fragments projecting from microtubules and neurofilaments within axoplasm are part of a three-dimensional lattice. This lattice or microtrabecular matrix of axoplasm was found to consist of an organized system of crossbridges between microtubules, neurofilaments (100Å filaments), cisternae of the smooth endoplasmic reticulum and the plasma membrane. Unlike conventional epoxy sections where the microtrabecular lattice of axoplasm appears as wispy, discontinuous fragments, these "tissue only" PEG sections show an axoplasm rich with a continuous crossbridging lattice.

Preparations derived from PEG embedded tissue may be examined in the SEM after removal of the embedding matrix. For these purposes we have found it most useful to cut a large block face, then save, not the sections, but the block face, removing the PEG and further processing the tissue as mentioned above. Where axons have been sectioned open, the fibrous nature of the axoplasm may be readily appreciated. At high magnifications neurofilaments and microtubules may be recognized as well as fine lateral projections corresponding to the trabecular elements described in our PEG thin sections. The preparation of PEG embedded blocks of tissue for SEM is far less difficult and more rapid than preparing sections of PEG embedded material for TEM.

Using a periodate-lysine-paraformaldehyde fixative (McLean & Nakane, 1974) that preserves antigenicity, we have shown that the lattice structure is maintained long enough to permit immunocytochemical localizations. Either sections or block faces from these preparations may be suitable for immunoelectron microscopy and may help circumvent antibody accessability problems.

Supported by NIH grant NS14718 and a grant from MDAA to M.H.E.

30.10 THE ULTRASTRUCTURAL SITE OF FAST AXONAL TRANSPORT INITIATION: ROLE OF THE GOLGI APPARATUS AND SMOOTH ENDOPLASMIC RETICULUM. James D. Lindsey and Mark H. Ellisman, Department of Neurosciences, University of California at San Diego, La Jolla, CA 92093.

Recent studies have shown that the initiation of fast axonal transport requires the presence of calcium and is selectively poisoned in the presence of 0.18 mM cobalt (Hammerschlag et al., 1977). To identify the cellular systems that might play important roles in transport initiation, frog spinal ganglia were incubated for 24 hrs. in media known to inhibit the export of glycoproteins from the soma to the axon, and then examined electron microscopically. When compared with incubation in normal frog Ringer's medium (NM), incubations in calcium free media (CFM), NM supplemented with 0.18mM cobalt (NM-Co), and CFM supplemental with 0.18mM cobalt (CFM-Co) had little effect on the morphology of most somal organelles. Striking changes, however, were seen in the smooth endoplasmic reticulum (smooth ER) and the Golgi apparatus.

Quantitative analysis showed that in order of effect, CFM, NM-Co, and CFM-Co produced increasing amounts of smooth ER coupled with decreasing densities of identifiable Golgi apparatus stacks. In the extreme case CFM-Co incubation resulted in a nearly 10-fold increase in the smooth ER volume as well as a virtually complete depletion of Golgi apparatus stacks. The accumulated smooth ER often appeared as clumps of rounded profiles and was strongly associated with rough ER. After NM-Co incubation, the remaining GA stacks usually exhibited swollen cis pole elements. Disruption at the cis pole was also noted after CFM incubation, however, it was much less pronounced than after NM-Co incubation.

Incubations in CFM-Co for 12 hr. produced results similar to the longer incubation except that some Golgi apparatus stacks remained. After processing 12 hr. CFM-Co preparations for the demonstration of thiamine pyrophosphatase, the trans elements of these Golgi apparatus stacks stained similarly to those of control ganglia. The accumulated smooth ER profiles, however, were not stained by this technique.

Axons originating within the ganglia were also examined. CFM and NM-Co incubation resulted in moderate vesiculation of the axonal smooth ER. CFM-Co incubation resulted in moderate swelling of the SER profiles. Otherwise, the incubations had little effect on axonal ultrastructure.

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30.11 HORSERADISH PEROXIDASE DETERMINATION OF BRAIN STEM AND DIENCEPHALIC INNERVATION OF THE LUMBAR AREA OF THE SPINAL CORD. S. L. Scharoun*, F. C. Barone and M. J. Wayner. Brain Research Lab, Syracuse Univ, 601 Univ Ave, Syracuse, NY 13210.

Horseradish peroxidase (HRP, Sigma Type VI, 50%) was injected into the second lumbar segment of the spinal cord of male hooded rats. After 36-48 hr the animals were perfused intracardially with a phosphate buffer plus a sucrose wash followed by glutaraldehyde and paraformaldehyde fixative. The whole brain and the injected portion of the spinal cord were removed and sectioned. Alternate sections were processed with DAB and counterstained with cresyl violet, and with BDH and counterstained with neutral red for the brown and blue reactions respectively. Sequential sections were examined and positively labeled neurons were identified and photographed by a microscope with both light and dark fields. Labeled neurons were identified in the lateral hypothalamic and ventromedial hypothalamic regions of the diencephalon. In addition, labeled neurons and axons were also observed in the brain stem. These results, based on the retrograde movement of HRP, indicate that direct innervation of spinal motor neurons occurs from some neurons in the ventral diencephalon and that these direct connections, together with polysynaptic connections through the descending reticular formation and other mesencephalic regions, are involved in the motor control functions of the hypothalamus. (Supported by NIH Grant NINCDS USPHS No. 13543)

31.1 HIPPOCAMPAL POLYMORPHE NEURONS PROJECT TO THE "COMMISSURAL" DENDRITIC ZONE OF THE DENTATE GYRUS. S. L. Semple-Rowland*, J.L. Bassett* and T. W. Berger (SPON: A. Mallinger). Psychobiology Program, Dept. Psych., Univ. of Pittsburgh, Pittsburgh, PA 15260.

A longitudinal association system in the dentate gyrus has been anatomically identified by Zimmer (J. Comp. Neurol., 142:393 1971). This projection terminates within the inner one-third of the dentate dendritic region, the same zone which receives commissural hippocampal input (Gottlieb, D.I. and Cowan, W.M., J. Comp. Neurol., 149:393, 1973). While Zimmer's evidence implicated the CA3c/CA4 pyramidal neurons as the cells of origin for this projection, hilar polymorphic neurons could not be ruled out (Lorente de No, R., J. Psychol. Neurol., Lpz., 46:113, 1934). We have begun analysis of the system using axonal transport techniques, and here report the conclusive identification of polymorphe neurons of the hippocampal hilus as the cells of origin for afferents to the inner one-third of the dentate stratum moleculare.

All studies were conducted in male, New Zealand white rabbits. Horseradish peroxidase (HRP-Sigma, type VI, concentration of 33%) in volumes of 0.02-0.10 ul was injected into various septo-temporal regions of the hippocampus proper. Tissue was prepared according to Mesulum (J. Histochem. Cytochem., 24:1273, 1976; ibid., 26: 106, 1978). For autoradiographic experiments, volumes of 0.1-0.2 ul of ^{3}H proline (New England Nuclear) in 50 uCi/ul concentration were injected into the hilar region of the temporal hippocampus, and material was prepared with established procedures (Cowan, W.M. et al., Brain Res., 37:21, 1972).

Examination of temporal hippocampal regions following septal hippocampal HRP injections revealed retrograde cell labeling of polymorphic neurons within the temporal dentate hilar area. In almost all cases, HRP-containing neurons were easily discriminated as lying directly beneath the dentate granule cell layer and regionally separate from CA4 hilar neurons. Only neurons in this location were ever observed as labeled, and these were seen only after injections involved the dorsal hippocampal dentate gyrus. Labeled polymorphe neurons were seen both ipsilaterally and contralaterally to the injection site.

When either HRP or tritiated amino acids were injected into the dentate/hilar region of the temporal hippocampus, dense terminal labeling was visible in the inner one-third of the molecular layer of the dentate gyrus, in a laminated pattern identical to that described by Zimmer. No other region of the hippocampus was seen to contain anterogradely transported product. We feel this evidence conclusively identifies hippocampal hilar polymorphe neurons as the cells of origin for afferents to the "commissural" zone of the dentate gyrus.

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31.2 ASSOCIATION CONNECTIONS BETWEEN POSTERIOR AND ANTERIOR LIMBIC CORTICES IN THE RABBIT. J. L. Bassett* and T. W. Berger (SPON: R. Jennings). Psychobiology Program, Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260

Association connections between subdivisions of cingulate cortex have been difficult to evaluate with degeneration anatomical methods (Domesick, V.B., Brain Res., 12:296, 1979). We have recently begun an examination of limbic cortical connectivity with axonal transport techniques, and here report the existence of reciprocal associational pathways between posterior cingulate/retrosplenial and anterior limbic cortical regions in the rabbit brain.

Terminology for limbic cortical regions refers to that of Rose and Woolsey (J. Comp. Neurol., 89:279, 1949) for the rabbit. That is, posterior limbic cortex is assumed to include granular cingulate and retrosplenial components; rostral agranular limbic areas of interest here are the anterior limbic and infralimbic cortical subdivisions. HRP (Sigma, type VI, concentration 33%) in volumes of 0.02-0.05 ul was injected into cingulate, retrosplenial, or anterior limbic cortical zones. Following a survival time of 24-48 hours, tissue was prepared according to Mesulum (J. Histochem., 24:1273, 1976; ibid., 26:106, 1978). For autoradiographic experiments, ^{3}H proline in 50uCi/ul concentration was injected into the above cortical regions and material was prepared with established procedures (Cowan, W.M. et al., Brain Res., 37:21, 1972).

After HRP injections into posterior limbic cortex, retrogradely labeled neurons were consistently seen within what Rose and Woolsey have termed the anterior limbic cortical region (i.e. ventral to the precentral agranular area). HRP-positive neurons were occasionally found within the infralimbic area as well. Injections producing this retrograde pattern typically involved the cingulate and dorsal retrosplenial cortical regions. Anterogradely transported product from these anterior limbic regions was concentrated in layer I of both cingulate and retrosplenial cortex.

Injections of HRP in anterior limbic cortex resulted in heavy retrograde labeling of posterior retrosplenial cortical cells of lamina V. Injections did not infringe on the cingulum, so the latter labeling should not have resulted from uptake and transport by ruptured corticofugal fibers of unknown termination, or from corticothalamic projections which arise from layer VI (Berger, T. W. et al., Neurosci. Abstr., 5:270, 1979). To insure this was not the case, however, tritiated amino acids were injected into posterior cingulate/retrosplenial cortex. Silver grains were visible in the anterior cingulum, as expected, but were also evenly distributed over lamina in the anterior limbic cortex.

In total, these results demonstrate reciprocal associational connections between major subdivisions of limbic cortex in the rabbit.

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31.3 DEVELOPMENT OF CA1 NEURONS IN RABBIT HIPPOCAMPUS. P. A. Schwartzkroin, L. H. Mathers, D. D. Kunkel, Dept. Neurological Surgery, Univ. Washington, Seattle, WA 98195 and Dept. Biological Structure, Stanford Univ., Stanford, CA 94305.

The development of hippocampal neurons in the CA1 region of rabbit has been studied using electrophysiological and neuroanatomical techniques. Intracellular recordings were obtained from cells in animals from fetal stage to the adult using an in vitro hippocampal slice preparation. At comparable ages, hippocampal tissue was taken for light and electronmicroscopic investigation.

Recordings from young rabbits revealed spiking activity and functional synaptic contacts. Healthy impalements were difficult to obtain, presumably due to the small cell size. Resting potential in good penetrations, however, was similar in mature and immature rabbits. Action potentials were somewhat broader in young animals than in the adult (> 2.0 msec at birth vs. 1.4 msec in adults), and cell input resistance was considerably higher (50-60 MΩ in newborn vs. 25 MΩ in adults). These differences were undetectable by the second to third weeks of age. Calcium spikes were seen in very young rabbits (4 days, probably younger), but in no case were calcium spikes seen before sodium spikes. Onset of spiking activity appeared to be 7-10 days before birth.

Synaptic events were first observed 2-5 days before birth, and events were almost always long duration depolarizations. These EPSPs were elicited by stimulation in strata radiatum and oriens, could trigger action potentials, and were associated with conductance increases. Repetitive stimulation at 3-10 Hz produced EPSP potentiation and in some cases led to seizure after-discharge. Animals 1-2 weeks old were particularly seizure-prone. IPSPs were rarely seen in animals less than 2 weeks old; IPSPs were apparent after this time, as were cell types tentatively identified as interneurons.

Anatomical studies provided parallel data. Light microscopic analysis indicated that the dendritic regions of CA1 undergo the greatest expansion during development. Dendritic length, number of branches, and spine density all increase to near adult levels at 3 to 4 weeks of age. Synapse number and location were analyzed in EM photomicrographs from CA1 pyramidale and radiatum regions, using both osmicated and E-PTA stained material. In both regions there was a monotonic increase in number of synapses in animals up to 1 month of age. Synapses in stratum pyramidale were rare in young animals, the earliest synapses being concentrated in dendritic regions. Symmetrical synapses first appeared in countable numbers in animals 5-10 days old, just prior to onset of physiologically-recorded IPSP activity.

31.4 NEUROGENESIS IN THE RAT AMYGDALA. S. A. Bayer. Dept. of Biol. Sci., Purdue Univ., W. Lafayette, IN 47907.

Neurogenesis in the rat amygdala was examined with ^{3}H-thymidine radiography. The animals were the offspring of pregnant females given two injections of ^{3}H-thymidine on consecutive days in an overlapping series: Embryonic day (E) 12+E13, E13+E14, ... E21+E22. On 60 days of age, the percentage of labelled cells and the proportion of cells added during each day of formation were determined at several anatomical levels within the amygdala.

All large and many small neurons originate in most nuclei between E13-E17, those in the intercalated masses between E15-E19, those in the amygdalo-hippocampal area between E16-E19. The anterior amygdaloid area, intercalated masses, central, medial, posterolateral cortical, posteromedial cortical, basomedial, basolateral and lateral nuclei have strong rostral to caudal intranuclear gradients. There are five additional intranuclear gradients: 1) medial to lateral in the central nucleus, anterior amygdaloid area, and anterior intercalated masses; 2) lateral to medial in the bed nucleus of the lateral olfactory tract and basolateral nucleus; 3) superficial to deep in the amygdalo-hippocampal area, posterolateral and posteromedial cortical nuclei; 4) ventral to dorsal in the medial nucleus; 5) dorsal to ventral between the small and large-celled parts of the lateral nucleus. Only the bed nucleus of the accessory olfactory tract and the anterior cortical nucleus do not have intranuclear gradients. Between 10-15% of the total cell population in most nuclei are very small neurons and/or glia which originate simultaneously between E18-E20. This population is absent in the ventral part of the medial, anterior cortical, and anterior basomedial nuclei; these contiguous areas may form a distinct subunit in the amygdala.

In contrast to the pronounced intranuclear gradient, internuclear gradients are weak. There are groups of early-originating neurons in the central, medial and basolateral nuclei located near the periphery of the amygdala. Each of these groups is surrounded by younger neurons farther within the interior. The youngest cells are in the centrally-placed intercalated masses. These settling patterns suggest that cells in the amygdala arise simultaneously from more than one neuroepithelial source during morphogenesis. The chronology of neurogenesis in the amygdala may be related to some of its anatomical connections; for example, rostral to caudal gradients in the corticomedial complex may be timed to coincide with early vs. late arrival of olfactory fibers.

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31.5 FURTHER OBSERVATIONS ON THE DEVELOPMENT OF THE CONNECTIONS OF THE HIPPOCAMPAL FORMATION. D.G. Amaral, B.B. Stanfield and W.M. Cowan. Dept. of Anatomy and Neurobiology, Wash. Univ. Sch. of Med., St. Louis, MO 63110.

The development of certain of the afferent and efferent connections of the hippocampal formation of the rat has been examined by making relatively small injections of ^{3}H-proline or horseradish peroxidase (HRP) into either the hippocampal region itself or the diencephalon, on the day of birth (day 0) or on postnatal day 1,2,3,5,7 or 9; in each case the post-injection survival time was 6 hours.

Following injections of HRP into the hippocampus on day 0, retrogradely labeled pyramidal and hilar cells were seen in the contralateral hippocampus in those areas which, in mature animals, are known to contribute to the commissural projection. Comparable injections of ^{3}H-proline at this stage resulted in substantial labeling in both the *stratum oriens* and the *stratum radiatum* of the contralateral *regio superior* and *regio inferior* of the hippocampus but failed to give rise to label in the contralateral dentate gyrus. However, both the ipsilateral associational and the entorhinal projections to the dentate gyrus were labeled by ^{3}H-proline injections on day 0.

On day 1, diencephalic afferents to the hippocampus (but not to the dentate gyrus) could be seen after ^{3}H-proline injections. The commissural fibers may have reached the septal pole of the dentate gyrus by day 1 but this projection was not clearly labeled until day 2. Hypothalamic injections on day 3 gave rise to labeled fibers within the developing *stratum moleculare* of the supra-pyramidal blade of the dentate gyrus.

Some observations were also made on the development of hippocampal efferents. The projections from the subicular complex to the thalamus and hypothalamus could be seen as early as day 0, as could the projection from the hippocampus to the lateral septum. The hippocampal and subicular projections to the entorhinal cortex (especially layer 4) were labeled on postnatal day 1.

These results demonstrate that the various hippocampal afferents investigated reach their sites of termination somewhat earlier than has previously been shown and, when taken together with developmental ultrastructual studies, indicate (at least for the dentate gyrus) the major afferent fiber systems are present and topographically arranged in a manner characteristic of their eventual lamination several days before significant numbers of morphologically-identifiable synapses can be seen in their zones of termination. Although the different fiber systems appear to be topographically segregated from the time of their arrival, we cannot exclude the possibility of some degree of overlap between them, when they first reach their target regions.

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31.6 NEURONS OF ORIGIN OF THE PERFORANT PATH. P.D. Coleman and S.P. Schwartz* (SPON: J. Ison). Dept. of Anatomy, Univ. of Rochester Sch. of Med. and Dent., Rochester, NY 14642.

The perforant path projection from entorhinal cortex to the hippocampal formation was first described over 70 years ago by Cajal. Yet, the identity of the neurons of origin of this pathway remains uncertain. Recent investigations utilizing the horseradish peroxidase (HRP) retrograde transport method have provided evidence that these neurons are in layers II and III of the entorhinal cortex. However, the unequivocal identification of these cells in terms of the cell types seen in Golgi-stained material could not be accomplished in these retrograde transport studies.

Diffuse filling of injured axons with HRP has been used to reveal Golgi-like pictures of dendritic and axonal arborizations. We have applied this method to determine the form of the neurons of origin of the perforant path by inserting an HRP-laden plug of Gelfoam directly into the dorsal hippocampal formation of Fisher 344 rats. After a survival of 22 hours, the animal was perfused in accordance with the procedure of Rosene and Mesulam (J. Histochem. Cytochem., 26:28, 1978). The brain was frozen-sectioned at 80μm horizontally and processed by a modified version of Adam's diaminobenzidine method (Neurosci., 2:141, 1977). Heavily labeled cells containing both the granular and diffuse reaction products were observed in layer II of the ipsilateral entorhinal cortex. These labeled cells were both stellate and fusiform neurons with the former predominating in pars medialis and the latter in pars lateralis. In agreement with Golgi descriptions of this region, the dendritic ramifications of both cell types were largely restricted to layers I and II. Less intensely labeled pyramidal cells containing only the granular form of reaction product were seen in layer III both ipsilaterally and contralaterally.

Our findings demonstrate for the first time that the layer II neurons of entorhinal cortex which project to the hippocampal formation (LaVail, J.H., et al., Brain Res., 58:470, 1973; Segal, M. and S. Landis, Brain Res., 78:1, 1974; Steward, O. and S.A. Scoville, J. Comp. Neur., 169:347, 1976) are stellate and fusiform cells.

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31.7 A DEVELOPMENTAL COMPARISON OF LTP IN AREAS CA1 AND FASCIA DENTATA OF THE RAT HIPPOCAMPAL FORMATION. K. M. Harris, T. J. Teyler, and W. L. R. Cruce. Program in Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Granule cells of rat dentate gyrus are known to proliferate postnatally with peak proliferation at or about postnatal day 17, while pyramidal cells of area CA1 show their peak proliferation by embryonic day 19 and are not known to proliferate postnatally. With these developmental phenomena in mind we have begun experiments to determine if functional plasticity also follows a differential time course in these two regions. We have used the in vitro hippocampal slice preparation to study long-term-potentiation (LTP) in both of these areas from postnatal day 1 to postnatal day 30 and are continuing studies of more mature animals. As a measure of response plasticity, LTP is observed in the CA1 pyramidal cell layer or dentate granule cell layer as an increase in extracellular evoked population response to monosynaptic activation following a brief tetanus applied to Schaffer collaterals or perforant pathway fibers, respectively.

For each age group, animals were randomly choosen from a litter so that several age groups were represented across litters, and littermates were compared across age groups. Young animals were killed by decapitation and the hippocampus was rapidly dissected free, sliced at 400μm thickness, and several slices transferred to different pools in an incubation chamber (Alger and Teyler, 1976, Brain Research, 110:463-480). In this system the slices remained healthy for at least four hours as shown by absence of decay in responsivity to afferent stimulation of either CA1 or dentate gyrus.

After the slices equilibrated in vitro for an hour, stimulating and recording electrodes were lowered into either area CA1 or dentate gyrus. Since several slices were available for use from each animal, measurements from area CA1 pyramidals were made from one slice and another slice from the same hippocampus was used to measure responses of dentate granule cells. To equate for length of in vitro incubation, area CA1 was examined first in some experiments, whereas the dentate gyrus was studied first in other experiments.

Preliminary results reveal a trend for area CA1 to lead in LTP magnitude until day 20. From day 20 to day 30 the pattern reverses, with the dentate gyrus showing more LTP, and CA1 LTP magnitude declining from earlier levels. Further studies will be required to determine whether the pattern will again reverse itself during maturation. For both areas the magnitude of potentiation is less than that reported for adults in previous studies.

31.8 NEURONAL ACTIVITY IN THE PREFRONTAL CORTEX, CAUDATE NUCLEUS AND MEDIODORSAL THALAMIC NUCLEUS DURING DELAYED RESPONSE PERFORMANCE OF IMMATURE AND ADULT RHESUS MONKEYS. G.E. Alexander, E.D. Witt and P.S. Goldman-Rakic. NICHD and NIMH, NIH, Bethesda, MD; and Yale School of Medicine, New Haven, CT.

For more than four decades it has been known that the dorsolateral prefrontal cortex (DLCx) in adult monkeys participates in the mediation of delayed response performance, since this capacity is impaired in animals which have undergone selective ablations or local cooling of this region. In immature monkeys, however, ablation or functional depression of the DLCx does not result in disruption of the capacity for delayed response performance, and recent studies have demonstrated that the monkey's dependence upon the DLCx in performing cognitive tasks such as delayed response develops gradually over the first three years of postnatal life. In contrast, the head of the caudate nucleus (HCN) and the mediodorsal thalamic nucleus (MDN), the principal subcortical nuclei which are known to participate in delayed response performance and which are connected anatomically to the DLCx, are of critical importance in both adult and infant monkeys. Lesions of either structure, regardless of age, result in impaired delayed response capacity.

We examined the activity of individual neurons in DLCx, HCN and MDN during delayed response performance in a total of 5 adult (36 months) and 7 immature (12 months) rhesus monkeys, using chronic extracellular recording techniques. Of the 261 DLCx units recorded from adult monkeys, 38% were activated during the delay interval between cue and response, whereas only 20% of the 268 units recorded from immature monkeys were so activated ($p<.001$). In contrast, there was no significant disparity between adults and infants in terms of the proportion of subcortical neurons activated during the delay interval. In the HCN 31% of 106 adult neurons and 32% of 140 infant neurons were activated during the delay interval. Similarly, in the parvocellular division of the MDN 34% of 76 adult neurons and 30% of 190 infant neurons manifested delay-related increases in firing.

The present data provide the first elecrophysiological evidence that subcortical mediation of delayed response performance in rhesus monkeys matures early in postnatal life--prior to one year of age. In contrast, the role of the DLCx in delayed response performance continues to grow throughout development and does not become prominent until 3 years of age. This age-dependent increase in the importance of the DLCx may be related to the two-fold expansion in the population of delay-activated prefrontal neurons which appears to occur during postnatal development.

31.9 PSEUDOPODIA BETWEEN MAMMALIAN NERVE TERMINALS ARE POTENTIALLY PLASTIC, PROBABLE POTASSIUM TRAPS. Alan F. Boyne & Sally B. Tarrant*, Dept. Pharm. & Exptl. Therap., Univ. MD, Sch. Med., Baltimore, MD 21201.

Stimulation of Torpedine ray electric organ can cause loss of synaptic vesicles and formation of pseudopodia. These extend from individual nerve terminals and penetrate reciprocal indentations in abutted terminals. Last year, we reported that similar structures occur in axodentritic terminals of unstimulated, perfused rat brains in the central nucleus of the amygdala.

Can significant concentrations of extracellular K^+ accumulate in the narrow space between pseudopodium and indentation during trains of action potentials? Literature values suggest release of 1 p'mole of K^+/cm^2/action potential. Assuming a 15 nm separation between the two membranes, this represents a concentration increment of 0.5 mM/action potential from either pseudopodium or indentation or of 1.0 mM if both fire simultaneously. In order to calculate the manner in which pseudopodial length and firing frequency would influence 'steady state' concentrations of K^+ achieved, the diffusional dissipation of K^+ from the pseudopodial base was calculated using equations for linear diffusion in a cylinder with an extended initial distribution and reflection at one end. The K^+ diffusion coefficient in the extracellular space of the brain has been reported to be 3.1×10^{-6} $cm^2 s^{-1}$. The more rapid the firing rate, the smaller the loss of K^+ by diffusion after each release and before the next and so the higher the 'steady state' achieved. As shown in the Figure pseudopodial length and firing rate interact in such a way that explosive accumulations of K^+ may be expected at physiological firing rates as the length increases above 1 micron.

The mean length in control amygdalae was 0.99 micron (n=6). Since stimulation in electric organ induces pseudopodia, it does not seem unreasonable that applied stimulation of rat brain may lengthen them (e.g., seizures or kindling). If so, subsequent trains of endogenously generated or of applied action potentials may liberate and trap enough K^+ to depolarize the synapses which are immediately adjacent to the pseudopodia. This would alter transmitter output.

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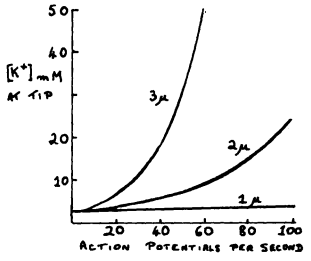

31.10 COINCIDENCE OF ACHE-CONTAINING AND SUPRAMAMMILLARY NUCLEUS PROJECTIONS TO THE SUPRAGRANULAR LAYER OF THE RAT DENTATE GYRUS. S.S. Lasher, R.C. Wilson, and O. Steward. Depts. of Psychology, Physiology, and Neurological Surgery, Univ. of VA Sch. of Med., Charlottesville, VA 22908.

Recent anatomical (Wyss , J.M., Swanson, L.W., and Cowan, W.M., Anat. Embryol., 156:165, 1979) and electrophysiological (Segal, M., Br. Res., 162:137, 1979) reports have identified a hitherto unrecognized projection from the supramammillary nucleus of the hypothalamus to the supragranular layer of the dentate gyrus in the rat hippocampal formation. Because the supragranular zone is also the site of termination of a prominent acetylcholinesterase-containing projection of enigmatic origin and because the supramammillary region contains AchE-positive neurons, we sought to determine whether this newly discovered projection did, in fact, have its origin in the AchE-containing cells of the supramammillary nucleus.

Two sets of experiments using adult, male Sprague-Dawley rats were performed. A first series of animals received unilateral injections of HRP (.02-.05ul, 25% solution) into the supramammillary region. Following a 2-day survival the animals were anesthetized and perfused, their brains removed and sectioned and alternate sections were processed for AchE histochemistry and for HRP anterograde (terminal) labeling using the tetramethyl benzidine procedure. Direct comparison of the HRP labeled terminal field and the AchE-positive zone in the dentate supragranular layer revealed that the terminal zones were largely coincidental.

To ascertain whether the cells of origin of the supramammillary projection were AchE-positive, a second series of rats received unilateral HRP injections (0.1-0.5ul) in the dentate gyrus. In these brains, combined AchE histochemical and HRP-TMB procedures were carried out on the same section to permit double-labeling of AchE-containing neurons which also had been filled with HRP transported retrogradely from the dentate gyrus. Numerous neurons were found in the supramammillary nucleus which were lightly stained for AchE and which also contained the HRP reaction product.

Although previous studies have demonstrated that most of the AchE in the hippocampus is derived from direct projections from the medial septal nucleus, evidence for a septal projection to the supragranular layer is not conclusive. While AchE staining of cell bodies is not positive evidence for a cholinergic projection, the present results suggest that some portion of the AchE staining in the supragranular zone may be attributable to the projection arising in the hypothalamic supramammillary nucleus.

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31.11 ELECTROPHYSIOLOGICAL IDENTIFICATION OF INTERNEURONES IN THE HIPPOCAMPAL CA1 PYRAMIDAL CELL LAYER: IN VITRO AND IN VIVO ANALYSIS. J.J. Miller, T.S. Miles* and R.W. Turner*. Dept. of Physiology, Univ. British Columbia, Vancouver, Canada V6T 1W5.

Stimulation of the schaeffer/commissural (Sch/Com) input results in a monosynaptic activation of pyramidal cells (P) in the hippocampal CA1 region. This activation is followed by a long duration inhibitory period on spontaneously firing P cells which is presumed to be mediated by basket cell interneurones. In the course of examining the plastic properties of the Sch/Com afferent to the CA1 region using the 'in vitro' hippocampal preparation and intact animals, recordings were obtained from a population of neurones displaying a number of characteristics which differentiate them from both P-cells and basket cells. These are: (1) multiple spike discharge to a single pulse stimulation consisting of 1-8 spikes firing at rates of up to 500 Hz; (2) a subsequent period of inhibition (50-200 msec) of those neurones which fire spontaneously; (3) activation at intensities less than that required to evoke either dendritic or population spike responses; (4) activation at shorter latencies (2.5-4.5 msec) than either the dendritic or population spike response; (5) ability to follow frequencies of up to 100 Hz but with significant jitter in latency; (6) progressive increase in the duration of discharge with increasing stimulus intensity and frequency; (7) alvear stimulation at intensities suprathreshold for the antidromic population spike activated these cells at latencies of 2.5 msec but did not elicit the high frequency response characteristic of basket cells; (8) in intact animals anaesthetized with urethane, single pulse stimulation of the commissural input evoked a spike discharge with comparable characteristics. These data suggest the presence of another population of interneurones in the CA1 pyramidal cell region which may be involved in an excitatory feedback loop that is normally inhibited by basket cells and excited by commissural afferents.

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31.12 EFFECTS OF EXTRACELLULAR CALCIUM ON POTENTIATION AND HABITUATION OF CA1 AND DENTATE EVOKED POPULATION RESPONSES IN THE 'IN VITRO' HIPPOCAMPAL SLICE PREPARATION. R.W. Turner* and J.J. Miller (SPON: T.W. Calvert) Dept. Physiology, Univ. British Columbia, Vancouver, B.C., Canada V6T 1W5.

Previous studies have demonstrated that low frequency stimulation (1Hz) of the schaeffer/commissural (Sch/Com) fibers elicits a frequency potentiation of the dendritic and population spike responses of CA1 hippocampal pyramidal neurones. In contrast, low frequency stimulation of the perforant path (PP) results in habituation of the dendritic and granule cell field responses in the dentate gyrus (DG). Inasmuch as these alterations in response amplitude reflect changes in synaptic efficacy and presumably transmitter release mechanisms, the present investigation was undertaken to examine the effects of a high extracellular calcium concentration on the plastic properties of CA1 and DG evoked potentials following low frequency stimulation of the Sch/Com and PP afferents respectively.

Transverse sections of the rat hippocampus were prepared for recording and incubated in a modified Ringer's solution perfused with a 95% O_2-5% CO_2 gas mixture. Incubation of the slices for 20 min in a perfusate containing a high calcium-low magnesium concentration (4 mM Ca^{2+}/1 mM Mg^{2+} vs the standard medium containing 2 mM Ca^{2+}/2 mM Mg^{2+}) resulted in a decrease or elimination of the potentiation of the CA1 dendritic and population spike responses induced by 1 Hz stimulation of the Sch/Com input. The habituation of the PP-evoked dendritic and spike responses in the DG was also decreased or eliminated in the presence of the 4/1 medium. Upon return to the standard medium these responses displayed partial or complete recovery to control levels. The inverse relationship between extracellular calcium concentration and the magnitude of potentiation or habituation induced by low frequency stimulation suggests that different processes may regulate calcium in the CA1 and DG regions and that these underly the selective evocation of opposing responses to low frequency inputs.

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31.13 Failure of sparing, or recovery,of recognition memory after early hippocampal resections in the rhesus macaque. L. Rehbein*, S. Zola-Morgan*, H. Mahut and M. Moss*. Psychology Dept., Northeastern Univ., Boston, MA 02115.

Most experiments with animals demonstrate that brain damage in infancy is less deleterious than in adulthood, though opposite effects were seen in patients (Hebb, 1942). The functional sparing is attributed to the plasticity of young brains. However, with a few exceptions (Harlow et al., 1968; Goldman et al., 1970) most studies have been concerned with single sensory or perceptual functions in rodents or carnivores.

In the present study, we assessed the effects of hippocampal lesions at 2 mos. of age, using several spatial and memory tasks. These were: (1) A series of left-right position reversals and (2) Retentions of easy, two-choice object discriminations at 1, 24 or 48-hr intervals. Operated infants and juveniles operated on at 2 yrs of age, together with normal juvenile and infant control groups, were tested post-operatively and twice again, at one year intervals. Post-operatively, infants were as impaired as were juveniles on both types of tasks. On the last re-test, two years after surgery, their performance recovered on the spatial, but not on the retention task, at both short and long intervals.

Long-term effects. (1) Five years after surgery, all operated juveniles, but only 4 of the 8 monkeys operated on in infancy, were impaired on a new spatial task, the one trial delayed - alternation. (2) However, unlike juveniles, monkeys with perinatal damage performed normally on a concurrent discrimination task which requires the learning of multiple (8) stimulus-object-reward associations in the presence of proactive and retroactive interpair interference. (3) In contrast, six years after surgery, monkeys operated on in infancy were as severely impaired as were the juveniles on the delayed non-matching-to-sample task, a trial-unique object recognition test. This deficit was found at 30, 70 and 130 sec, but not at 10 sec, delays between the presentation of the sample and the match. They were also significantly impaired when the number of object samples shown prior to pairing each one with a new, unfamiliar object numbered 1, 3, 5 or 10 (lists of differing lengths).

Thus, early hippocampal damage produced significant and long-lasting impairments one of which became attenuated and the other recovered with practice and time. The most enduring one, however, was the selective deficit in recognition memory.

31.14 BEHAVIORAL AND BIOCHEMICAL CHANGES AFTER HIPPOCAMPAL DAMAGE. D.K. Reinstein*, J.H. Hannigan, Jr.*, and R. L. Isaacson. Dept. of Psychology and Center for Neurobehav. Sci., SUNY-Binghamton, Binghamton, NY 13901.

The hippocampal formation's projections to the nucleus accumbens may act in a modulatory fashion on forebrain mesolimbic mechanisms. In addition, there are parallels between the behavior of animals with hippocampal lesions and those with experimentally-induced alterations of nucleus accumbens. It may be that the striatal-mesolimbic changes are of primary importance for some of these behavioral alterations. Hippocampal lesions may produce certain of its usual behavioral effects through secondary alterations of activity in nucleus accumbens. The present studies were undertaken to explore the possible functional relationship between the hippocampal formation and the nucleus accumbens. Rats were prepared with bilateral destruction of the hippocampal formation, lesions of the overlying cortex, or sham operations. The animals were behaviorally tested at 7, 14, and 28 days after surgery. On each of these testing days animals were examined in two different situations. These were: (1) an activity and exploration measuring apparatus (a rectangular open field with 16 holes in the floor), and (2) a smaller circular open field. All animals were also prepared with cannulae into the nucleus accumbens. Before Test 2, (3,4-dihydroxyphenylamino)-2-imidazoline HCl (DPI) or saline was injected via the cannula. The DPI was always given at a dose of 10 µg in 0.5 µl of fluid.

In the first apparatus the animals with hippocampal lesions had an increased frequency of both peripheral and central locomotion, as well as increased exploratory responses into centrally located holes. An increased number of explorations into peripherally located holes were evident at 28 days after surgery. A decreased response duration of this behavior was found at 14 and 28 days. These animals also showed decreased frequency of rearing relative to controls. The duration of episodes of both rearing and grooming were decreased. In the circular open field the hippocampally-lesioned animals showed increased frequencies of rotations in both ipsilateral and contralateral directions relative to the site of injection. The frequency of rearing was also increased in this apparatus, whereas there was a decrease in the frequency of grooming episodes at 7 and 14 days. The durations of rearing and grooming epsidoes were also decreased. Injections of the dopamine agonist, DPI, tended to normalize the behavior of the animals with hippocampal lesions on the measures of rotation and grooming in animals 7 days after the lesion but not at later times.

31.15 REPETITIVE STIMULATION OF THE SCHAFFER-COMMISSURAL SYSTEM: HOMOSYNAPTIC LONG TERM CHANGES OF POSTSYNAPTIC POTENTIALS. G. Barrionuevo*, K. Negishi* and G. Lynch. (SPON: S. Gerling) Dept. of Psychobiology, Univ. of California, Irvine, CA 92717

Field potential studies have shown that repetitive stimulation of hippocampal pathways produces long term potentiation (LTP) of synaptic responses. Intracellular recording studies using in vitro "slices" indicate that the EPSP is increased for several minutes following repetitive stimulation but there is confusion surrounding the presence or absence of heterosynaptic effects, changes in membrane potentials, and alterations in IPSP's. Since most of the studies on this issue followed the "potentiated" cells for relatively brief periods, it is possible that some of these effects represent transient changes induced by repetitive stimulation which are not directly related to LTP. In the experiments to be discussed intra- and extracellular responses were monitored from 14 cells in field CA1 (resting membrane potentials: 50-80 mV) for at least 30 minutes and up to 2 hours after 3 brief bursts of high frequency stimulation delivered to the Schaffer collateral-commissural projections. In several experiments a second collection of inputs was used to test for generalized changes in the target cell. Pair pulse tests were used to rule out any overlap in the population of axons activated. Background stimulation rate was 0.1 sec^{-1} and the stimulation current was set at levels below that required to spike the target cells.

Three types of effects were observed which persisted without evident change for the duration of the recording session: 1) an increase in the slope and amplitude of the EPSP, typically to a level which triggered the action potential; 2) the appearance in most cells of a depolarizing after potential (DAP) and 3) a depression of the IPSP's which often accompany Schaffer collateral-commissural stimulation. While various other effects were observed in the period immediately after the high frequency train, these were not in evidence at 30 minutes poststimulation. In particular we found no lasting changes in the membrane potential or in the heterosynaptic responses. This pattern of results is reasonably acounted for by the hypothesis that LTP is caused by an enduring facilitation of synaptic transmission restricted to stimulated pathways; if large enough this will produce a DAP which may obscure the IPSP. Other effects are sometimes triggered by the potentiating train but these do not appear to possess the stable character of LTP.

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31.16 LONG-TERM POTENTIATION IN THE DENTATE GYRUS FOLLOWING LOW FREQUENCY (0.1 Hz) STIMULATION OF THE PERFORANT PATH IN CHRONIC RATS. Ronald W. Skelton, James J. Miller and Anthony G. Phillips, (SPON: John P.J. Pinel). Depts. of Physiology and Psychology, Univ. of Brit. Columbia, Vancouver, B.C., Can., V6T 1Y7.

Tetanic stimulation of the perforant path (PP) results in a long-term potentiation of the population spike recorded extracellularly in the dentate gyrus (DG). Several reports have indicated that potentiation is produced by tetanic stimulation between 5 and 20 Hz, while lower frequencies may produce either no effect or depression. In our laboratory, control stimuli delivered at < 1 Hz have been observed to produce an immediate enhancement of the DG population spike in chronic rats. The present investigation sought to determine the magnitude and duration of this potentiation following stimulation of the PP at 0.1 and 0.2 Hz.

Under Nembutal anaesthesia, electrodes were implanted in the DG and PP and fixed with dental cement at depths chosen to maximize the evoked population spike in the DG cell layer. After a three week recovery period, input/output functions of the PP-DG response were determined on the freely-moving animals. Evoked potentials were recorded in response to a series of 15 stimulation intensities ranging from 10 to 500 µA, presented in an ascending, logarithmic series. The stimulation frequency was either 0.1 or 0.2 Hz and no stimulations other than those in a series were delivered. Five responses at each intensity were averaged and plotted. The amplitude of the population spikes, plotted against the current intensities of a single series, constituted a single input/output function. Immediately (3-5 min) following the first series, all intensities were retested in the same order. Comparisons of the spike amplitudes recorded in the first and second series revealed large increases at most intensities (often > 500%). Largest percentage increases occurred at lower intensities (20-150 µA). Subsequent stimulation series at 1,24, 48,72, and 96 hrs revealed persistent, although somewhat reduced levels of potentiation. Dramatic reductions in the thresholds for activation of the population spikes (as much as 50%) accompanied the potentiation.

These data demonstrate that stimulation of the PP at frequencies well within physiological ranges of neuronal activity can potentiate subsequent evoked potentials in the DG. They indicate further the extreme sensitivity of this system and the ease with which information transmission through it may be facilitated.

31.17 NORADRENALINE LEVELS AFFECT LONG-TERM POTENTIATION IN THE HIPPOCAMPUS. G. V. Goddard, T. V. P. Bliss*, H. A. Robertson, and R. S. Sutherland*. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1.

Rats were depleted of hippocampal noradrenaline by systemic injection of reserpine or by 6-OH-dopamine destruction of the dorsal noradrenergic bundle. Verification was by radioenzyme liquid scintillation count and all experimental levels were less than 7.5% normal. Brief high-frequency electrical stimulation of perforant path fibers was used to cause short-term and long-term potentiation of the synapses onto granule cells as measured by the field potential response to test shocks in the perforant path. Short-term potentiation of the field excitatory post-synaptic potential (EPSP) was unaffected by noradrenaline depletion whereas long-term potentiation of the EPSP was reduced by more than 50%.

In agreement with earlier work (Bliss, T. V. P. and Lømo, T., J. Physiol., 232:331, 1973; Douglas, R. M. and Goddard, G. V., Brain Res., 86:205, 1975) long-term potentiation of the field EPSP and long-term potentiation of the population spike are not always tightly coupled. Careful measurement of input-output curves indicated a lasting shift to the left (greater spike per unit EPSP) following the high-frequency trains, indicating a lasting increase in cellular excitability. This increase in excitability was just as great in the noradrenaline depleted animals as it was in controls, even though the depleted animals showed much less long-term potentiation of the EPSP.

31.18 IMPAIRMENT OF LONG-TERM POTENTIATION FOLLOWING CHRONIC ETHANOL CONSUMPTION IN RATS. D. Durand, P.L. Carlen, P. McMullen. Depts. Medicine, Biomedical Engineering, Physiology, Addiction Research Foundation, Playfair Neuroscience Unit, Toronto Western Hospital, University of Toronto, Toronto, Ontario.

Chronic alcoholic brain damage with impairment of learning and memory has been associated with both malnutrition and alcohol consumption. Long-term-potentiation (LTP) in the hippocampus has been suggested as a neural correlate of memory and learning and has been shown to be impaired in aged animals. The effects of chronic ethanol consumption on hippocampal LTP have been investigated in the rat using the in-vitro hippocampus slice preparation.

For 8 to 9 months, male Sprague-Dawley rats were provided ad-libitum but measured access to a liquid diet containing 35% of its calories as ethanol, while a control group received isocaloric amounts of the same diet with ethanol replaced by maltose-dextrins. In a double blind study, 400 µm slices were prepared and placed in an in-vitro recording chamber. Stimulation was applied to the Schaffer collaterals in 4 trains of 200 pulses each, separated by 10 minute intervals at 25, 50, 100, 200 Hz respectively. The extracellular recording electrode was placed in the CA1 cell layer. The percent increases in the amplitude of the population spike at each frequency were measured 10 minutes after each applied tetanus and labelled P_{25}, P_{50}, P_{100}, P_{200}.

Table 1 shows the means and standard error means at each frequency and the total percent increase, P_T. There was a significant impairment (Mann-Whitney test, see Table 1) of the long-term potentiation in the ethanol-fed animals at P_{25}, P_{50}, P_{100}, P_{200} and P_T. The number of slices with a total potentiation of less than 10% increased from 16 to 85% in the ethanol group.

Morphological analysis of Golgi stained CA1 cells in the contralateral hippocampus of the same animals revealed a significant decrease in the branching (Scholl analysis) and total length of the dendrites in the ethanol group (see abstract, 1980, by McMullen et al).

	P_{25}	P_{50}	P_{100}	P_{200}	P_T
Control	23.1±15.9	13±7	16.7±7.7	9.2±3.7	90.7±30.5
Ethanol	1±3.5	2.5±2.12	-.2±2.85	.8±2.4	2.5±5.7
Probability	<.02	<.12	<.05	<.04	<.004

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31.19 A MORPHOMETRIC STUDY OF THE EFFECTS OF POSTNATAL LEAD EXPOSURE ON HIPPOCAMPAL DEVELOPMENT IN RATS. Janelle C. LeBoutillier*, Dennis P. Alfano and Ted L. Petit. Dept. Psychol., Univ. Toronto, West Hill, Ont., Canada, M1C 1A4.

Increased exposure to inorganic lead (Pb) has been observed to have severe consequences on the normal postnatal development of the central nervous system in experimental animals. Recent evidence has suggested that hippocampal dysfunction may possibly underly the behavioral alterations seen in postnatally Pb exposed animals. This study was undertaken to investigate the possible effects of Pb on the development of the mossy fibre pathway of the hippocampal formation.

Timm's method for the histochemical staining of heavy metals results in a precipitate confined selectively to the terminals of mossy fibres, providing a useful means of assessing the development of this hippocampal pathway.

On postnatal day 1 (P1) all litters were cross-fostered, randomly assigned to either a Pb or control group and reduced to 5 and 10 male pups, respectively. Mothers were maintained ad lib on either 4% Pb carbonate or a sodium carbonate control diet from P1 to P25. On P25, 9 animals per group were selected and sacrificed under ether anaesthesia. Consecutive horizontal sections from the ventral portion of the medial hippocampus were collected and stained.

All measurements were performed on a Leitz Image Analyzer. Five sections per brain were measured. Significant reductions were observed in the maximal width of the hippocampus, and the maximal width and length of the dentate gyrus in Pb exposed animals. These reductions in overall hippocampal dimensions are consistent with our previous observations of reduced hippocampal weight following early Pb exposure. Measurements made on the mossy fibre pathway: 1) at it's point of emergence from the hilus of the dentate gyrus; 2) at the point of maximum curvature; 3) just prior to it's termination, and 4) it's overall length, indicated significant reductions for all measures in Pb animals.

These results indicate a severe effect of postnatal Pb exposure on normal hippocampal development. Alterations in the development of this pathway, resulting in a possible disruption of the internal circuitry of the hippocampus, may contribute to the behavioral changes observed in postnatally Pb exposed animals.

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31.20 ALTERATIONS IN DENTATE GRANULE CELL DENDRITIC DEVELOPMENT FOLLOWING POSTNATAL LEAD EXPOSURE. D. P. Alfano and T. L. Petit. Dept. Psychol., Univ. Toronto, West Hill, Ont., Canada, M1C 1A4.

Several lines of evidence have suggested the possibility of hippocampal involvement in the behavioral deficiencies observed in experimental animals following postnatal lead (Pb) exposure. This study was thus conducted to discern the possible effects of Pb on the dendritic development of hippocampal dentate granule cells.

Long Evans hooded rat pups were exposed to Pb from postnatal day 1 (P1) to P25 via the maternal milk. Mothers were fed diets containing either 4% $PbCO_3$ (High Pb), .4% $PbCO_3$ (Low Pb) or 2.2% Na_2CO_3 (Control-10 and Control-5). On P1, High Pb and Control-5 litters were culled to 5 males while Low Pb and Control-10 litters were culled to 10. The use of differential litter size resulted in minimal pup weight differences between Pb treated and Control-10 litters prior to P25.

On P30, pups from at least 3 litters per group were selected and their hippocampi processed with the Rapid Golgi method. Both Pb treated groups had brain weights lower than either Control group. Low Pb hippocampi weighed less than Control-10 hippocampi.

For analysis of dendritic development, granule cells from the infrapyramidal limb of the dentate gyrus were chosen. Five cells from 6 High Pb, 5 Low Pb, 5 Control-10 and 5 Control-5 brains were drawn. Only fully impregnated cells from horizontal sections located at the dorsal aspect of the middle third (medial) portion of the hippocampus were chosen. Dendritic branching was evaluated by the method of Scholl. No differences were observed in the maximal width, however both Pb treated groups showed a reduction in the maximal length of their dendritic fields as compared to either Control group. High Pb animals showed greater dendritic branching at 20 µm from the cell body as compared to either Control group. Low Pb animals showed increased branching at 20 µm as compared to Control-10 animals. Marked reductions in dendritic branching was evident in Low Pb animals at distances from the cell body greater than 100 µm as compared to Control-5 animals and at 180, 200 and 220 µm as compared to Control-10 animals. High Pb animals showed reduced branching at 180 µm as compared to Control-5 animals.

The results of this study indicate increased dendritic branching proximal to the cell body, but both a reduced length of the dendritic field and a reduction in the number of dendritic branches distal to the cell body of dentate granule cells following postnatal Pb exposure. These findings are consistent with a pattern that might be expected as a result of a retardation in the temporal sequence of dendritic development. As such, these results support the suggestion that hippocampal dysfunction may be a contributory factor in producing the behavioral deficiencies observed in experimental animals following postnatal Pb exposure.

32.1 NEURAL INFLUENCE ON MUSCLE HYDROLASE ACTIVITY. R. J. Boegman and T. W. Oliver*. Dept. of Pharmacology, Faculty of Medicine, Queen's University, Botterell Hall, Kingston, Ont. K7L 3N6.

The specific activity of several lysosomal enzymes increases in skeletal muscle undergoing atrophy due to denervation, tenotomy or genetic dystrophy. Axonal degeneration products, increased endocytosis, muscle inactivity and neurotrophic factors have been suggested as being responsible for activation of these hydrolases. In an attempt to avoid direct muscle damage in studying the neural influence on muscle hydrolases we made use of specific neurotoxins which block impulse transmission. Inactivity of mouse soleus or extensor muscles following neural application of 1 µl of either Tetrodotoxin (TTX 1 x 10^{-9}M) or Batrachotoxin (BTX 1 x 10^{-12}M) to the common sciatic nerve innervating the right leg resulted in an increased (2-3 fold) activity of N-acetylgalactosaminidase and acid protease (pH 3.5) over that found in the contralateral sham injected leg. The non-lysosomal enzyme alkaline protease (pH 9.0), did not show a similar increase following neural application of either toxin, indicating that the increased hydrolase activity was not due to invasion of the inactive muscle by macrophages. Since a direct effect of the toxins on muscle has been shown to be unlikely (Boegman et al., Brain Research (1980) 187: 183-196) our results are best explained as a response of the muscle hydrolases to an induced state of functional inactivity.

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32.2 RESULTS OF CROSSING ELECTRICALLY SILENT NERVES TO FAST AND SLOW MUSCLES IN THE CAT HINDLIMB. L. Eldridge*, M. Liebhold* and W.F.H.M. Mommaerts* (SPON:P.A. Black-Decima). Dept. of Physiology, School of Medicine, Univ. of California at Los Angeles, CA. 90024.

In order to help determine whether the reversal of properties of fast (F) and slow (S) muscles after cross-reinnervation (NX) is brought about by the differential activity patterns imposed on the muscles by the F and S nerves or, alternatively, by trophic specifying substances released by the nerves, cross-reinnervation of F and S hindlimb muscles was conducted in a cat preparation in which the crossed nerves had been rendered chronically electrically inactive by a surgical spinal cord isolation (SI). In this procedure, all dorsal roots between two cord transections enclosing segments lumbar 6 through sacral 4 were severed, leaving the muscles involved in the nerve cross innervated but in permanent flaccid paralysis. At 5 and 8 month intervals after the simultaneous SI and NX operations, the muscles were compared to self-reinnervated or untouched SI muscles, as well as to crossed and self-reinnervated muscles from normal (non-paralyzed) cats. The biochemical, histochemical and contractile characteristics which we tested included myosin light chains, myosin and myofibrillar adenosine triphosphatase activity, lactate dehydrogenase isozymes and activity, calcium^{++} uptake by microsomes or muscle homogenates, histochemical fiber type, time to peak twitch tension, tetanus fusion frequency, and fatigue resistance. On all tests, the nerve-crossed paralyzed muscles consistently failed to show any reversal of F-S properties. All SI slow muscles eventually acquired fast characteristics as a result of the paralysis itself, but the nerve-crossing had no effect on the rate of this change. In contrast, the normal nerve-crossed muscles showed significant reversals of most of these properties even at the comparatively short crossing-test intervals. The failure of electrically silent nerves to effect reversal of F and S muscles in these experiments is in agreement with our previous long-term studies in which nerve crosses performed more than a year after the SI caused no changes even after nerve cross-test intervals of up to 20 months.

32.3 EFFECT OF ESTROGENS ON DENERVATED MUSCLE. Stephen R. Max. Dept. of Neurology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Denervation of rat extensor digitorum longus (EDL) muscles results in a nerve stump length-dependent increase in glucose 6-phosphate dehydrogenase (G6PD) activity (Wagner and Max, Brain Res. 170:572, 1979). However, the rate of increase of G6PD activity in EDL following transection of the sciatic nerve is strikingly different in muscles from adult male and female rats. In females G6PD activity was 170% control 24 h after denervation. In males this increase was delayed; G6PD activity was the same as control 24 h after denervation of EDL from male rats but increased to the level observed in females by 48 h. By day 4 G6PD activity was the same in both sexes (200% control). Pharmacological studies with hormone receptor agonists and antagonists showed this effect to be attributable to stereospecific interaction of estrogens with their receptors; androgens do not make a significant contribution (Max and Knudsen, Brain Res., In press). We investigated this phenomenon further by assessing the effects of castration and hypophysectomy on the denervation-induced increase in G6PD activity. Ovariectomy (OVX) for 4 weeks eliminates the significant increase in G6PD observed 24 h after denervation of EDL in intact female rats. Administration of estradiol-17β in silastic implants for 5 days into OVX rats restores the increase in G6PD activity 24 h following denervation. Estradiol-17α has no effect, indicating that estradiol action is mediated via a stereospecific receptor. Hypophysectomy (Hypox) of female rats for 1 week also eradicates the denervation effect on G6PD activity 24 h after denervation. Estradiol-17β restores the denervation effect on G6PD activity in Hypox rats, further supporting the role of estrogens. Treatment of female rats with MER-25, an estrogen antagonist, prevents the increase in G6PD 24 h after denervation and reduces the extent of the increase in enzyme activity by 40% 4 days after denervation. Similarly, treatment of male rats with MER-25 reduces the denervation-mediated increase in G6PD on day 4 by 40%. These data demonstrate that estrogens enhance the increase in G6PD activity following denervation of muscle and provide a model system which will be valuable for further studies on integration of neural and hormonal effects on muscle. (Supported by NIH grants, NS-15760 and NS-15766).

32.4 CONTRALATERAL DENERVATION CAUSES INCREASED SYNAPTIC EFFECTIVENESS IN FROG SARTORIUS. Albert A. Herrera and Alan D. Grinnell. Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA., 90024.

It has been reported that unilateral denervation of the frog cutaneous pectoris muscle causes nerve terminal sprouting and a concomitant increase in polyneuronal innervation (PI) in the intact contralateral muscle (Rotshenker, S. J. Physiol. 292: 535, 1979; Reichert, F. & Rotshenker, S. Brain Res. 170: 187, 1979). In similar experiments on the frog sartorius we also see an apparent increase in PI but little evidence for sprouting. Further, we find that unilateral denervation causes markedly enhanced transmitter release from nerve terminals in contralateral muscles.

In 23 adult Rana pipiens the left sartorius was denervated by several methods involving crushing the sartorius nerve or the 8th spinal root. The different operative techniques did not produce detectably different results. After 5-16 weeks we stained the intact right sartorius with nerve terminal and cholinesterase stain, and tested synaptic strength. Normal muscles from similar but unoperated frogs were also studied.

The incidence of PI, detected by the usual intracellular recording techniques in curarized muscles, was higher at experimental end plates (EPs) (14%) than at normal EPs (6%). However, this increase did not appear to be due to sprouting. EP size, measured as total postsynaptic gutter length, was not significantly different in experimental (413 ± 13 µm SEM; 258 EPs, 10 muscles) and normal muscles (430 ± 19 µm; 78 EPs, 4 muscles).

In normal muscles lowering [Ca] to 1.0 and 0.6 mM causes nerve-evoked twitch tension to drop to 42 and 4%, respectively, of twitch tension in normal Ringer containing 1.8 mM Ca. Tension falls when all the synapses on some fibers become subthreshold. Experimental muscles showed enhanced synaptic strength, falling only to 70% and 17%, respectively. In Ringer containing 0.3 mM Ca and 1 mM Mg, we also measured quantal content at identified junctions whose morphology was later analyzed. Experimental muscles showed nearly a 4-fold increase both in total quanta released and release per unit terminal length as compared to normal muscles. This enhanced release might explain why PI appeared to increase. If the very weak polyneuronal inputs known to occur in the sartorius (Grinnell, A.D. & Herrera, A.A., Soc. for Neurosci. Abstr., 1980) were made stronger, then these inputs would no longer be obscured by the curare used to assess PI. We conclude that unilateral denervation causes a marked increase in contralateral synaptic effectiveness due to a change in the inherent properties of transmitter release. (Supported by USPHS grant NS06232, a MDA grant, and a NIH fellowship to AAH.)

32.5 CONTRACTILE AND HISTOCHEMICAL PROPERTIES OF THREE RAT MUSCLES IMMOBILIZED IN STRETCHED, RELAXED AND NEUTRAL POSITIONS. C.P. Simard*, S.A. Spector*, M. Fournier*, E. Sternlicht*, J. Vallieres*, and V.R. Edgerton. Neuromuscular Research Lab., UCLA, Ca. 90024.

While it has been shown that muscle immobilized under stretch results in initial muscle weight (MW) enhancement, knowledge of the muscle's functional speed and force-related capacity is sparse. Therefore, this study investigates the effects of muscle maintained in chronic shortened, neutral or stretched positions on isometric contractile and histochemical properties of the soleus (SOL), medial gastrocnemius (MG) and tibialis anterior (TA) of immobilized female rats. A brace consisting of transversely inserted steel pins through the femur and tibia and externally fixed at a specified angle was used to bilaterally fix the hindlimbs, thus stabilizing the joint while permitting normal vascularization. The three muscles were studied under four conditions: maximal stretch (S), moderate stretch (N), maximal relaxation (R) and sham control (C), each group containing seven animals.

Effects of imposed degrees of stretch on MW and tetanic tension (P_o) at 35±1°C are shown in Tables 1 and 2. The SOL, MG and TA of S demonstrated the least decline in MW (13, 16 and 28%, respectively, of C), while greater wasting was seen in all muscles of N and R. While P_o of the SOL, MG and TA was the least in R, about two-fold greater P_o was observed in N, whose P_o values were about half of C. P_o of the SOL and MG of S was similar to that of N, in contrast to P_o of the TA of S, which was only 55% of N values. No changes were seen in time to peak tension (CT) of the MG and TA for all groups. CT of the SOL of R (46.2±10.0) and S (48.3±6.2) were similar to C (47.6±4.8), while a decrease in CT of N (39.9± 4.2) was seen. In parallel, histochemical fiber type conversion of SO (65%) to FOG (35%) of the SOL of N was found, compared to 16% (SO) and 84% (FOG) normally seen in rats of similar age.

Therefore, in the absence of stretch (R), extreme atrophy occurs, and is manifest in decreased tension produced by the muscle. While extreme stretch (S) is a stimulus to maintain MW of the SOL, MG and TA at nearly normal levels after four weeks of fixation, its potency for maintaining muscle force is not great (about 50% for SOL, MG; 32% for TA, compared to C), indicating that elongation of muscle fibers and not increased cross sectional area, is the direct result of fixation under stretch. NIH #10423; MRC #MA-5715.

	C	S	N	R	
SOL	.164±.019	.142±.029	.094±.011	.082±.016	Table 1: Wet
MG	.835±.130	.696±.151	.410±.065	.360±.050	muscle weight
TA	.602±.044	.433±.070	.373±.076	.394±.096	(grams).

SOL	142± 35	75± 23	84± 19	35± 26	Table 2: Te-
MG	1113± 327	575± 209	546± 91	264± 85	tanic tension
TA	773± 165	247± 78	444± 57	273± 145	(grams).

32.7 STRESS-INDUCED NEUROLOGICAL IMPAIRMENTS AFTER 6-HYDROXYDOPAMINE EFFECT OF LESION SIZE AND AGE. Abigail M. Snyder*, Edward M. Stricker, Michael J. Zigmond, Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

Large dopamine (DA)-depleting brain lesions produce severe behavioral deficits in rats. For example, animals show both akinesia and catalepsy. Although these impairments gradually abate, they are seen again when lesioned rats are exposed to acute regulatory stress (Stricker et al., J. Comp. Physiol. Psychol. 93:512-521, 1979). In the present experiments, selective DA depletions were produced in adult rats by intracisternal administration of 6-hydroxydopamine (6-HDA, 200, 250, or 2 x 250 µg) 30 min after pretreatment with desmethylimipramine (DMI, 25 mg/kg, i.p.) and pargyline (50 mg/kg, i.p.). Following recovery several weeks later, animals were given 2-deoxy-D-glucose (2-DG, 500 or 750 mg/kg, i.p.), a drug which blocks the transport and utilization of glucose producing a functional glucoprivation. Neurological performance was examined 30 and 120 min after 2-DG administration. Measurements included latencies to move 4 limbs when placed on a table (to test for akinesia) and latencies to return all 4 paws to the table when either front or rear limbs were placed up on a 10 cm high block (to test for catalepsy). Those animals with less than 7% DA left in the striatum showed significant akinesia and catalepsy after 500 mg/kg 2-DG, and were even more debilitated after 750 mg/kg 2-DG. In contrast, animals with 7-20% DA left showed no deficits after 500 mg/kg 2-DG, and only moderate deficits with the higher dose. Those animals with more than 20% DA left did not differ from controls at either dose. These results indicate that the debilitating effects of acute glucoprivation occur only when central DA depletions are large, and are pronounced only when glucoprivation is severe.

Additional rats were given intracisternal 6-HDA with DMI in the first week of life and were tested for neurological performance after 2-DG treatment (500 mg/kg, i.p.) as adults. In contrast to the above observations no impairments were seen in most animals even when striatal DA was less than 7% of control values. These results suggest that adaptive compensatory changes may have occurred in animals brain damaged during infancy.

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32.6 IN VITRO DEMONSTRATION OF PARASYMPATHETIC DENERVATION SUPERSENSITIVITY OF DISSOCIATED PAROTID ACINAR CELLS. Barbara R. Talamo (with the aid of J.R. Kaltenbach). Depts. of Neurology and Physiolog. Chem., Johns Hopkins Medical Sch., Baltimore, MD.21205

Parasympathetic denervation of parotid glands in the rat produces supersensitivity to cholinergic agonists administered in vivo without increasing the number of muscarinic receptors in glandular membranes (Talamo, Adler and Burt, Life Sci. 24,1573 1979). Since the cholinergic response which is monitored is salivation rate, it is not clear whether the supersensitive target is the acinar cell or a more distal one which might alter blood flow or hormone release. A dissociated acinar cell suspension has been prepared which shows an increased rate of $^{22}Na^+$ uptake in response to carbachol. The effect of carbachol is dose-dependent and is completely blocked by atropine or quinuclidinylbenzilate (QNB). Epinephrine also increases 22Na influx; this effect is blocked by phentolamine. Membranes prepared from dissociated cells have 122 fmol of (^{3}H)QNB receptors/mg membrane protein (n=3) as compared to membranes prepared from intact tissue, with 126.1 ±8.0 fmol/mg membrane protein (n=6). The K_d of the (3H) QNB receptor in dissociated cell membranes is not significantly different from that on membranes of intact glands. Dissociated acinar cells prepared from parasympathetically denervated glands show an enhanced sensitivity of ^{22}Na flux to stimulation by carbachol, indicating that the cells are supersensitive. The EC_{50} for activation is 1.26 x 10^{-7}M for denervated cells (n=3) compared to 5.62 x 10^{-7}M for control cells (n=5). In paired experiments, the EC_{50} for control relative to denervated cells is 3.61 ± 0.28 (P 0.001). Thus, these cells show changes in responsiveness and are useful for studies of the mechanism of muscarinic supersensitivity.

33.1 REINNERVATION OF FROG MUSCLE. P.A. DeCino. UCLA School of Med., Dept. of Physiol., Ahmanson Lab. of Neurobiology, and the Jerry Lewis Neuromuscular Research Center, Los Angeles, CA 90024.

The recovery and reinnervation following nerve crush in the frog cutaneous pectoris (cp) muscle has been described previously by Dennis & Miledi (J. Physiol., 1974, 239:553,571). How the nerve returns to its normal state was examined in more detail by employing intra- and extracellular recordings correlated with histological techniques which visualize pre- and postsynaptic structures from physiologically identified endplates. Reinnervated adult frog (Rana pipien) cp muscles were examined 4-30 days postcrush.

Morphologically, reinnervation occurred quickly with more than 90% of the postsynaptic membrane covered by regenerated neural processes within 10 days. Functional recovery, however, lagged behind. At 10 days miniature endplate potential (mepp) frequency was 26% and quantal content was 30% of control levels. By 30 days both of these parameters approached normal adult values. The time difference in the return of function after the nerve has regenerated might be explained by 1) nonfunctioning portions of the nerve terminal, 2) a proximal-to-distal gradient of spontaneous and evoked release with the more distal portions of a terminal branch releasing less frequently, or 3) overall immaturity of the nerve terminal process. Because low mepp frequency prevented accurate determination of the presence of spontaneous release along an entire branch, the first possibility could not be excluded. However, when mepps were detected, there were no consistent gradients in their frequency or amplitude. 29% of the terminal processes examined showed the expected gradient in mepp frequency and only 13% for mepp amplitude. Similar variability was also seen in adult cp muscles. Once the nerve was capable of evoked release, which often occurred when less than half of the postsynaptic membrane was covered by regenerated nerve, the entire length of a given branch would release in response to nerve stimulation. In a limited number of experiments on 8-12 day reinnervated muscles, it appeared that there was a slight gradient in the probability of evoked release along a branch. Thus, it would appear that early in reinnervation the entire terminal is basically immature, but capable of transmitter release, with gradients in evoked release.

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33.2 EXTRACELLULAR STRUCTURES AT THE NEUROMUSCULAR JUNCTION DIRECT THE GROWTH OF REGENERATING AXONS. Duane R. Edgington*, Damien P. Kuffler and U.J. McMahan, Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Several anatomically well defined extracellular structures are associated with motor nerve terminals at skeletal neuromuscular junctions: 1) the myofiber basal lamina sheath which extends through the synaptic cleft and thus separates the nerve terminal from the myofiber, 2) the basal lamina that overlies the nerve terminal's Schwann cell cap, and 3) the reticular lamina, a layer of collagen fibrils which is situated external to the Schwann cell basal lamina. To this list we add another extracellular structure of the frog neuromuscular junction: a band of filamentous material, 0.05-1.0 μm thick, extends from the Schwann cell basal lamina into the reticular lamina. This layer of extracellular material blankets the entire axon terminal and surrounds the unmyelinated pre-terminal axon, but it does not cover the parent myelinated axon within the nerve trunk, as does the Schwann cell basal lamina and the reticular lamina. Like the glycoprotein-rich basal laminae, the filamentous material stains intensely with ruthenium red in osmium.

Damaged motor axons regenerate to establish neuromuscular junctions at original synaptic sites on myofibers. In previous studies it has been demonstrated that factors associated with synaptic basal lamina influence the differentiation of regenerated nerve terminals. We examined the role of extracellular structures in directing the growth of regenerating motor axons to the synaptic sites by removing the cells of the frog's cutaneous pectoris muscle prior to reinnervation. After damaging the nerve, the region of innervation was frozen, resulting in degeneration and phagocytosis of all cellular elements at the neuromuscular junctions (myofibers and Schwann cells) and in the intramuscular nerve trunk (perineurial and Schwann cells). Extracellular structures, including the basal lamina sheaths of myofibers, Schwann cells and perineurial cells, as well as the layer of filamentous material, remained structurally intact. Within 2 weeks after damage myofibers had regenerated within the basal lamina sheaths of original myofibers. Regenerating axons grew through the original Schwann cell and perineurial basal lamina sheaths of the nerve, and made synaptic contact precisely at the original synaptic sites on the myofiber basal lamina sheaths even though none of the original cells of the synaptic sites were present. Thus, factors contained in or connected to extracellular material direct the growth of axons to the original synaptic sites. (Supported by a Muscular Dystrophy Association Postdoctoral fellowship to D.R.E., a National Paraplegia Foundation and a USPHS NRSA Postdoctoral fellowship to D.P.K., and USPHS Grant NS 14506.)

33.3 MOTOR AXON REGENERATION IN CRAYFISH LIMBS: DISTAL STUMP ACTIVATION FOLLOWED BY SYNAPTIC REFORMATION. M. S. Bouton*, and G. D. Bittner. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Previous reports have disagreed as to the mechanism of motor axon regeneration in crayfish limbs. These reports have suggested that the proximal ends of these severed motor axons may "fuse" with severed distal processes which almost always survive for at least 70-90 days (Hoy, et al., 1967; Kennedy and Bittner, 1974). Other reports have suggested that outgrowths from the proximal severed stump may reform synapses on target muscles in a process similar to that shown to occur in vertebrates (Nordlander and Singer, 1972). We now suggest that regeneration initially occurs by activation of the severed distal stump from the proximal stump followed by synaptic reformation on the target muscles via continued outgrowth from the proximal stump.

Nerves were cut in the proximal portion of crayfish limbs and examined ultrastructurally at various post-operative times. According to our behavioral tests, 31% of the denervated muscles returned function within 70 post-operative days. Muscles near the cut did not regain function sooner or more frequently than more distal muscles. Two muscles which are located in different limb segments but share a common excitatory axon always regained function simultaneously rather than sequentially.

This return of function to various limb muscles was correlated with ultrastructural events. At one week post-operative, when no function has returned to the denervated muscles, outgrowing processes (satellite axons) from the proximal stump are not seen in the glial sheaths of the surviving distal stumps. At one month, some limbs regain function and satellite axons are seen from 1-2 mm proximal to the cut to 2-5mm distal to the cut. These satellite axons are small in diameter and up to 200 in number within a given motor axon glial sheath. Small axon-like processes are seen to cross the lesion site. From 1-2 months, these outgrowing processes from the proximal stump continue to grow to distal target muscles, but do not reach these muscles until 3-4 months post-operatively. Satellite axons continue to be found in the distal sheaths of regenerated axons at one year after initial regeneration, at which time the original distal stumps can no longer be identified.

We conclude that the severed proximal ends of crayfish peripheral motor axons grow out and contact appropriate distal stumps. The distal stumps are then activated by one of a number of possible mechanisms. We have found no evidence for gap junctions, and little evidence for axonal fusion between satellite axons and the surviving distal stumps. Our data indicates that the surviving distal stumps are ephaptically activated by satellite axons arising from the proximal stumps and that these satellite axons then continue to grow to the target muscles to reform synapses.

33.4 CAN A REGENERATING ALPHA MOTONEURON INNERVATE TWO DIFFERENT TYPES OF INTRAFUSAL MUSCLE FIBRE SIMULTANEOUSLY? R. Butler, Dept. Anat., McMaster Univ., Hamilton, Ontario, L8N 3Z5.

Previous studies indicate that following nerve injury, regenerating sensory and motor fibres can reinnervate muscle spindles, eventually restoring normal function (Brown and Butler (1976) J. Physiol. 260, 253). During reinnervation, an extensive transient, but 'inappropriate' fusimotor innervation by branches of α motoneurons (called β fibres) occurs which diminishes with time as it is functionally replaced by the 'appropriate' γ innervation. A further series of experiments is underway to study in detail this transient innervation by β fibres and their apparent competitive replacement by γ fibres. At selected times following crush or cut injuries of the nerve to the cat's tenuissimus muscle, the state of reinnervation is assessed by recording the response of single spindle afferents while stretching the muscle during stimulation of single fusimotor fibres which innervate that particular spindle. As expected, α fibres with β branches were found sooner following the lesions than γ fibres and they produced either dynamic or static effects on primary endings. When an α fibre with β branches affects more than one spindle, the primary afferent responses are usually all consistent in action, i.e., either all dynamic or all static. Since dynamic and static effects are believed to be due to selective innervation and contraction of two types of intrafusal muscle fibre (slow twitch and fast twitch, respectively), it follows that branches of an α motoneuron which produce dynamic effects probably innervate slow twitch muscle fibres selectively while α motoneuron branches producing static effects probably innervate fast twitch muscle fibres selectively. An interesting result concerns a single α fibre found in a 6 week post-crush regenerate whose collateral β's had static actions on three spindles and, surprisingly, a dynamic action on a fourth spindle. The implication is that this single α motoneuron, through its β branches, functionally innervated two different types of muscle fibre simultaneously. (Supported by grants from the Ontario March of Dimes and the Medical Research Council of Canada).

33.5 Numbers of nerve terminals per motor axon in sartorius and ext. l. dig. IV muscles of the frog. Y.M. Yao* and J.N. Weakly, Dept. of Physiology, Univ. of North Carolina at Chapel Hill, Chapel Hill, N.C. 27514

Histological, histochemical, and electrophysiological techniques were used to estimate the mean number of nerve endings elaborated by motor axons innervating the sartorius or ext. l. dig. IV (toe) muscles of the frog, R. pipiens. The sartorius muscle contains 700-900 extrafusal muscle fibers; the toe muscle, 50-75. Retrograde labeling of motoneurons by horseradish peroxidase applied to the muscle nerves indicates that the sartorius muscle is supplied by 10-14 motoneurons; 4-8 motoneurons innervate the toe muscle. Cholinesterase staining suggests that, on average, each sartorius muscle fiber has about 2.5 end-plates (range, 2-5), whereas the average number of end-plates per fiber in the toe muscle is about 1.3 (range, 1-3). Graded stimulation of the motor nerve revealed that about 7% (range, 1.3-16%) of the end-plates in normal sartorius muscles were polyneuronally innervated, most receiving connections from two motor axons. In contrast, no focal polyneuronal innervation was observed in normal toe muscles. These data suggest that each sartorius motoneuron, on average, gives rise to 135-240 nerve terminals, whereas each toe muscle motoneuron may elaborate as few as 8-24 endings. These observations in normal muscles provide the basis for transplantation experiments designed to determine whether, following reinnervation, the number of terminals per motor axon depends upon alterations in the peripheral field (e.g., numbers of muscle fibers and "old" end-plates) into which the regenerating axons must grow.

33.6 THE RATE OF AXONAL REGENERATION DIFFERS BETWEEN THE CENTRAL AND PERIPHERAL PROCESS OF THE DORSAL ROOT GANGLION (DRG) CELL. J. R. Wujek* and R. J. Lasek, Dept. of Anat., Case Western Reserve Univ., Cleveland, OH 44106

The DRG cell sends out a single axon which bifurcates within the ganglion; one axon proceeds centrally into the spinal cord and the other proceeds peripherally. In the peripheral branch of the DRG cell, the rate of slow axonal transport and the amount of material transported is greater than in the central branch. Furthermore, Black and Lasek (Exp. Neurol., 63: 108, 1979) have hypothesized that the slow axonal transport (SCb) rate will, in part, determine how rapidly regenerating axons will elongate. The DRG cell provides a unique system to test whether the regeneration rates of the two branches differ. If so, will the rates of regeneration vary accordingly with the SCb rates in the DRG axons?

Slow component b of axonal transport was labeled by injection of ^{35}S-methionine into the lumbar five (L5) DRG of albino rats. The radioactivity of a protein (43K daltons), indicative of SCb, was measured and the distance from the DRG to the wave peak and leading edge was used to determine the SCb transport rates. In the regeneration experiments, either the L5 dorsal root or the sciatic nerve was crushed with a jeweler's forceps; then a ^{3}H-proline/lysine mixture was injected into the L5 DRG. The axonally transported radioactivity served as a marker for the regeneration distance of the fastest growing axons.

The regeneration rates of the DRG cell axons were 2.0 mm/day in the dorsal root (central branch) and 4.6 mm/day in the sciatic nerve (peripheral branch). This difference offered the opportunity to further test the hypothesis of Black and Lasek by measuring the SCb rate in two branches of the same nerve cell. Measurements of SCb demonstrate that the transport rate was 1.4 - 2.0 mm/day in the dorsal root and it was 3.9 - 5.5 mm/day in the sciatic nerve. Thus, the rates of axonal regeneration correlated with the rates of SCb in the central and peripheral processes of the DRG cell. These data support the hypothesis that SCb may be a limiting factor influencing the rate of axonal regeneration.

33.7 THE ROLE OF SLOW COMPONENT a (SCa) IN DETERMINING AXON CALIBER: CHANGES DURING REGENERATION. P.N. Hoffman* J.W. Griffin* and D.L. Price* (SPON: S.E. Poduslo). Laboratories of Neuropathology and Neuromuscular Diseases, Johns Hopkins School of Medicine, Baltimore, Md 21205.

Several studies have documented persistent reductions in the caliber of regenerating axons proximal to the site of axotomy. The reversibility of this reduction appears to depend on successful reconnection with target cells in the periphery. Recently Hoffman and Lasek (Brain Res., in press) have demonstrated a decrease in the amount of labeled neurofilament protein and tubulin transported in SCa in regenerating motor neurons of the rat sciatic nerve. The reduced labeling of these proteins was specifically confined to SCa since it was accompanied by an enhanced labeling of proteins in SCb. This reduction in SCa may be related to changes in axon caliber during regeneration because previous studies have shown that axon cross-sectional area is directly proportional to the number of microtubules and neurofilaments. Thus, a decrease in the number of microtubules and neurofilaments entering the regenerating axon, via SCa, could represent at least one mechanism responsible for the reductions in axon caliber which occur during regeneration. If this hypothesis is correct, then axon shrinkage should start near the cell body and spread distally at a rate equal to the velocity of SCa (1.7 mm/day).

In order to test this idea we have analyzed axon caliber at three different levels of the L_5 ventral roots following crush injuries to the rat sciatic nerve. We have found that axon diameter at the root entry zone was significantly reduced in relation to contralateral controls at 1 week post-axotomy. By 3 weeks this reduction was present along the entire 25 mm length of the ventral roots. Clearly, these findings are consistent with our hypothesis that the reduced transport of neurofilaments and microtubules in SCa plays an important role in reduction in axon caliber during regeneration. They also suggest that SCa is a major intrinsic determinant of axon caliber.

33.8 THE INFLUENCE OF AXOTOMY ON GLUCOSE UTILIZATION IN RAT SUPERIOR CERVICAL GANGLION EXPLANTS IN VITRO. C. W. Montgomery*, A. Jerkins*, A. Dombrowski* and F. C. Kauffman., Univ. MD, Sch. Med. Pharm. & Exptl. Therap., Baltimore, MD 21201.

Axotomy of the rat superior cervical ganglion produces marked alterations in metabolism associated with the accumulation of lipid, protein and nucleic acids during the initial period of the retrograde response (Harkonen & Kauffman, Brain Res. 65: 127-157, 1974). Mechanisms underlying these anabolic responses are poorly defined and difficult to study in vivo. Consequently, comparative biochemical studies of explants from normal and axotomized rat superior cervical ganglion were carried out under conditions that promote outgrowth of neurites from these tissues in vitro. Explants obtained from adult rat superior cervical ganglion display consistent growth of neurites when cultured in Eagle's minimum essential medium supplemented with 1% bovine serum albumin and nerve growth factor (100 units/ml). In the present study, ganglia which were axotomized for three days in vivo and contralateral intact ganglia were prepared for explant culture and maintained in vitro for 24 h in the presence or absence of 0.5 mM glycerol. In the absence of glycerol, glucose utilization and lactate production were the same in explants of control and 3-day axotomized ganglia. Addition of glycerol to the media increased glucose utilization nearly two-fold in axotomized ganglion explants (1.68 + 0.07 vs 2.95 + 0.16 μmol/mg dry wt/24 h) but had no effect on glucose utilization in explants from intact ganglia (1.26 + 0.45 vs 1.28 + 0.14 μmol/mg dry wt/24 h). The increase in glucose utilization in the axotomized ganglion explants was accompanied by a significant increase in lactate production after the addition of glycerol (4.62 + 0.38 vs 6.34 + 0.59 μmol/mg weight/24 h). Stimulation of glucose utilization in explants from axotomized ganglia by glycerol does not appear to be related to gross alterations in energy state of the tissue since ATP concentrations in explants from control and axotomized ganglia were the same in both the presence and absence of glycerol. However, in the absence of glycerol concentrations of α-glycerophosphate in explants from axotomized ganglia were only one-half the values noted in control ganglia (1.39 + 0.24 vs 0.66 + 0.08 μmol/mg dry wt). Addition of glycerol to the media increased concentrations of α-glycerophosphate in explants from axotomized ganglia but did not alter concentrations of this metabolite in explants from control ganglia. Thus, in the absence of exogenous glycerol, lipid synthesis may be limited in explants from axotomized ganglia, by the glycerol moiety as suggested by lower concentrations of α-glycerophosphate in this tissue. Addition of glycerol to the culture medium may enhance lipogenesis and thereby stimulate glucose utilization.

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33.9 Axonal Transport of Adenyl Cyclase in Frog Sciatic Nerve: Alterations Following Axotomy

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Orthograde axonal transport of adenyl cyclase occurs in normal, intact, frog sciatic nerves. There is no evidence for retrograde transport in the same nerves. Transport was measured as a progressive increase in adenyl cyclase activity occurring in the 5 mm nerve segment just proximal to a constricting suture placed around the nerve. The increase in enzyme activity reached 179% of control values 24 hours following nerve constriction. Activity in the 5 mm segment distal to the tie was not different from control values.

Twenty four hour accumulation experiments were also performed in sciatic nerves transected 1,3,5,7 and 9 days previously. While control, presumably non-mobile, enzyme activity levels remained equivalent to those in intact nerves, transported enzyme activities were altered following nerve transection. Orthograde adenyl cyclase transport declined progressively after axotomy. Twenty four hour accumulation of activity proximal to a constriction reached 141% of control in nerves transected 3 days previously, 119% 5 days after transection, and was not different from control values in nerves 7 and 9 days postaxotomy. In contrast to normal, intact, nerves, accumulation of enzyme activity distal to a constriction increased significantly ($p<.05$) above control values in nerves transected 3 and 5 days previously. Activity increased to 132% of control in 3 day axotomized nerves and to 113% in 5 day nerves. Distal accumulation was not different from control 7 and 9 days after transection. The time course of this novel appearance of retrograde adenyl cyclase transport in injured nerves compares closely to our previous demonstration of a transient increase in cyclic AMP concentration in axotomized spinal roots (Exp. Neurol. 66:556, 1979). We are now attempting to determine what function the normal orthograde transport of adenyl cyclase subserves, and that "signal" may be associated with the transient retrograde transport appearing in injured nerves (Supported by NIH NS15065).

33.10 PROTEIN SYNTHESIS AND AXONAL TRANSPORT FOLLOWING PERIPHERAL NERVE DAMAGE. G.W. Perry* and David L. Wilson. Dept. of Physiology and Biophysics, U. of Miami, Sch. of Medicine, Miami, FL. 33101.

Previous studies have suggested that following peripheral nerve damage some changes can be detected in rapidly transported protein during subsequent regeneration.[1,2] These studies employed one-dimensional gel electrophoresis for analysis of rapidly transported proteins. However, such techniques were unable to distinguish differences arising as a result of changes in relative amounts of transported proteins from differences caused by the appearence of new species of transported protein. Also, previous studies have shown that changes in the synthesis of specific proteins occurs in sympathetic ganglia as a result of axotomy.[3] Here we explore these issues utilizing the excellent resolution afforded by two-dimensional gel electrophoresis.

Sciatic nerves of the Bullfrog, Rana catesbeiana, were severed or crushed unilaterally in vivo, approximately 30 mm from the 8th and 9th dorsal root ganglia (DRG). One, two or four weeks after nerve damage the sciatic nerves together with the 8th and 9th DRG were removed and the ganglia selectively labelled with ^{35}S-met. for 8 hrs in vitro (see ref.4). Rapidly transported proteins were collected at a ligature placed just proximal to the site of nerve injury (usually 30 mm), and at a similar distance in the unoperated contralateral control nerves. After 24 hrs at 18°C the labelled ganglia, and protein which had accumulated in the 3 mm segment proximal to the ligature, were subjected to two-dimensional gel electrophoresis. Qualitative and quantitative comparisons between experimental and control ganglia and rapidly transported proteins were made from fluorographic patterns of the dried gels. Qualitative comparisons of changes after 1,2 and 4 weeks revealed similar patterns; consequently these data were pooled.

No new rapidly transported protein species were detected following either nerve crush or cut. However, significant changes in the relative abundance of label in a few transported proteins were observed. These changes were present irrespective of whether the sciatic nerve had been crushed or cut. Also, the relative synthesis rates of several proteins in the ganglia were changed. Furthermore, qualitative comparison between control and normal, unoperated animals detected no differences.

These results support the hypothesis that peripheral neurones are capable of effecting regeneration without gross readjustment in their protein synthesis and transport.

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33.11 SPONTANEOUS ACTIVITY IN PRIMARY AFFERENT NEURONS PRIOR TO REINNERVATION OF THEIR MUSCLE. M. DeSantis and J. W. Duckworth*. Dept. of Biological Sciences and WAMI Medical Program, Univ. of Idaho, Moscow, ID 83843

Discharge properties of primary afferent neurons were studied for up to 20 days after freezing the nerve to the medial head of the gastrocnemius (MG) muscle with dry ice in adult male and female cats anesthetized with pentobarbital. Units were recorded in the L7 and S1 dorsal roots, after acute denervation of the hind limb save for the previously lesioned MG nerve, and from the MG nerve itself. Units recorded in the dorsal root were identified by the all-or-nothing, time-locked action potential elicited on stimulation of the MG nerve proximal to the lesion. Comparison was made with recordings from cats in which the muscle nerves were unoperated, sham-operated and crushed. The last category gave the same results as when the lesion was made by freezing.

As the MG nerve was frozen, the neurons being damaged fired a barrage of spikes, but then no more spikes occurred during recordings of up to several hours. From 2 to 20 days after the lesion, there was spontaneous discharge in some MG primary afferents, although an even greater number had none. About half of the active units discharged continuously. The other half fired trains of spikes in a cyclic pattern. The train duration varied for a given unit and in an independent fashion for units recorded simultaneously. The interspike interval was constant and typical for a given cyclic unit regardless of the variability in train duration.

Except for a few units recorded at 20 days after the lesion, neither the quiet nor the spontaneously active units were responsive to static lengthening of the MG muscle or to succinylcholine. Most units, whether quiet or spontaneously active, responded to dynamic lengthening of MG; a majority of only the spontaneously active ones fired a series of action potentials to stimulation of the MG nerve.

In recordings from dorsal roots, most MG units with spontaneous discharge continued to be spontaneously active after we cut the MG nerve and then the sciatic nerve at the hip. Lidocaine placed on the spinal ganglion quieted these units. In other cats, recordings were made from the proximal and distal ends of the MG nerve cut between the ganglion and the previous freeze lesion. Multi-unit activity was greater in the proximal than in the distal cut end of the MG nerve. Subsequent lumbosacral laminectomy followed by cutting the dorsal and ventral roots had no effect on the spontaneous activity in the proximal cut end. Lidocaine placed on the spinal ganglia abolished the discharge.

Thus, some primary afferent neurons from muscle show spontaneous activity after a lesion of their peripheral processes. The spikes may be initiated in the cell body region.

33.12 A COMPARISON OF REINNERVATION OF CAT CAROTID BODY BY CAROTID SINUS AND LINGUAL NERVES. L.J. Stensaas, B. Dinger*, S.J. Fidone. (SPON: K. Horch). Dept. Physiol., Sch. Med., Univ. Utah, Salt Lake City, UT 84108.

The relative contributions of afferent nerve fibers and carotid body type I and type II cells in chemosensory transduction have not been completely clarified. One possible approach to this problem is to compare the chemosensory function of the carotid body following reinnervation by foreign or normal nerves. Previous authors have found chemosensory activity elicited from carotid bodies reinnervated by the superior laryngeal n. (s.l.n.). The relevance of these results to the issue of the specific functions of type I and type II cells has been criticized because the s.l.n. contains fibers innervating receptors in the upper respiratory tract which respond to 100% CO_2. Another interesting result following reinnervation of the carotid body by the s.l.n. is that these foreign fibers did not form normal anatomical relationships with type I cells of the carotid body. We have readdressed these questions by reinnervating the carotid body with either the lingual branch of the IXth n., or with the carotid sinus n. (c.s.n.) so as to provide necessary control information.

Quantitative electron microscopic examination of carotid bodies from normal and cross-anastomosed animals 2-19 months following surgery reveals a 90% reduction in the number of afferent terminals contacting type I cells (n = 5). Similar studies of tissue reinnervated by the c.s.n. shows that the number of synaptic contacts per type I cell nucleus is reduced by only 30% (n = 3). That these differences are not due to failure of foreign fibers to invade the carotid body is demonstrated by several types of data. (1) Application of tritiated amino acids to the petrosal ganglion results in the build-up of labeled material in the carotid body over a period of several days in cross-anastomosed animals. (2) Light microscope autoradiography of this tissue has shown that nerve fibers are distributed around the lobules in very much the same manner as in normally innervated tissue. (3) Further examination of the tissue with the electron microscope has demonstrated many unmyelinated fibers invading the lobules, but few of these contact type I cells because of interposed slender processes of type II cells. The histological results from both groups of animals are correlated with the physiological findings: chemoreceptor activity is present following foreign innervation but the threshold is greatly elevated with respect to natural and pharmacological stimuli; carotid bodies reinnervated by the c.s.n. respond similarly to normal carotid bodies.

Our results demonstrate specific properties of c.s.n. afferents and, in addition indicate an important role for carotid body parenchymal cells in chemosensory transduction. Supported by USPHS research grants NS 12636 and NS 07938.

33.13 EFFECT OF THYROID HORMONE ON NERVE REGENERATION.
W.H.A. Yu* and R. Srinivasan* (Sponsor: J.Shriver). Department of Anatomy, Mount Sinai School of Medicine, New York, N.Y. 10029.

We have reported previously (Anat. Rec. 196: 212A, 1980) that administration of testosterone enhanced nerve regeneration. The action of testosterone could be mediated by the nerve growth factor (NGF) even though it is controversial whether testosterone could raise the level of NGF in rats. Since thyroxine treatment significantly increased the NGF concentration in the rodent brain (Walker et al., Science, 204: 427, 1979), the present study was undertaken to examine the effect of thyroxine (T_4) on axonal outgrowth during nerve regeneration. The right hypoglossal nerve was transected in female rats aged 5 weeks. From the 1st postoperative (PO) day the experimental group received 50 µg of T_4 daily s.c. for a week and then 10 µg daily thereafter. The control group received normal saline. Animals from both groups were killed at the end of 2nd, 3rd and 4th PO weeks. 24 hours prior to sacrifice 20ul of 10% HRP solution was injected into the tongue. After suitable fixation 50µ serial frozen sections of the caudal brain stem were cut and reacted histochemically for demonstration of HRP activity. Comparative quantitative analysis of the HRP labeled neurons of the right and left hypoglossal nuclei revealed that during the 2nd PO week few axons had regenerated in both the control and T_4-treated animals. During the 3rd PO week there was a substantial increase in the number of axons regenerated in control animals but none in T_4-treated animals. Both the control and T_4-treated animals showed almost complete regeneration of axons during the 4th PO week. These results indicate that thyroxine at the dose used had no effect on the rate of axonal elongation in the transected hypoglossal nerve.

36.1 GATING KINETICS OF THE TRANSDUCTION ELEMENT IN A VERTEBRATE HAIR CELL: EVIDENCE FOR A THREE-STATE MODEL. D. P. Corey and A. J. Hudspeth, Division of Biology, California Institute of Technology, Pasadena, CA 91125

A large variety of ionic channels in cell membranes can be thought of as existing in either open or closed states. The probability of a channel being in a given state is determined by the relative energy of that state, and the relative energies are changed - the channel is gated - by factors such as voltage and binding of ligands. We have evidence that the opening of transduction channels in vertebrate hair cells involves a redistribution between states; here the energy change is effected by displacement of the hair bundle.

An in vitro preparation was used, wherein a small portion of the bullfrog's sacculus was stimulated and the transepithelial receptor current was measured. Step displacements of the otolithic membrane were complete within 80 μs, and the voltage clamp included series-resistance compensation to reduce its response time to <10 μs. The preparation was maintained at 4 °C to slow the gating kinetics.

Following a 0.1-μm step displacement of the otolithic membrane in the positive direction - toward the kinocilium - the current increases with a roughly exponential time course with a time constant of 400-500 μs. Larger displacements elicit larger currents and shorter time constants; with displacements larger than 0.5 μm the current saturates but the time constant continues to decline. Negative displacements decrease the current but also decrease the time constant; the negative saturation occurs with displacements of >0.1 μm. The relationship between steady-state current and displacement (displacement-response curve) is roughly sigmoidal, consistent with a probability distribution between two states. The roughly exponential approach to steady state and the bell-shaped variation of time constant with displacement imply an energy barrier to transition between the states of ∿12 kcal/mol whose height is influenced by bundle displacement. Thus energy states of the transduction channel appear to be directly and continuously modified by hair bundle position.

The two-state model is only qualitatively satisfactory, however: a sigmoidal rise at the onset of current, a two-phase fall at the offset, and an asymmetry in the displacement-response curve are all inconsistent with two states and a single transition. Good quantitative agreement requires a model with three states. The transition from the first (nonconducting) state to the second (nonconducting) state is relatively slow and very displacement-sensitive; the subsequent transition to the third (conducting) state is faster and less displacement-sensitive.

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36.2 ELECTROPHYSIOLOGY OF SOLITARY CONES FROM THE TIGER SALAMANDER RETINA. P. R. MacLeish and M. Tachibana*. Dept. of Neurobiology, Harvard Med. Sch., Boston 02115.

Solitary cones, obtained by enzymatic dissociation of the retina of the tiger salamander, could be maintained in vitro for several days during which time their responses to light and their electrical properties could be studied using intracellular recording techniques. These solitary cones retained the morphological features of cones and were easily distinguished from solitary rods, whose physiological properties already have been reported (Bader, MacLeish and Schwartz (1979), J. Physiol. 296:1).

The average resting potential of dark-adapted solitary cones was -35 ± 8 mV (mean, ± S.D.); the range was from -19 to -50 mV. In response to a brief flash of white light, dark-adapted solitary cones gave hyperpolarizing responses whose time-course was similar to that of cones in the intact retina but faster than that of solitary rods. In the intact retina, a similar difference in time-course between responses of cones and rods has been reported (Lasansky and Marchiafava (1974), J. Physiol. 236: 171). The amplitude of the responses in solitary cones increased over about 3 log units of light intensity and reached a saturating value, Vmax, of 18 ± 3 mV. The relationship between the peak amplitude of the response, V, and light intensity, I, was described by the equation V/Vmax= I/(I + K), where K is the light intensity that produces a response with a half-maximal peak amplitude. A reversal potential for the light response was found in the range of 0 to +10 mV.

In the fully dark-adapted state, solitary cones were less sensitive to light than were solitary rods; the light intensity that gave a just barely detectable response in cones was 2.2 log units brighter than that in rods. At this higher light intensity the responses in rods were near saturation.

Both solitary cones and rods had a transient component in the response to a bright flash but this was less prominent in that of cones (11-17% of peak response amplitude) than in that of rods (33-47% of peak response amplitude). Voltage- and time-dependent mechanisms generate the transient in the case of rods, and are likely to generate at least part of the transient in the case of cones; passage of rectangular pulses of hyperpolarizing current in solitary cones gave rise to an on-transient that was similar in amplitude and time-course to the transient seen in the responses to bright flashes. The presence of voltage-dependent mechanisms in solitary cones is further demonstrated by the steady-state current-voltage relationship which revealed outward-going rectification. The number and characteristics of these voltage- and time-dependent mechanisms remain to be determined.

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36.3 TEMPERATURE DEPENDENCE OF A VOLTAGE DEPENDENT JUNCTIONAL CONDUCTANCE. A.L.Harris, D.C.Spray, and M.V.L. Bennett, Div. Cellular Neurobiology, Dept. Neuroscience, A. Einstein Col.Med.,BX,NY 10461

The conductance of electrotonic junctions between Ambystoma blastomeres is strongly voltage dependent (Spray et al., Science 204: 432,'79). The effect of temperature between 17.5 and 25°C on steady state and kinetic properties was studied by means of a dual voltage clamp technique. Previous data are consistent with mediation of this conductance by intercellular channels exhibiting first order transitions between open and closed states and energy differences between open, closed and transition states linearly affected by transjunctional voltage. The junctional conductance measured at the onset of a small transjunctional voltage step decreased with decreasing temperature with a Q_{10} of 1.2. The voltage sensitive component of the steady state conductance decreased with decreasing temperature with a Q_{10} of 1.7 at 15 mV and a Q_{10} of 2.9 at 27 mV. The time constant of the exponential decline of junctional conductance at each transjunctional voltage increased with decreasing temperature. At 15 mV, the time constant had a Q_{10} of 2.35, and at 27 mV, the time constant had a Q_{10} of 2.15. Since a Q_{10} of 1.2 is expected for an electrolyte filled pore, we conclude that at V=0 temperatures over the range studied do not significantly affect the distribution of channels between open and closed states. The larger Q_{10}'s are reasonable for conformational changes in macromolecules. The steady state conductance during voltage steps permits calculation of the ratio of open to closed channels which is equal to the equilibrium constant of the transition between states. If the voltage sensitivity of the energy difference between states is assumed temperature independent, the Q_{10} of the equilibrium constant permits calculation of the change in enthalpy (ΔH) between the open and closed states from the integrated form of the van't Hoff equation. By this method, ΔH equals approximately 11 kcal/mole. From ΔH, together with ΔW, about 2 kcal/mole, the difference in energy between open and closed states at V=0 derived from analysis of the voltage dependence (Spray et al., ibid.), one obtains the difference in entropy of the two states as approximately 31 cal/°C/mole. At 27 mV transjunctional voltage the time constant is dominated by the closing rate constant. Application of the Arrhenius equation yields an energy of activation (E_a) of 13 kcal/mole at this voltage. From the voltage sensitivity of opening and closing rates and their linearity with transjunctional voltage, one calculates that at V=0 the E_a for closing is 15.3 kcal/mole and the E_a for opening is 13.3 kcal/mole. These calculations assume that the temperature changes act on the free energy of the channel molecule directly and not via secondary mechanisms.

36.4 CONDUCTANCE OF GAP JUNCTIONS IS HIGHLY SENSITIVE TO CYTOPLASMIC pH. D.C.Spray, M.V.L.Bennett & A.L.Harris. Div. Cellular Neurobiology, Dept. Neuroscience, A. Einstein Col.Med., BX,NY 10461

Conductance of gap junctions (g_j) between mechanically dissociated pairs of embryonic cells from axolotl (Ambystoma mexicanum) and killifish (Fundulus heteroclitus) was measured directly with a dual voltage clamp technique or calculated from current clamp data using the π-T transform. Intracellular pH (pH_i) was measured in one cell of the pair using a microelectrode with sealed pH-sensitive glass recessed inside the tip (Thomas type). Response time constants of the pH electrodes were hundreds of msec for voltage changes and seconds for pH changes. A pH electrode was considered to be intracellular only if it recorded the same steady state voltage during current pulses as a KCl filled electrode in the same cell. Normal internal pH for isolated pairs of morula stage Ambystoma blastomeres was 7.75 ± 0.06 (SD;n=9) and for 32-64 cell stage Fundulus blastomeres pH_i was 7.67 ± 0.06 (SD; n=9). On addition of saline equilibrated with 100% CO_2 to the bath, pH_i and g_j decreased; the relationship between pH_i and g_j was sigmoid. There was little hysteresis for values obtained during intracellular acidification and recovery on washing. The sigmoid g_j vs.pH_i relations for both species are well fit by titration curves of the form $G_j=K^n/K^n+H^n)$ where G_j is normalized g_j, K is about 50nM (pK about 7.3) for both Ambystoma and Fundulus, and n, the equivalent number of titratable sites, is 4 to 5 for both species. Bathing for up to 10 min with strong acids or impermeant buffers at pH 6 had no effect on g_j. Bathing with the weak organic acids propionate, acetate, and lactate (120 mM, pK's 4.87; 4.75; 3.08 respectively) could reduce pH_i and g_j. The speed and degree of reduction depended inversely on the external pH and their effects at low external pH (6.5) could be reversed by treatment with the same solutions at higher pH (7.5). We conclude that these weak acids decrease pH_i by crossing the cell membrane in the undissociated form. Kinetic parameters of voltage dependence of Ambystoma junctions were unaffected over the entire titration curve for g_j. This observation suggests that separate sites mediate pH and voltage sensitivity of the gap junction channel macromolecule. The precise dependence of g_j on pH_i and the absence of hysteresis during relatively rapid changes support the hypothesis that hydrogen ions act directly on the channel macromolecule and not through changes in cytoplasmic concentration of some other substance such as Ca^{++}. Constancy of pCa_i during marked changes in g_j induced by weak acids was reported previously (Biol. Bull. 155: 428, 1978). DCS is a McKnight Scholar in Neuroscience.

36.5 INTERNAL PERFUSION OF NEUROBLASTOMA CELLS AND THE EFFECTS OF DIPHENYLHYDANTOIN ON VOLTAGE-DEPENDENT CURRENTS. Fred N. Quandt* and Toshio Narahashi (Spon: A.I. Farbman). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Neuroblastoma cells represent an excellent material for the pharmacological study of neurons. They are mammalian cells, and can be subjects to a variety of measurements including those of ionic conductances, receptor binding and ionic fluxes. We have successfully applied the internal perfusion and voltage clamp techniques originally developed for snail neurons by Lee et al. (J. Gen Physiol. 71, 489, 1978) to neuroblastoma cells (N1E-115) using one or two suction pipettes. This technique offers significant advantages over the two microelectrode voltage-clamp technique. First, current through the Na, K, or Ca channels can usually be observed for over an hour without much deterioration. This is critical for any drug action to be monitored over a certain period of time. Second, better resolution of the fast currents can be obtained with this technique than with the conventional two microelectrode method. Third, current through the K channel can be blocked by replacing internal as well as external K^+ with Cs^+. This enables a more detailed analysis of the currents through Na^+ and Ca^{2+} channels to be made. Normal internal perfusion had the following composition: 100 mM K glutamate, 100 mM sucrose, 20 mM Na HEPES (pH 7.25). Na currents were recorded at 10°C. Currents through the Ca channel were examined at 30°C after equimolar replacement of Na^+ with either 20 mM Ca^{2+} or 60 mM Sr^{2+} in order to increase their amplitude. Residual Na currents were blocked with 0.5 µM tetrodotoxin. As an example of the use of neuroblastoma cells for pharmacological study, the effects of the anticonvulsant diphenylhydantoin (DPH) on the action potential and voltage-dependent ionic currents of these cells were examined. Repetitive firing of action potentials often occurred following the termination of anodal polarization at 30°C. External application of 20 to 50 µM DPH suppressed the repetitive firing obtained under these conditions. However, the initial anodal break action potential appeared normal in the presence of DPH within this range of concentrations. Peak inward Na currents in response to step depolarizations from a holding potential of -80 mV were reduced only 10% by DPH. Peak current through the Ca channel appeared to be reduced 25% in the presence of DPH. This latter effect may underly the ability of DPH to block repetitive firing in these cells. DPH did not reduce either current to a greater extent during repetitive depolarizing voltage steps which is characteristic of compounds exhibiting "use-dependent" block.

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36.6 SOME ELECTROPHYSIOLOGICAL PROPERTIES OF PITUITARY TUMOR CELLS. A. R. Martin. Dept. of Physiol., Univ. Colorado Med. Sch.,Denver, CO 80262.

Electrophysiological measurements were made on mouse anterior pituitary tumor cells (AtT-20/D-16v) which secrete ACTH/endorphin (B.A. Eipper & R.E. Mains, Endocrine Rev., 1: 1, 1980). Dishes containing low density cultures were mounted on the stage of a compound microscope and perfused with a saline solution containing 10% modified Eagle's medium and kept at 36°C. Cells were observed with interference contrast optics at 320X. Cell penetrations were made with microelectrodes of 150 - 200 MΩ resistance. Standard techniques were used for single electrode recording and current passing. Successful penetrations lasted for up to 2 hours.

Resting potentials of the cells ranged from 40 to 80 mV, with a mean (±S.D.) of 57 ± 10 mV (n = 34). Input resistances and time constants were measured by passing current pulses through the recording electrode. Time constants ranged from 3.5 to 24 msec with a mean of 10 ± 6 msec; mean input resistance was 250 ± 130 MΩ. When input resistance was plotted against time constant, the points were scattered around a straight line corresponding to a cell capacitance of about 40 pF. Morphology of the cells varied considerably, but the "average" cell had a surface area of about 2300 μm^2. Thus the specific membrane resistance averaged about 5750 Ωcm^2 and the specific capacitance 1.7 $\mu F/cm^2$. When resting potential was plotted against time constant, there was, if anything, a slight negative correlation rather than the positive correlation expected if the variability of the passive properties were due to cell damage by the micropipette.

All but three of the 34 cells studied had some sort of regenerative response to depolarization, ranging from a barely detectable "hump" to repetitive action potentials. Two cells displayed spontaneous activity. Regenerative responses were unaffected by tetrodotoxin (10^{-6}gm/ml) and blocked by $CoCl_2$ (0.2 mM). In general the active and passive properties of the cells varied between two extreme states. At one extreme, cells had low resting potentials, long time constants and well-developed action potentials ("active"); at the other, resting potentials were high, time constants short and regenerative responses small or absent ("inactive"). Transitions from the active to the inactive state were observed during the period of recording in four cells; no transitions were observed in the opposite direction.

AtT-20 cells secrete about 15% of their content of ACTH/endorphin per hour. Norepinepherine (10^{-5}M), which approximately doubles this basal rate of secretion, had no electrophysiological effect on six cells to which it was applied.

36.7 ELECTRICAL ACTIVITY IN CONTINUOUSLY CULTURED SMALL CELL CARCINOMA OF THE LUNG (SCCL). Frances V. McCann, Olive S. Pettengill* and Jerome A.G. Russell*. Depts. of Physiology and of Pathology, Dartmouth Medical School, Hanover, NH 03755.

Electrical activity in cultured pulmonary small-cell carcinoma cells has been measured with intracellular electrodes coupled to a current injection bridge (WPI-Ks 700). Electrode and current monitor signals were digitized and stored on floppy discs by a New England Digital Corp. Able-40 computer. Determinations of cell membrane resistances, capacitances and time-constants were computed from the digitized records using a special curve fitting program.

DMS 53, a continuous cell line, was isolated from a primary SCCL tumor (Pettengill, et al., Cancer 45:906, 1980). Cells were plated at 10^5 cells/ml in Waymouth's medium with 20% FCS and buffered with 25 mM Hepes. Experiments were carried out at 36° ± 0.5°C, four days after plating. Only isolated cells, independent of contact with their neighbors were impaled.

Patterns of electrical behavior were of three types: passive, local and regenerative. Ranges of passive characteristics measured in 50 cells were:

Resting potential	-34 to -58 millivolts
Time-constant	5.37 to 8.87 milliseconds
Input resistance	25 to 115 megohms
Input capacitance	60 to 116 x 10^{-12} Farads

For the calculation of surface area the cell was assumed to be an oblate spheroid whose thickness is 1/2 its projected diameter. Surface areas ranged from 216 to 1951 μ^2. A typical value for specific membrane resistance was 1480 Ωcm^2 and specific capacitance was 6 $\mu F/cm^2$.

Local responses in many cells were elicited by injecting depolarizing current pulses, and the resulting peak voltages were proportional to the magnitude of the injected current. Delay in the appearance of the peak voltage was inversely related to the magnitude of injected current. A depolarizing rectification was also found to be characteristic of these cells.

In some cells, action potentials could be evoked by anodal break excitation. The amplitude and latency of these spikes were related to the level of membrane polarization.

These data strongly suggest that SCCL cells in this culture exhibit neural-like transmembrane electrical behavior.

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36.8 IDENTIFICATION OF THE LINEAR PROPERTIES OF A NEURON. C.I. Valenzuela.* Neural and Behavioral Biology Program, University of Illinois, Urbana, IL 61801.

The aim of this work is to characterize (identify) the linear electrical behavior of a neuron composed of a spherical soma concatenated to a cylindrical axon (or dendrite). The neuron input function is defined as the electrical current injected into the neuron soma (by means of a microelectrode) and the neuron output function is defined as the transmembrane voltage response recorded at the neuron soma (by means of a microelectrode).

The following parameters of the neuron in question are identified (the membrane is assumed to be homogeneous regarding its specific parameters):

1. τ_{sm}: soma time-constant (τ_{sm}=membrane time-constant, τ_m).
2. $\{\tau_n, n=1,2...\}$: axon "equalizing time-constants".
3. L: axon electrotonic length.
4. ρ: axon to soma conductance ratio.
5. R_n: neuron input resistance (as "seen" from the soma's microelectrode).
6. R_{ax}: axon input resistance (as "seen" from the soma's microelectrode).
7. R_{sm}: soma resistance.
8. C_{sm}: soma capacitance.

Also, the following functions of the neuron are identified:

1. $h_{sm}(t)$: soma impulse response.
2. $h_{ax}(t)$: axon impulse response (as "seen" from the soma).
3. $h_n(t)$: neuron impulse response (as "seen" from the soma).

The identification process employs the following techniques:

1. Rall's classical analysis of passive neuronal models (c.f. W. Rall, Biophys. J. 9:1483, 1969 and W. Rall, In "Handbook of Physiology: The Nervous System I, E.R. Kandel, ed., 1977).
2. Provencher's algorithm and program for the analysis of multicomponent exponential decay data (S.W. Provencher, J. Chem. Phys. 64:2772, 1976 and S.W. Provencher, Biophys. J. 16:27, 1976).
3. A program to deconvolute the output function and the impulse response function of a linear system. Written by the author.

The relevance of this work to the study of the non-linear (active) properties of neurons in non-isopotential experimental situations as well as to voltage clamp studies of somata of whole neurons is discussed. Supported by a scholarship awarded to C.I. Valenzuela by the Consejo Mexicano de Ciencia y Tecnologia (CONACYT) and by PHS-NS15186 to J.A. Connor.

36.9 A PRACTICAL METHOD FOR INDIRECT ESTIMATION OF THE BIAS OF AN EXTRACELLULAR RECORDING EXPERIMENT TOWARDS LARGE NEURONS.
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Despite the overwhelming preponderance of small neurons in most of the mammalian CNS, when extracellular recording techniques are used the larger neurons are represented in the sample to a much greater extent than is warranted by their numbers alone. The excessive representation of large neurons reflects the bias of extracellular recording techniques in favor of large neurons. In a previous communication(Abzug, C.(1978). Society for Neuroscience Abztracts, 4: 387) I reported on the participation of several significant parameters, some neuronal(i.e., anatomical and biophysical) and some experimental(e.g., speed of electrode advancement), in determining the chances of detecting neurons of different size. The present communication describes a technique for indirect experimental measurement of the bias. The probability of detection of a neuron in a single electrode track can be expressed as:

$$P(\delta) = k \cdot D^{j} \cdot V_{min}^{-m},$$

where D is the neuron's diameter, V_{min} is the smallest size of extracellular "action potential" that can be reliably detected with the particular electrode and recording system in use, and k, j and m are positive constants subject to the constraint that $j = 2m$. The value of V_{min} can be experimentally manipulated to any arbitrary value larger than the smallest possible value, V_{spv}, by rejecting all action potentials observed whose amplitudes are smaller than V_{min}. Thus, in general we can have $V_{min} = n \cdot V_{spv}$. $N(D)$ is defined as the distribution of neurons of size D in the structure to be examined. The expected number of neurons to be encountered during a single track is given by:

$$E_n(N) = \sum_D N(D) \cdot kD^{j} (V_{min})^{-m},$$

and therefore:

$$E_n(N) = n^{-m} \cdot E_1(N).$$

Thus, by plotting $E_n(N)$ as a function of n the value of m, and hence also of j, can be determined. The above derivation implies the assumption that the speed of microelectrode advancement is uniform throughout the experiment. Thus, determination of the value of j must be made by means of a motorized microdrive.

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37.1 INTERACTIONS OF LOCUS COERULEUS TRANSPLANTS WITH POSTNATAL DEVELOPING CEREBELLUM: DOES TARGET AGE INFLUENCE GROWTH?

R.H.Schmidt, U. Stenevi* and A. Björklund*, Dept. Histology, Univ. Lund.

Locus coeruleus afferents to the cerebellum have a marked capacity for regenerative growth after 6-hydroxydopamine treatment, but only during a limited developmental critical period. This growth period may be regulated to an important extent by the target tissue because it is temporally independent of developmental and regenerative growth of locus coeruleus collaterals to other CNS targets. To test this hypothesis we are looking for age-dependent differences in cerebellar reinnervation by locus coeruleus transplants to postnatal rat hosts.

Hosts consisted of postnatal rat pups between 2 and 20 days old, denervated 1-3 days earlier by an 80-100 µg intracisternal dose of 6OHDA. Brain stem tissue containing the locus coeruleus was obtained from 14-25 mm CRL rat embryos, and was implanted into the cerebellum using a glass needle inserted via the cisterna magna. After 4-12 weeks survival the rats were processed for catecholamine histofluorescence. Transplants were successful to host cerebella at all ages attempted, and contained up to 400 noradrenergic neurons with normal morphology and visible dendrites. These were usually found partially within the IVth ventricle fused to the vermis, with axons distributing toward the host accross the sites of fusion, thus leading to at least partial reinnervation of the cerebellum. Rats receiving transplants at younger ages generally had considerably higher densities of cerebellar noradrenergic innervation, even profound hyperinnervation, than rats transplanted when greater than 7 days old. In control material processed in parallel without transplants a variable quantity of endogeneous innervation was present in younger hosts, thus precluding a definative conclusion of the extent of transplant mediated reinnervation. The fact that detectable growth from the transplants has occurred has been shown with cerebellar injections of a fluorescent tracer compound, propidium iodide, which lead to retrograde labelling of noradrenergic neurons within the transplants.

In a related study, embryonic cerebellar tissue transplanted to the cerebellum of hosts at various ages does become innervated by host locus coeruleus axons, regardless of host age. These experiments represent a feasible strategy by which the source of axonal growth control during development and regeneration, ie. source neuron or target, can be identified.

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37.2 AXONAL TRANSPORT AND TRANSCELLULAR TRANSFER OF NUCLEOSIDES AND POLYAMINES IN THE GOLDFISH VISUAL SYSTEM: A MODEL FOR AXONAL REGULATION OF PERIAXONAL CELL METABOLISM.

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The axonal transport (AT) and transcellular transfer of uridine, adenosine, and polyamines have been compared in intact and regenerating optic axons of goldfish. Both optic nerves of fish were crushed and 18-30 ds later, as regenerating fibers are reentering the tectum, ^{3}H-uridine, or ^{3}H-adenosine, was injected into the right eye. At various times after injection, right and left tecta were analyzed for trichloroacetic acid (TCA) soluble and insoluble radioactivity. AT was determined by comparing left-right difference in radioactivity, and since the majority of incorporation of small molecules into macromolecules occurs in periaxonal cells, transcellular transfer was indicated by the levels of TCA insoluble radioactivity, and confirmed by light autoradiographic analysis. Results demonstrated AT, transcellular transfer, and periaxonal cell utilization of both nucleosides in intact axons and several fold increases of all of these processes in regenerating axons. Following lyophilization of TCA soluble tectal radioactivity, approx. twice as much radioactivity was lost from the right tecta (which receives only blood borne radioactivity) compared with radioactivity in the left tecta, (which primarily receives axonally transported material). These results suggest that axonally transported uridine and adenosine are protected from degradation and thus relatively more available for periaxonal cell utilization than radioactivity reaching these cells via the blood.

In order to determine if axonally transported polyamines are transferred to periaxonal cells, regenerating optic axons were labelled with ^{3}H putresine or spermidine for 3 or 6 ds before removing the axons from the tectum by cutting the optic nerve. After allowing one week for degeneration to occur, approx. 55% of the radioactivity following ^{3}H-putrescine injections and 74% following ^{3}H-spermidine injections was still present in the tectum, suggesting that following AT ^{3}H-putrescine and ^{3}H-spermidine are transferred to periaxonal cells.

On the basis of these and previous findings, we propose that the AT and transcellular transfer of uridine, adenosine, the polyamines and perhaps other small molecules, is a means of communication between axons and periaxonal cells, that the axon can effect RNA and protein synthesis in periaxonal cells by regulating the availability of these small molecules, and that during nerve regeneration the increased metabolic needs of periaxonal cells, are met by increased axonal supply of precursors (adenosine and uridine) and other molecules (polyamines) critical for protein synthesis. Supported by EI-02887 from NIH.

37.3 MUSCARINIC-NICOTINIC CHANGES IN THE CHOLINERGIC RECEPTORS INVOLVED IN IRIS ACTIVATION DURING DEVELOPMENT.

R. Nuñez*, G. Pilar, and Ken Vaca*, Physiology Section, Bio. Sci. Grp., Univ. of CT, Storrs, CT 06268.

The mature avian iris is formed by striated muscle cells multiply innervated by ciliary ganglion neurons making nicotinic junctions. They respond to repetitive nerve stimulation (20-30 Hz) with a maintained contracture accompanied by action potential activity for the stimulation period. It was previously shown that profound alteration of the cells' ultrastructure occurs during development (Pilar, Landmesser, Burstein, 1980, J. Neurophys); they originate from the inner retinal layer, and migrate to iris location to form myoepithelial cells at St 34; and later, under nerve influence, myofibrils are organized. In the present study the mechanical properties of the iris muscle were investigated, as was its synaptic activation from Stage 36 (the time of formation of neuromuscular contacts) until reaching maturity after post hatching.

At St 36 repetitive nerve activation (30 Hz) causes a slowly developing contracture, elicited after 1.2 sec of the beginning of the electrical pulse, and maintained after the termination of the stimulation. This nerve mediated response was blocked by quinuclidinyl benzilate (QNB) (0.5 nM) and atropine (10^{-7} g/ml), but was unaffected by αBTX (1ug).

One day later (Stage 38) the mechanical response showed two distinct components, a fast initial deflection followed by a later slow response, which overlasts the stimulus, a twitch is elicited after single stimulation. The initial component is sensitive to αBTX, leaving unmodified the slow component which is sensitive to atropine. Postsynaptic electrical responses disappear after αBTX. Acetylcholine and carbachol elicited contractions in the superfused iris. αBTX and atropine each partially block this mechanical response, and in combination both cholinergic antagonists cancelled the ACh response altogether. It was therefore concluded that the iris cells are initially similar to the smooth muscle type and synaptically activated by muscarinic receptors; later the mechanical response becomes fast as in skeletal twitch muscle, and dependent on nicotinic receptors. The changeover occurs between St 38 and 1 day after hatching.

The changes described in the iris during embryonic development provide a clear example of normal transdifferentiation in the absence of experimental intervention. In the accompanying abstract (Vaca, Nuñez, Pilar this volume) evidence is presented showing that the switchover of the mechanical contracture as well as its receptor activation is dependent on the presence of innervation. Supported by NIH NS10338, Muscular Dystrophy Assn. of America & the Univ. of Conn. Research Foundation.

37.4 NEURAL CONTROL OF ACETYLCHOLINE RECEPTOR AND CONTRACTION IN AVIAN IRIS.

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At the earliest stages of neuromuscular transmission in the chick iris (St. 34-36), no specific binding of ^{125}I-α-bungarotoxin (αBTX) can be detected, just as the mechanical response of the iris to nerve stimulation is insensitive to αBTX (see abstract by Nuñez, Pilar and Vaca this volume). Two days later a small amount of specific ^{125}I-αBTX appears and increases progressively to maturity. The specific ^{125}I-αBTX binding is displaced by unlabeled αBTX, is saturated at a concentration of 10 nM and reaches equilibrium within 1 hr of incubation. At St. 36, when neuromuscular transmission is completely blocked by quinuclidinyl benzilate (QNB) or other muscarinic antagonists, irises specifically bind ^{3}H-QNB (close to 20 fmoles per iris). ^{3}H-QNB binding has an apparent dissociation constant of approximately 1nM, is saturated at 4nM, is displaced by unlabeled QNB or atropine and reaches equilibrium in less than an hour. However, while neuromuscular transmission becomes less sensitive to QNB, specific ^{3}H-QNB binding increases with the mass and protein content of the iris.

At two days post-hatching, iris muscle exhibits highly organized sarcomeres and pupillary constriction is blocked by αBTX but not QNB. If the iris is denervated at this time and acetylcholine (ACh) sensitivity of the mechanical response examined seven days later, it is found that the response to ACh is virtually eliminated by QNB but little affected by αBTX. However, the specific binding of ^{3}H-QNB is unchanged while specific binding of ^{125}I-αBTX increases slightly in the denervated iris. In response to the denervation, the ultrastructure of the iris shows a marked disintegration of the sarcomeres which are replaced by a loose filamentous cytoplasm not unlike that of smooth muscle. If the ciliary ganglion is denervated by section of the oculomotor nerve, mydriasis is maintained as in the case of denervation of the muscle, but changes in sensitivity of the iris to αBTX or QNB are not observed. It is concluded that the presence of the ciliary nerve can influence the cholinergic receptors and the muscle mechanical response by some means other than activity. Supported by NIH NS10338, Muscular Dystrophy Assn. of America, and The Univ. of Conn. Research Foundation.

37.5 RETROGRADE AXONAL TRANSPORT OF PROTEINS SYNTHESIZED BY CILIARY GANGLION TARGET TISSUES. I. A. Hendry* and C. E. Hill* (SPON: R. Porter). Department of Pharmacology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T. 2601, Australia.

The normal developmental pattern of the neurones of the ciliary ganglion requires the presence of target tissues derived from the optic cup. Removal of the target tissues during early development results in the death of the neurones (Landmesser, L. and Pilar, G., Fedn. Proc. 37:2016, 1978). Media conditioned by various non-neuronal tissues contain factors that result in the survival of dissociated ciliary neurones in culture. The chick iris, ciliary body and choroid contain large amounts of such factors (Adler, R., Landa, K., Manthorpe, M. and Varon, S., Science. 204:1434, 1979). It is likely that these factors are the target tissue derived trophic factors that ciliary neurones require for their in vivo survival.

We have grown combined cultures of iris, choroid and ciliary body in medium containing tritiated amino acids. The medium from these cultures contained labelled proteins synthesized by and released from the tissues. The medium was concentrated and separated from labelled amino acids by pressure dialysis against 154 M NaCl. The concentrated labelled proteins were introduced into one eye of chickens to come into contact with the intraocular terminals of the ciliary ganglion. The chicks were killed 16 hours later, both ciliary ganglia removed and the radioactivity in each ganglion estimated by scintillation counting. There was an accumulation of radioactivity on the injected side showing the retrograde axonal transport of labelled proteins by the neurones of the ciliary ganglion. Isoelectric focusing of the accumulated radioactivity in the ganglion showed five major protein species. It is likely that at least one of these proteins synthesized by the target tissues and accumulated by neurones of the ciliary ganglion after retrograde axonal transport represents the factor required for the target tissue dependent survival of neurones of the ciliary ganglion in vivo. This technique may provide a convenient method for the demonstration of these factors (retrophins) in other neuronal systems.

37.6 MASSIVE DESTRUCTION OF DORSAL ROOT GANGLION NEURONS PRODUCED BY IN UTERO EXPOSURE TO MATERNAL ANTIBODIES TO NERVE GROWTH FACTOR (ANTI-NGF). E. M. Johnson, P. D. Gorin, L. D. Brandies* and J. Pearson. Dept. of Pharmacol., Washington Univ. Med. School, St. Louis, MO 63110 and Dept. of Pathology, New York Univ. Med. Center, New York, New York 10016.

Nerve growth factor has been shown to enhance the survival of embryonic avian and mammalian dorsal root ganglion (DRG) neurons in vitro. The role of NGF in sensory neurons in vivo is unclear. Adult rats and guinea pigs immunized against mouse NGF produce antibodies which cross react with their own NGF and which are transferred to developing fetuses in utero. Adult rats which had been exposed to anti-NGF in utero or in milk (cross-foster protocol) showed a 90% reduction in numbers of sympathetic neurons in the superior cervical ganglion (SCG). Sensory neurons in the 8th cervical DRG were reduced 70% as a result of in utero exposure to maternal anti-NGF but were unaffected by postnatal exposure in milk. The effects of in utero exposure to maternal anti-NGF in guinea pigs is more extensive, probably due to the fact that guinea pigs achieve higher serum titers of anti-NGF and their offspring receive much more antibody prenatally than do rats. Newborn guinea pigs exposed to maternal anti-NGF had a greatly increased pain threshold, developed corneal opacities within two days of birth, and have failed to gain weight and thrive. Neuronal numbers in newborn guinea pigs exposed to anti-NGF in utero were reduced by over 99% in the SCG and 80% in the 8th cervical DRG. Neuronal numbers in nodose ganglia were unaffected. In both affected rats and guinea pigs size-frequency histograms of DRG neurons were very similar to those of control animals, indicating a loss of sensory neurons across the size spectrum. These results indicate that at least 80% of sensory neurons in DRG go through a phase of NGF dependence during embryonic development. This experimental approach may prove useful in studies of the development and physiology of the sensory and sympathetic nervous system. (Supported by the March of Dimes, the Dysautonomia Foundation, NIH grants HL20604 and HD12260. E. Johnson is an Established Investigator of the American Heart Association.)

37.7 NERVE GROWTH FACTOR STIMULATES DEVELOPMENT OF SUBSTANCE P IN THE EMBRYONIC SPINAL CORD. J.A. Kessler and I.B. Black. Dept. of Neurology, Cornell Univ. Medical College, New York, NY 10021.

Development of the putative neurotransmitter, substance P (SP), in the embryonic rat dorsal root ganglion (DRG) and spinal cord was defined in vivo. SP was not detectable by radioimmunoassay before day 17 of gestation (E17). On E17, cervical sensory ganglia contained 4 pg SP/ganglion, rising to 49 pg/ganglion at birth. The dorsal horn of the cervical spinal cord contained 0.75 ng SP/mg protein on E17, rising to 6 ng SP/mg protein on postnatal day 3. The ventral horn of the spinal cord contained approximately 20% of the SP content in the dorsal horn at each gestational age. Intrauterine forelimb amputation partially prevented the normal developmental increase of SP in sensory ganglia destined to innervate that limb, suggesting that target structures regulate the development of peptidergic neurons. Conversely, treatment with nerve growth factor (NGF) stimulated development of SP in the DRG. Moreover, NGF treatment increased SP in the dorsal horn of the spinal cord, suggesting that NGF can modulate development within the CNS, as well as peripheral structures. However, treatment with antiserum to NGF failed to significantly inhibit development of ganglion SP. The system of SP-containing neurons in the DRG may provide a convenient model for defining events regulating peptidergic maturation.

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37.8 CHRONIC EXPOSURE TO NERVE GROWTH FACTOR ANTIBODIES IN THE ADULT RAT: EVIDENCE FOR ATROPHY AND DEATH OF LONG ADRENERGIC NEURONS. Pamela D. Gorin and Eugene M. Johnson. Dept. of Pharmacology, Washington University School of Medicine, St. Louis, Mo. 63110

An autoimmune method was used to determine the effects of long-term nerve growth factor (NGF) deprivation on a number of cell types in the mature mammalian nervous system. Adult rats were immunized and boosted with 2.5S mouse NGF in complete Freund's adjuvant for a 5-6 mo. period. In the superior cervical ganglion, a paravertebral ganglion which contains the cell bodies of long adrenergic neurons, there was a marked neuronal atrophy and an apparent decrease in neuronal number, which was accompanied by a pronounced biochemical atrophy (80 and 60% reductions in tyrosine hydroxylase and dopamine-β-hydroxylase, respectively). Significant reductions of these adrenergic synthetic enzymes were also measured in the celiac ganglion, a prevertebral ganglion. Norepinephrine was reduced by approximately 90% in heart and brown fat and by 50% in the pineal gland. Thus, neuronal terminals, as well as cell bodies, were affected by chronic exposure to anti-NGF. No effects were observed on short adrenergic neurons innervating the vas deferens, adrenal medullary chromaffin cells, peripheral sensory neurons (which share a common embryological origin with long adrenergic neurons in the neural crest), or on central noradrenergic neurons.

In order to determine whether the biochemical changes observed in sympathetic ganglia and in their innervated targets represented neuronal atrophy or neuronal death, morphometric studies were carried out in the superior cervical ganglion. These studies showed a 35% reduction in cell number ($p < .001$) and a 50% decrease in cell volume.

The results reported here suggest that although NGF deprivation is less devastating for the adult mammal than for the immature mammal, mature long sympathetic neurons remain dependent of NGF throughout life for survival, as well as for maintenance of normal biochemical function. The autoimmune approach described here may be useful in elucidating the role of NGF (or other polypeptide growth factors) in the maintenance of other cell types and in providing an animal model for pathophysiological processes.

(Supported by Natl. Federation March of Dimes, NIH Grant HL 20604 and by NIH Training Grant HL 07275. E.M.J. is an Established Investigator of the American Heart Association.)

38.1 DISTRIBUTION OF SUBSTANCE-P (SP), METHIONINE-ENKEPHALIN (ENK) AND SOMATOSTATIN (SOM) IMMUNOREACTIVITIES IN THE SPINAL CORD OF THE DOMESTIC FOWL. A.L. LaValley* and R.H. Ho. (SPON: D. Clark) Dept. Anat., Coll. Med., The Ohio State University, Columbus, Ohio, 43210.

The indirect antibody peroxidase anti-peroxidase method of Sternberger was used to study the distribution of SP, ENK and SOM immunoreactive elements in the spinal cord of the domestic fowl, Gallus domesticus. Immunohistochemical localization was performed on 10μm sections of spinal cords that were fixed by intracardiac perfusion with Bouin's fluid. Immunoreactivities for all three peptides could be localized in the gray matter, except for laminae III as described by A.H. Martin (Acta Morphol. Neerl.-Scand., 17: 105-117, 1979). SP immunoreactive elements were densest in laminae I, II, IV, V and VI and around the central canal. In contrast, the ventral horn exhibited only sparse staining. The distribution of ENK immunoreactive elements was similar to that just described for SP. However, ENK immunoreactivity was most prominent around the central canal, within the intermediate gray matter and the ventral horn. Sparse SOM immunoreactive elements were present in laminae I, II, X and the ventral horn. The specificity of immunostaining was established in control experiments in which the primary antiserum for each peptide, pretreated with an excess of the corresponding synthetic antigen, failed to demonstrate the aforementioned structures on adjacent sections. We conclude that SP, ENK and SOM immunoreactivities are present in the domestic fowl spinal cord. (Supported by the Snyder Fund, The Graduate School and Department of Anatomy, The Ohio State University.)

38.2 PRELIMINARY STUDIES OF 5 HYDROXYTRYPTAMINE-LIKE IMMUNOREACTIVITY IN THE SPINAL CORD OF THE DOMESTIC FOWL. R.H. Ho, A.L. LaValley* and F.J. DiTirro. Dept. Anat., Coll. Med., The Ohio State University, Columbus, Ohio, 43210.

Spinal 5 hydroxytryptamine (5HT)[+] projections have been implicated in centrally induced analgesia and motoneuronal activity. To obtain baseline data for developmental studies, we examined the distribution of 5HT elements in the adult domestic fowl spinal cord. Animals were fixed by intracardiac perfusion with either Zambonie's or 4% paraformaldehyde solution. The indirect immunofluorescent technique of Coons and collaborators was utilized to localize 5HT on 10μm thick cryostat sections taken from representative spinal cord levels. With the exception of lamina III as described by A.H. Martin (Acta Morphol. Neerl.-Scand., 17: 105-117, 1979), a moderate density of 5HT containing elements are present throughout the gray matter. These very fine elements are interpreted to be axons and terminals. 5HT varicosities are densest around the central canal and in areas adjacent to the glycogen body. Immunoreactive varicosities also abut on large motoneurons. The specificity of immunostaining was established in control experiments in which anti-5HT serum, pretreated with an excess of 5HT, either prevented or greatly diminished such staining on adjacent sections. We conclude that 5HT is present in the spinal cord of the domestic fowl. (Funded by the College of Medicine Progress Endowment Fund and the Snyder Fund, The Ohio State University, College of Medicine; The Graduate School and the Department of Anatomy, The Ohio State University; Upjohn's Company and N.I.H. Grant NS-10165. We thank Dr. Robert P. Elde for the anti-5HT serum.)

[+]For convenience, the indolamine's immunoreactivity was referred to by its name.

38.3 SEROTONIN (5HT) IMMUNOREACTIVITY IN THE SPINAL CORD OF THE NORTH AMERICAN OPOSSUM, DIDELPHIS VIRGINIANA. F.J. DiTirro, R.H. Ho, and G.F. Martin. Dept. Anat., Coll. Med., The Ohio State University, Columbus, Ohio, 43210.

The opossum spinal cord, like that of the cat, receives projections from brainstem areas known to be indolaminergic, at least in part. Such connections have been implicated in endogenous opiate analgesia. Also, intrinsic enkephalin-containing elements within the dorsal horn are thought to function as an intermediate in this brainstem-spinal system. To date, we have shown that the spinal cord of the North American opossum contains enkephalin and substance P elements in various regions of the spinal gray including the dorsal horn. We have since examined the adult opossum spinal cord for the presence and distribution of 5HT immunoreactivity. Antibodies against serotonin were raised in rabbits; tissues fixed with 4% paraformaldehyde were processed by the indirect immunofluorescent method of Coons. Cryostat sections from all spinal levels were examined. Immunoreactive fibers were present in the dorsolateral funiculus. Immunoreactivity in presumed terminals and fibers was present throughout the gray matter with the exception of lamina III. Immunofluorescent staining was densest in the intermediolateral cell column at thoracic and rostral lumbar levels. Staining exhibiting moderate density was observed in lamina X, specific areas of the ventral horn, including laminae VIII and IX, and in the intermediate zone. The dorsal horn exhibited an even but sparse distribution of 5HT elements at all levels. The specificity of immuno-staining was established in control experiments wherein anti-5HT serum, pretreated with an excess of 5HT, failed to localize the aforementioned structures on semi-adjacent sections. We conclude that 5HT-containing elements are present throughout the rostral-caudal extent of the adult opossum spinal cord and that the coexistence of 5HT and ENK elements within the dorsal horn suggests their possible participation in endogenous opiate analgesia. (Funded by the College of Medicine Progress Endowment Fund and the Snyder Fund, The Ohio State University, College of Medicine; the Graduate School and the Department of Anatomy, The Ohio State University; Upjohn's Company and N.I.H. Grant NS-10165 and U.S.P.H.S. Grant NS-07410. We thank Dr. Robert P. Elde for the anti-5HT serum.)

38.4 ORIGINS AND TERMINATIONS OF SEROTONERGIC PROJECTIONS TO THE SPINAL CORD. R.M. Bowker*, K.N. Westlund, H.W.M. Steinbusch*, and J.D. Coulter. Marine Biomedical Inst., and Dept. of Psychiat. and Physiol., Univ. of Texas Med. Branch, Galveston, TX. 77550 & Dept. of Anat. & Embryol., Univ. of Nijmegen, Nijmegen, The Netherlands.

The distribution of serotonin (5HT) containing neuronal cell bodies and terminals was localized in the brainstem and spinal cord of monkeys by means of immunohistochemistry using an antiserum to serotonin (Steinbusch et al., 1978). Animals were perfused and tissue sections were processed for immunohistochemistry using the peroxidase-antiperoxidase method (Sternberger, 1974). Cell bodies demonstrating 5HT-immunoreactivity were widely distributed through the medial medulla, pons and caudal midbrain. In the medulla, 5HT-containing neurons comprized a relatively homogeneous population of small to medium-sized cells located in the nucleus raphe pallidus and raphe obscurus. In addition, many cells were found in the adjacent medial third of the nucleus gigantocellularis and extending in a band laterally over the inferior olive. In the pons, 5HT-containing cells were concentrated in the nucleus raphe magnus and adjacent medial reticular formation. Substantial numbers of cells were also in the medial half of the pontine-central grey and extended laterally and ventrally over much of the reticular formation to the lateral margin of the brainstem. In the caudal midbrain, large concentrations of 5HT-containing perikarya were located in the ventral half of the periaqueductal grey and the adjacent medial reticular nuclei ventrally (nucleus supratrochlearis and nucleus centralis superior of Olszewski and Baxter, 1954). In the rat, which has a similar distribution of 5HT neurons, cells projecting to the spinal cord were identified by retrograde transport of either HRP or wheat germ agglutinin using $CoCl_2$ in the reaction to yield a black punctate reaction product. Sections were then stained with 5HT antiserum to give a brown reaction product. Spinally projecting neurons containing 5HT, identified by the presence of both the black and brown labels in the same cell, were found in the raphe nuclei and reticular formation of the medulla and pons and in the ventral periaqueductal grey of the midbrain. In the monkey, spinal cord 5HT immunoreactivity was in fiber bundles in the white matter, including the dorsal lateral funiculus of all cord levels, with fibers and terminals having a wide distribution in the spinal grey matter. The most dense concentrations of 5HT-containing fibers and varicosities were localized in the ventral horn surrounding motor cells in the region corresponding to Rexed's (1952) lamina IX, in the dorsal horn (lamina I, II, IV), in the intermediolateral cell column of the thoracic and sacral cords, and in the zone surrounding and dorsolateral to the central canal. (Supported by NIH Grants NS12481 and NS11255).

38.5 ORIGINS AND TERMINATIONS OF DESCENDING NORADRENERGIC CELL GROUPS IN RAT. K.N. Westlund, R.M. Bowker*, M.G. Ziegler*, and J.D. Coulter. Marine Biomedical Institute, Depts. of Psychiat., Physiol., and Pharm., Univ. of Texas Med. Branch, Galveston, TX. 77550.

The origins of descending noradrenergic projections to the spinal cord were demonstrated using retrograde transport of antibody to dopamine-β-hydroxylase (DβH), the enzyme converting dopamine to norepinephrine. DβH antiserum produced in goat was prepared against DβH purified from bovine adrenal medulla and formed one band on immunoprecipitation (Jacobowitz et al., 1975). Four µl of a 50% solution of DβH antiserum was injected into various levels of the spinal cord in rats. After survival for 3-4 days, animals were perfused with 3% paraformaldehyde. Frozen sections were processed using rabbit-anti-goat IgG and goat peroxidase-antiperoxidase (PAP) as modified from the method of Sternberger (1974). In the brainstem, cells showing DβH immunoreactivity contained punctate granules and a diffuse light staining of the proximal dendrites and cell cytoplasm, exclusive of the nucleus. Labeled neurons were confined to known noradrenergic cell groups. DβH immunoreactive cells were localized mainly in the nucleus locus coeruleus, the nucleus subcoeruleus, the medial parabrachial nucleus and the Kölliker-Fuse nucleus. Cells in these nuclei were seen following injections of DβH antiserum at all spinal cord levels including thoracic and sacral-coccygeal segments. Localization of DβH immunoreactivity in terminals was mapped in the spinal cord using the Sternberger PAP method. While fibers possessing many large, darkly-staining varicosities course diffusely over much of the spinal grey matter at all spinal cord levels, labeled fibers and varicosities were concentrated in specific regions: the ventral horn, around large motoneurons in the region corresponding to Rexed's (1952) lamina IX; in the marginal layer of the dorsal horn (lamina I) and extending into laminae II and III of the substantia gelatinosa; and around the central canal in lamina X. Very heavy fiber and terminal labeling was also seen in the intermediolateral spinal grey matter, around preganglionic sympathetic and parasympathetic motoneurons, extending medially in a band toward the central canal. Although most of the terminal label observed with immunocytochemical localization agrees with histofluorescence data presented previously, DβH localization associated with the sacral autonomic preganglionic motoneurons has not been reported. Together, the results suggest a major role of pontine noradrenergic cell groups in the mediating descending influences on autonomic, somatic sensory and motor cell groups of the spinal cord.
(Supported by NIH Grants NS12481 and NS11255).

38.6 BRAIN-STEM RETICULAR FORMATION CONNECTIVITY IN THE WHITE RAT. A STUDY UTILIZING RETROGRADE FLUORESCENT TRACERS AND MONOAMINE TECHNIQUES. W.R. Buck and A.O. Humbertson, Jr. Dept. Anat., Coll. Med., The Ohio State University, Columbus, Ohio, 43210.

Recently the investigation of systems possessing axonal collateralization have received much attention in the neuroanatomical community. Through the use of retrograde fluorescent tracers combined in double labelling experiments neurons that project to more than one area can be positively identified. By combining monoamine techniques with fluorescent tracers one can also speak to the aminergic nature of these neurons.

Classically, the reticular formation has been described as a system whose organization within the brain-stem is diffuse in nature. Our experiments suggest a somatotopical organization at least to the medullary reticular formation. Our results indicate that cell which project to lumbar and cervical areas of spinal cord area clustered along median areas (raphe obscurus) and paramedian areas (nucleus reticularis paramedians, nucleus interfascicularis nervus hypoglossi, nucleus reticularis gigantocellularis) of the ventral reticular formation. Also, at pontine levels there appears to be a somatotopical organization to the lateral vestibular nucleus and to locus coeruleus. Preselected cross-sectional plots through the medulla and pons will be presented and discussed in detail. (Supported by U.S.P.H.S. Grant NS-07410.)

38.7 ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL CHARACTERIZATION OF NORADRENERGIC AND PEPTIDEGIC NEURONS AND SYNAPSES WITHIN THE AREA POSTREMA OF RAT. D.M. Armstrong, V.M. Pickel, R.J. Miller, T.H. Joh, D.J. Reis, Laboratory of Neurobiology, Dept. of Neurology, Cornell Univ. Med. College, New York, NY 10021. (RJM) Dept. of Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago, IL.

By light microscopic immunocytochemistry, using antibodies to the catecholamine synthesizing enzymes and two neuropeptides, we have shown that the area postrema (AP) of rat contains processes of neurons containing catecholamine, predominately noradrenalin, and the neuropeptides (Leu^5)-enkephalin ((Leu^5)-enk) and substance P (SP). The AP also contains perikarya of noradrenalin and enkephalin neurons (Armstrong et al., JCN in press). We sought to characterize the ultrastructure by electron microscopic immunocytochemistry of catecholaminergic and peptidergic neurons in the AP.

Specific antisera to tyrosine hydroxylase (TH), (Leu^5)-enk, and SP were raised in rabbits. Antisera were localized by the peroxidase-antiperoxidase method in vibratome sections of brains fixed by vascular perfusion with 4% paraformaldehyde and 0.2% glutaraldehyde.

Electron micrographs were taken throughout the rostro-caudal and dorsoventral extent of the AP. Although light microscopy showed peptidergic and catecholaminergic processes and perikarya close to the ventricle and/or blood vessels, electron microscopy showed no such association. In the AP, TH was contained only within neurons including perikarya, dendrites, axons, and axon terminals. TH-labeled dendrites and dendritic spines formed asymmetric contacts with unlabeled axon terminals containing both large dense vesicles (LDVs) and small clear vesicles (SCVs). Occasionally two TH labeled dendrites were in apposition, suggesting dendrodendritic contacts. Enkephalin-like immunoreactivity (ELI) was usually localized in dendrites, axons, and axon terminals and rarely in perikarya. The labeled dendrites formed asymmetric axodendritic contacts with unlabeled terminals containing predominately SCVs. Axons and axon terminals with ELI contained many SCVs and few LDVs and were more heavily labeled than dendrites. SP immunoreactivity was localized only to axons and axon terminals. The SP labeled terminals contained numerous SCVs and a few LDVs and formed synaptic contacts with dendrites which received unlabeled axon terminals containing numerous LDVs and SCVs. We conclude that in the AP dendrites and axon terminals of neurons containing noradrenalin and enkephalin and axons of neurons containing SP form asymmetric synapses. These are predominately axodendritic and are upon other neurons in the region.

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38.8 IMMUNOHISTOCHEMICAL LOCALIZATION OF GLUTAMATE DECARBOXYLASE IN CELL BODIES OF THE SUBSTANTIA NIGRA AND OTHER AREAS OF RAT BRAIN. W.H. Oertel, D.E. Schmechel*, D.H. Ransom*, M.L. Tappaz*, E. Mugnaini and I.J. Kopin (SPON: D.C. Jimerson). Laboratory of Clinical Science, NIMH, Bethesda, MD 20205.

Antiserum prepared in sheep against rat brain glutamate decarboxylase (GAD; E.C.4.1.1.15), the biosynthetic enzyme for γ-amino butyric acid (GABA), has been employed to localize GAD-containing neurons in the rat CNS using the indirect (PAP) immunohistochemical method of Sternberger.

Rats were perfused with 0.1M sodium phosphate buffer (pH 7.3) containing 4% paraformaldehyde and 0.1% glutaraldehyde and the brains postfixed in phosphate buffered 4% paraformaldehyde for 1 h at 25°C and 4 h at 4°C. 20-25 µm Vibratome sections were treated with 0.25% TX 100 in Tris buffered saline (pH 7.6) for 5 minutes and then processed for the indirect PAP method (GAD-antiserum 1:1500 or 1:4000; rabbit antisheep IgG 1:50; goat PAP complex 1:60). With primary antiserum (1:4000) nerve endings were stained in the cerebellum, olfactory bulb, hippocampus and the substantia nigra in agreement with previous reports using an anti-mouse GAD antiserum. Electron microscopic studies in the cerebellar cortex revealed reaction product in nerve endings of stellate, basket and Golgi cells and in Purkinje cell axon recurrent collaterals. Mossy and climbing fibers, granular neurons and glia cells were not stained.

With GAD-antiserum diluted 1:1500, GAD-immunoreactive somata of Purkinje, basket, stellate and Golgi cells in the cerebellar cortex, periglomerular and granule cells in the olfactory bulb and basket cells in the hippocampus were faintly stained in light microscopy. In addition lightly stained medium-sized somata were distributed throughout the substantia nigra. After injection of colchicine (5 µg in 0.5 µL) into the rostro-medial substantia nigra pars compacta, the number and intensity of GAD-immunoreactive cell somata in the substantia nigra was substantially increased.

This provides morphological evidence for the existence of GABAergic neurons in the substantia nigra previously postulated on the basis of electrophysiological, behavioral and biochemical studies.

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38.9 SUBSTANCE P IN HUMAN DORSAL HORN AND PALLIDONIGRAL AXIS --A COMBINED SILVER IMPREGNATION AND IMMUNOPEROXIDASE STUDY. R. Defendini,* G. Nilaver,* E.A. Zimmerman, V.M. Tennyson. Div. of Neuropathology and Depts. of Pathology, Neurology, and Anatomy, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

The combination of silver impregnation and immunoperoxidase staining for substance P (SP) on the same section demonstrates new details about the anatomic relation of SP fibers to target structures.

Paraffin embedded lumbosacral and pallidonigral blocks obtained 4-16 hr post mortem were sectioned in sagittal oblique, horizontal oblique, and transverse planes. Bielschowsky's silver method defined large and medium axons consistently and the soma and dendrites of neurons variably. Brown immunoreactive products to rabbit anti-SP serum (1:500/30 min; PAP method) stood out against the black silver stain. Preincubation of the immune serum with lug of SP prevented staining.

In the human dorsal spinal cord, SP is mostly in Rexed's laminae I,II, and III. Most prominent in the marginal zone (I), SP products form cords with punctate accentuations, representing terminals on thick, long, occasionally branched dendrites of Waldeyer's marginal neurons (MC). The SP-innervated dendrites run rostrocaudally in I but also transversely into ths substantia gelatinosa (SG) proper (II & III) and Lissauer's tract (LTx). The irregular outline of the cords suggests SP contacts on long dendritic spines. Some MC bodies too are superficially studded by SP terminals. In transverse section, the dense SP band on the lateral (sometimes also medial) margin of the horn, alongside nucleus proprius (IV,V,VI) levels, corresponds to embracing extensions of I. Except for penetrating MC dendrites, the SP pattern in SG is much finer and includes many fine beaded axons oriented rostrocaudally.

The SP patterns in nigra and pallidum are identical. Punctate SP products in great profusion outline a forest of thick, long, poorly branched dendrites. Their appearance as hollow tubes of uniform caliber suggests SP contacts on spineless membranes. In the nigra, they are concentrated rostrally and in the pars reticulata. They also run tangentially in the internal capsule. In the pallidum, they are restricted to the internal segment, stopping abruptly at the internal medullary lamina. These observations raise the possibility that SP-innervated pallidal dendrites are continuous with those of the nigra across the capsule.

39.1 INFLUENCE OF BRAIN MONOAMINES ON BARBITURATE SENSITIVITY
W. M. Bourn and C. E. Reigel, Jr.,* Div. of Pharmacology and Nuclear Pharmacy, School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209.

Evidence exists which suggests that central monoamines play a role in barbiturate tolerance and/or dependence. Although these findings would allow reasonable speculation that monoamines are directly involved with the acute effects of barbiturates, the relationship may be strictly of a secondary nature. For example, the previous finding that depletion of brain catecholamines results in an increase in the intensity of barbiturate withdrawal convulsions could be explained by the fact that norepinephrine appears to be a "modulator" of many types of experimentally induced convulsive seizures.

In the present study agents which enhanced or reduced the activity of monoaminergic systems in the brain were administered to rats which were subsequently tested for sensitivity to sodium methohexital (MH) using EEG burst suppression as an indicator of barbiturate effect on the CNS.

RO4-1284, a drug which produces a drastic reduction of brain norepinephrine, dopamine and serotonin, decreases MH sensitivity. 6-Hydroxydopamine, which selectively depletes catecholamines, produced a slight increase in MH sensitivity. Neither parachlorophenylalanine, an agent which selectively depletes brain 5-HT, nor α-methyltyrosine, an agent which selectively depletes catecholamines altered sensitivity to MH. Likewise, the uptake blockers desipramine and fluoxetine, agents which selectively block uptake of norepinephrine and serotonin, respectively, did not alter MH sensitivity.

Since the alterations in MH sensitivity produced by RO4-1284 and 6-hydroxydopamine are in conflict and may possibly be explained on a basis of peripheral drug distribution, these results support the hypothesis that the acute effects of barbiturates are not a result of a direct effect on catecholaminergic nor serotonergic system.

39.2 PSYCHOPHYSICAL ASSESSMENT OF PHENCYCLIDINE AND KETAMINE ON SENSORY AND MOTOR PROCESSES IN THE BABOON. Scott E. Lukas, Robert D. Hienz* and Joseph V. Brady*. Dept. of Psychiatry and Behavioral Sciences, The Johns Hopkins University, Baltimore, MD 21205.

Only limited research has been conducted in the area of behavioral pharmacology to experimentally determine the precise effects of pharmacological agents on basic sensory function and motor performance in animals. Drugs that are self-administered by humans (e.g. phencyclidine (PCP) and ketamine) are of particular interest because of the potential relationship between drug-induced changes in sensory and motor processes and relative abuse potential. Baboons (Papio anubis) were placed on a restricted feeding schedule and trained to perform under a standard psychophysical procedure to determine auditory and visual thresholds and reaction times (RT). Subjects were housed in double-walled sound attenuating chambers and were trained to hold a lever depressed for a variable interval until a 1.5 sec white light flash on a 1" radius stimulus patch or a 1.5 sec tone burst occurred, signaling the availability of food reward (190 mg banana pellet) following a lever release. Auditory and visual thresholds were determined by systematically varying the intensities by the method of constant stimuli and interpolating from percent correct detections at each intensity to that intensity which produced a detection score halfway between the false alarm rate and 100%. From each test session four independent within-session estimates of sensory thresholds were obtained. Drugs were administered intramuscularly one-half hour before testing with visual tests beginning after 30 min of dark adaptation. PCP produced a slight decrease in visual sensitivity with a corresponding 7% increase in RT at the 0.032 mg/kg dose. There was, however, no effect on auditory sensitivity in the dose range of 0.0032-0.1 mg/kg though there was a 29% increase in RT at 0.1 mg/kg. At doses of 0.32 mg/kg and above, PCP completely disrupted performance. In contrast, ketamine impaired auditory sensitivity by 40-60% at 0.32, 1.0, and 3.2 mg/kg. Motor performance was impaired only at the highest dose of 3.2 mg/kg (28% increase in RT). PCP was ten times more potent than ketamine in disrupting sensory and motor performance. Analysis of the individual within session trials revealed that ketamine produced maximal disruption of performance within one hour after injection and complete recovery was evident by 1½-2 hr. In contrast, PCP impaired performance throughout the entire session (approx. 2 hr) with no indication of recovery during the session. Thus, the present study has shown that while PCP and ketamine are qualitatively similar in their effects on motor performance, they may not be similar in regards to their effects on auditory and visual acuity. This difference may be attributable to PCP's greater potency and longer duration of action. (Supported by DEA contract #78-9).

39.3 ACUTE BEHAVIORAL EFFECTS OF PHENCYCLIDINE IN THE DOG. James L. Boren* and Paul Consroe. Dept. of Pharmacol. and Toxicol., Univ. of Arizona, Tucson, AZ 85721.

The behavioral effects of phencyclidine (PCP) in humans are complex and varied, ranging from intense agitation and excitement in some cases to a deep coma accompanied by respiratory depression in others. In addition to these extremes of excitement and depression, other commonly reported behavioral effects of PCP in humans include a "blank-stare" appearance, ataxic gait, stereotyped head and limb movements, muscular rigidity, horizontal and vertical nystagmus, excessive salivation, repetitive jaw snapping, opisthotonic posturing, and clonic and tonic seizures. A similar diversity of behavioral effects has not been reported in other species. In general, PCP elicits predominately excitatory effects in rodents and mainly depressant effects in most other laboratory animal species.

In the present investigation, the behavioral effects of intravenous doses of .25, .5, 1, 2.5, and 5 mg/kg of PCP were examined in 8 male mongrel dogs tested in an open field arena. The order of injection of doses was randomized for each dog with a minimum of 2 days between injections. Injection of PCP at each dose produced initial sedative effects with a dose-related latency to onset (ranging from means of 390 to 40.5 sec in the .25 and 5 mg/kg groups, respectively) and duration (ranging from means of 32.6 to 312.7 min in the .25 and 5 mg/kg groups, respectively). During this phase, animals rapidly lost the ability to stand, fell to their side, and exhibited an apparent lack of visual, auditory and tactile responsiveness. Other effects soon merged with these including tremors, rigidity, stereotyped limb movements and head weaving, tail stiffening, and copious salivation. In addition to the above effects, larger doses (1, 2.5 and 5 mg/kg) of PCP produced opisthotonic posturing, clonic and tonic convulsions, and repetitive jaw snapping. Termination of the first phase of PCP action was indicated by successful execution of a righting response, after which animals entered a phase of behavioral hyperactivity which was monitored for 2 hours after righting. Although an ataxic gait was initially apparent, animals evidenced dramatic increases in locomotor activity at all doses. In addition, dose-related increases in the duration of stereotyped circling, in latency to rear, and in latency to vocalize were observed. No deaths occurred at any dose and animals appeared completely normal 24 hrs later.

These results demonstrate that the behavioral effects of PCP in the dog bear a striking similarity to those observed in humans and suggest that this species may be an excellent laboratory animal model for studying the pharmacology and toxicology of PCP. (Supported by NIDA Grant DA 02137).

39.4 DISTRIBUTION OF AMPHETAMINE AND ITS EFFECTS UPON GLUCOSE UTILIZATION IN DISCRETE AREAS OF RAT BRAIN DURING CONTINUOUS AMPHETAMINE ADMINISTRATION. Michael S. Eison, Arlene S. Eison*, and Gaylord Ellison, Dept. Psychology, UCLA, Los Angeles, CA 90024.

A regimen of continuously administered amphetamine (AMPH) induces in rats a behavioral syndrome characterized by initially uninterrupted stereotypy (2 days) followed by aberrant social behavior (5 days). The late stage of this continuous AMPH syndrome can serve as a useful animal model of amphetamine psychosis. Biochemical alterations in the brain as animals undergo the behavioral transitions observed during continuous AMPH may model neurological changes associated with this psychosis. The regional distribution of 3H-d-AMPH and accumulation of 3H-deoxy-d-glucose (2DG) were therefore studied in 22 regions of rat brain during continuous AMPH administration using liquid scintillation and microdissection techniques.

AMPH counts were highest in the cortex and sensory regions of naive rats acutely administered label, and were lowest in the brainstem. AMPH derived counts in the accumbens, olfactory bulbs, hippocampus, and frontal cortex increased progressively relative to other brain regions as chronicity of exposure to AMPH increased from acute to 2 or 5 days of continuous administration, while retention by the other cortical samples, thalamus, and caudate decreased. The accumbens retained more AMPH than any other region following 5 days of continuous AMPH administration.

Altered regional differences in glucose utilization 45 mins after 2DG administration paralleled changes in AMPH distribution for many regions studied. 2DG counts observed in mesolimbic regions (accumbens, olfactory tracts, amygdala, hippocampus, frontal cortex) increased progressively with increasing duration of exposure to AMPH; after 5 days of continuous AMPH, 2DG counts in the accumbens increased more than any other region following any other regimen of amphetamine administration while decreasing in many non-limbic brain structures, such as the caudate. These changes did not persist 90 days after cessation of continuous amphetamine administration.

39.5 INHIBITION OF INTRAVENOUS COCAINE SELF-ADMINISTRATION BY RATS AFTER MICROINJECTION OF SPIROPERIDOL INTO THE NUCLEUS ACCUMBENS. A.G. Phillips and C.L. Broekkamp. Department of Psychology, University of British Columbia, Vancouver, B.C. Canada, V6T 1W5.

Numerous experiments have confirmed that rats will self-administer psychomotor stimulants (amphetamine, cocaine) via chronically indwelling intravenous (iv.) catheters. The reinforcing property of amphetamine is attenuated in a dose related manner by pretreatment with neuroleptic drugs (Yokel & Wise, 1976). Neuroleptics are known to serve as dopamine (DA) receptor antagonists and therefore it has been hypothesized that the psychomotor stimulants maintain self- administration behavior by a direct action on the ascending DA pathways in the brain. Further confirmation of this hypothesis comes from the disruption of cocaine self-administration after 6-hydroxydopamine lesions of the mesocorticolimbic DA pathway at the level of the nucleus accumbens (Roberts et al, 1977). In the present experiment, rats were trained to barpress for an iv. injection of cocaine (0.75 mg/kg/injection) during a daily 2 hr. test session. Following the establishment of stable iv. self-administration rates (25-40 presses/2 hrs.), one group of rats (N=5) received bilateral cannulae implants into the nucleus accumbens under stereotaxic control. A second group (N=6) was prepared with bilateral implants into the head of the caudate nucleus. Control tests after stereotaxic surgery confirmed that iv. self-administration rates remained at pre-operative levels. The following doses of spiroperidol (0.25, 0.4, 0.5, 0.6 µg/0.5 µl) were microinjected bilaterally into either the nucleus accumbens or caudate 30 min. prior to a self-administration test. Cocaine self-administration was blocked by 0.5 µg and 0.6 µg spiroperidol into the nucleus accumbens. This effect was locus specific, as no dose of spiroperidol attenuated the behavior when injected into the caudate nucleus. In a control experiment, microinjections of spiroperidol (0.25, 0.5 µg) into the nucleus accumbens had no significant effect on barpressing for food. These data provide further support for the involvement of the mesocorticolimbic pathway in the rewarding effects of psychomotor stimulant drugs.

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39.6 EFFECTS OF HYPOTHALAMIC KAINIC ACID LESIONS ON SELF-ADMINISTRATION OF HEROIN AND COCAINE. Michael D. Britt* and Roy A. Wise. Center for Research on Drug Dependence, Dept. Psychol., Concordia Univ., Montreal, P.Q., Canada H3G 1M8.

The posterior lateral hypothalamic area (LHA) has been suggested (M.E.Olds, Brain Res., 168:351, 1979) as a possible site of action for the reinforcing effects of opiates on the basis of the finding that rats would lever-press for direct opiate microinjections into this region. The present study reports the effects of kainic acid (KA) induced LHA cell loss on the intravenous self-administration of heroin. If LHA opiate receptors mediate the rewarding properties of heroin, then selective damage to the bed nucleus of the LHA should eliminate the reinforcing effects of intravenous injections. The effects of LHA cell damage on cocaine self-administration was also assessed as a control for non-specific debilitating effects of KA; cocaine self-administration is thought to depend on dopamine fibers of passage, but not the bed nucleus of the LHA.

Rats implanted with jugular catheters were allowed to lever press for cocaine (1.0 mg/kg/infusion) in daily 4h sessions. After stabilization of this behavior they were given access to heroin (50 µg/kg/inf.). Once stable responding for heroin was observed rats were tested in an alternating daily sequence of heroin or cocaine for six days (3 days each). Kainic acid was then injected stereotaxically (flat skull coordinates: AP -3.5, V 8.4, L 1.5) with 0.2 µg of kainic acid in 0.5 µl of saline. Following a one week recovery period the rats were again allowed access to heroin or cocaine in an alternating daily sequence for six days.

Comparison of mean pre- vs post-lesion drug intake for cocaine (7.5 -1.7 vs 7.1 -1.6:means and SDs in mg/kg/hour) and heroin (4.4 -1.9 vs 4.8 -1.5 µg/kg/h) revealed no significant difference for either drug. Histological analysis of pilot animals revealed major loss of cells in the bed nucleus of the LHA with the injection regimen used; histological analyses of animals tested behaviorally are in progress.

The failure of LHA kainic acid injections to disrupt intravenous cocaine or heroin self-administration suggests that the bed nucleus of the lateral hypothalamic area plays no critical role in either the rewarding effects of opiates or the response capacity for opiate self-administration. Confirmation of this suggestion awaits detailed histological analysis of the brains of the animals tested, and the demonstration that damage to cells in other regions can effectively disrupt this behavior.

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39.7 OPIATE EFFECTS ON SOCIAL COMFORT AND IMPRINTING. P. Bishop*, J. Panksepp, T.L. Sahley*. Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403.

These experiments were designed to evaluate the proposal that brain opioids participate in the mediation of social affect and social attachments (Biol. Psychiat. 1978, 13, 607). When young animals are separated from their normal social environment they distress vocalize (DV), and this response can be inhibited by stimuli to which the animal has been imprinted. For instance, young chicks which have been communally housed DV less in the presence of their own image (i.e., in a mirrored environment) than in one which has no reflective surfaces. Pharmacological analysis of this inhibition of DVs in young chicks indicated that blockade of opioid (1 mg/kg naloxone, i.p.), serotonin (0.5 mg/kg methysergide), and cholinergic (5 mg/kg atropine) systems could attenuate the inhibition of DVs produced by mirrors. Morphine (1 mg/kg) could amplify this comfort response, while quipazine and pilocarpine (at 10 mg/kg) did not. Formal imprinting experiments were conducted in which dark-reared chicks were exposed to mirrored environments for 10 mins at 1 day of age and retested at 4-5 days of age. Following naloxone (1 mg/kg i.p.) chicks DVed as much in mirrored chambers as in plain ones, while under control conditions mirrors reduced DVs by 42%. When chicks were imprinted to mirrors under various drug conditions at 1 day of age, retrieval testing indicated that saline trained animals DVed 57% as frequently at 3 days of age in a mirrored environment as in a plain one, morphine (1 mg/kg) trained animals cried 72% as much as controls and naloxone trained birds (2.5 mg/kg) cried at 97% of control levels. These results suggest that opioid systems participate in mediating the comfort which young birds derive from social stimuli, and suggest that the process of imprinting may be partially due to endogenous opioid release.

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39.8 SIMILARITIES AND DIFFERENCES IN DEPENDENCE AND ABSTINENCE PROFILES DURING SELF-ADMINISTRATION OF CYCLAZOCINE, PENTAZOCINE, NALBUPHINE, AND BUTORPHANOL IN MORPHINE POST-ADDICT RATS. G.F. Steinfels, G.A. Young, and N. Khazan. Univ. of Maryland School of Pharmacy, Dept. of Pharmacology and Toxicology, Baltimore, Maryland 21201.

Adult, female Sprague-Dawley rats were prepared with chronic EEG and EMG electrodes and intravenous cannulae. Rats were first made tolerant to and physically dependent on morphine by a series of automatically administered i.v. injections. The rats were then trained to lever press on a fixed ratio 20 schedule of reinforcement to receive morphine injections. After one week of stabilized responding rats were removed from the experimental cages and allowed to withdraw from morphine for two weeks. Each rat was then returned to the experimental cage and given the opportunity to establish self-administration of morphine (10 mg/kg/inj), cyclazocine (0.5 mg/kg/inj), pentazocine (1 mg/kg/inj), nalbuphine (5 mg/kg/inj), or butorphanol (0.5 mg/kg/inj). When morphine post-addict rats were given the opportunity to relapse to the self-administration of these narcotics, the mean daily number of self-injections gradually increased over days and was stabilized within a week. With all the narcotics REM sleep was suppressed during the first two days of the relapse period and then increased in amount and stabilized by days 5-7. A comparison of the total REM sleep times on day 7 of dependence with control values demonstrated that rats that self-administered cyclazocine, pentazocine, nalbuphine, or butorphanol had daily REM sleep times that were not significantly different from control values; however, the values during the dependence period were in the lower range for control. Withdrawal was initiated on day 8 by substitution of saline for each narcotic and was accompanied by increased numbers of self-injections in all animals. During withdrawal REM sleep was suppressed in rats previously dependent on morphine and pentazocine. In contrast, REM sleep times increased in cyclazocine, nalbuphine, and butorphanol rats. Abstinence from morphine and pentazocine was associated with behavioral symptoms such as diarrhea, wet-dog shakes, irritability, while abstinence from cyclazocine, nalbuphine, and butorphanol was not. These findings suggest that there was considerable physical dependence in rats dependent on morphine and pentazocine but relatively less physical dependence on cyclazocine, nalbuphine, and butorphanol. The above data also seem to differentiate between drugs which induce both psychological and physical dependence such as morphine and pentazocine, and drugs which produce a similar psychological and apparently less physical dependence such as cyclazocine, nalbuphine, and butorphanol.

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39.9 NEUROPHARMACOLOGIC EFFECTS OF N-ALLYLNORMETAZOCINE (SKF-10047).
Edgar T. Iwamoto. Dept. of Pharmacology, Univ. of Kentucky, Lexington, KY 40536.

N-allylnormetazocine (SKF-10047; Win-19,631) possesses potent psychotomimetic activity in man and also is an effective antagonist of meperidine analgesia in the rat having little or no detectable antinociceptive properties itself. In the chronic spinal dog, SKF-10047 caused mydriasis, tachypnea, tachycardia and mania, effects which were quite unlike those induced by morphine or ketocyclazocine (Martin and co-workers, 1976).

The bizarre behavior--consisting of mild ataxia, lateral head movements, pivoting of hindpaws, walking backwards and peculiar circular movements--induced by cyclazocine in the rat is purportedly caused by its σ (for SKF-10047) activity, activity which is also shared by other opioids such as nalorphine, levallorphan and pentazocine in addition to SKF-10047. The present study was designed to characterize further some of the pharmacologic effects of N-allylnormetazocine.

Locomotor activity in Sprague-Dawley male rats was stimulated for periods up to 90 minutes after 2.5, 5 and 10 mg/kg of SKF-10047. The locomotion induced by 10 mg/kg of SKF-10047 was diminished by: 20 mg/kg of naltrexone by 69 percent; 0.15 mg/kg of spiperone by over 94 percent; 0.03 mg/kg of spiperone by 50 percent; and a low dose of 0.1 mg/kg of apomorphine, which presumably causes presynaptic inhibition of dopamine-containing neurons, by 54 percent. SKF-10047 did not produce analgesia as assessed by the 49.5°C hotplate; significantly, however, 1.5 mg/kg s.c. of SKF-10047 did antagonize the analgesic effects of both morphine and ketocyclazocine.

Using the unilateral 6-hydroxydopamine-induced mesostriatal lesion model of circling behavior, SKF-10047 administration caused high intensities of ipsilateral circling behavior; circling towards the lesioned side is also caused by the indirect-acting dopamine-mimetic, d-amphetamine. SKF-10047-induced circling was antagonized by 0.15 and 0.05 mg/kg of spiperone; the same circling behavior, on the other hand, was not altered by 0.01 mg/kg of spiperone.

The data suggest that the pharmacologic effects of SKF-10047 include indirect activation of the mesostriatal and/or "mesolimbic" ascending dopamine pathways which result in enhanced central dopamine receptor activity expressed as locomotor activity and circling behavior; this action can be blocked by presynaptic inhibition of the ascending dopaminergic pathways after 0.1 mg/kg of apomorphine or by postsynaptic dopamine receptor blockade after spiperone.

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40.1 DIETARY TRYPTOPHAN REVERSAL OF PATHOLOGICALLY ELICITED SHOCK-INDUCED FIGHTING. Kathleen M. Kantak, Linda R. Hegstrand and Burr Eichelman. Univ. of Wisconsin and William S. Middleton Veterans Administration Hospital, Madison, WI 53706.

Using two pathological procedures known to facilitate shock-induced fighting and muricide,ie septal lesions and intraventricular 5,7-dihydroxytryptamine (5,7-DHT) infusions, male rats were tested for the reversibility of these lesion effects by the addition of 5% tryptophan to the diet. Both septal lesions and 5,7-DHT infusions produced significant increases in shock-induced fighting within a few days following surgery. In animals fed the 5% tryptophan loaded diet, which was initiated 4 days prior to surgery, the level of shock-induced fighting did not differ in the septally lesioned or 5,7-DHT infused groups compared to sham operated or vehicle infused groups and compared to their own baseline levels of fighting. In confirmation of our earlier findings, a 5% tryptophan loaded diet failed to modulate shock-induced fighting in non-lesioned control animals. When tested for muricide, both septal lesions and 5,7-DHT infusions facilitated the incidence of predatory killing which could not be reversed by the addition of 5% tryptophan to the diet. Body weight and food intake substantially decreased following the septal lesions and 5,7-DHT infusions regardless of chow or tryptophan feeding regimens. These data demonstrate that under normal conditions a 5% tryptophan loaded diet produces no observable changes in shock-induced fighting or muricide but that under pathological conditions a 5% tryptophan loaded diet reverses the aggression enhancing effects of septal lesions and 5,7-DHT infusions on shock-induced fighting but not muricide. This reversal of shock-induced fighting following 5% dietary tryptophan loading is postulated to occur via a serotonergic mechanism.

40.2 HOW GO-NO GO DISCRIMINATION LEARNING AFFECTS BRAIN MONOAMINES AND ENZYME ACTIVITY IN CATS. Anne Kitsikis and Andrée G. Roberge, Laval University, School of Medicine, Que, G1K 7P4, CANADA.

The aim of this investigation was to determine whether learning produces changes in brain monoamine content and where these changes take place. Twelve cats were trained five days a week on a go-no go discrimination task. On go trials animals learnt to displace, within 5 sec, a black (S^+) cover in order to retrieve a piece of meat. On no go trials, they had to refrain from displacing a white (S^-) cover for 7 sec. Correct no go responses were not rewarded. A group of 6 trained and 3 untrained cats (group 1) was sacrificed on day 5 for biochemical assays. Another equivalent group was sacrificed after reaching criterion performance of 90% correct response on 5 consecutive days (group 2).

Cats in group 2 took between 500 and 950 (mean 766) trials to reach criterion performance. In both groups, practically all errors were made on no go trials and significantly ($P<0.001$) more go and no go errors were made by cats in group 2 during the first 5 days of training compared to the period between day 6 and criterion performance. Response latencies were significantly ($P<0.01$) longer on no go (error) trials than on go trials, suggesting that cats did not always respond " blindly ".

The biochemical effects observed in group 1 revealed a significant increase in tryptophan hydroxylase activity in the raphe nuclei of pons and medulla whereas in group 2, the enzyme activity was significantly increased in all raphe nuclei of the brainstem. Moreover, the increase observed in group 2 was significantly higher ($P<0.01$) than in group 1. Corresponding to this enzymatic activity, the concentrations of serotonin (5-HT) and 5-hydroxyindoleacetic acid(5-HIAA) were measured in 12 different brain structures. The 5-HT content in group 1 was found decreased in the mesencephalon, thalamus and frontal cortex whereas the 5-HIAA content remained constant. In group 2, however, the 5-HT content did not change significantly but the 5-HIAA content was significantly decreased in all structures of the brainstem, thus suggesting a low turnover rate. The concentration of noradrenaline was also measured in 12 structures and was found to be significantly decreased only in the pons and hypothalamus. The present findings reveal that go-no go discrimination induced an important reactivity of the serotoninergic metabolism in the brainstem and a well localized involvement of the NA metabolism. Moreover, biochemical changes occurring at criterion performance (group 2) differed markedly from those observed after a 5-day training period (group 1). The step by step acquisition of the go-no go task seems therefore to be mirrored by the deployment of biochemical mechanisms.

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40.3 Differential effects of three learning tasks on brain amine synthesis in cats. Andrée G. Roberge, L. Vachon, C. Roberge and Anne Kitsikis, Laval Univ. Sch. of Medicine, Quebec, G1K 7P4, Canada.

Several studies have indicated the involvement of biogenic amines in different learning situations. Recently, we have shown that in intact, undrugged cats learning is accompanied by changes in monoamine concentrations, turnover rate and synthetising enzyme activities. Using a modified WGTA apparatus for conditioning, three different learning situations were successively used in an attempt to define the respective roles of biogenic amines in respect to behavioral parameters. The delayed response (DR) situation was a visual discrimination learning with interposed delays (0,9, 27 and 54sec). The successive discrimination (SD) situation was a 2-choice successive learning, implicating the association of a color with the direction of the response and the third situation was an asymmetrically reinforced go-nogo visual discrimination (VD).

No significant biochemical change was observed between a non-manipulated baseline group and a manipulated no learning group that was put through the experimental procedure but did not learn the task. All trained animals that reached criterion performance revealed the involvement of 5-HT metabolism whatever task learned. In go-nogo VD, a low turnover rate was demonstrated in all dissected brainstem structures including the raphe nuclei of mesencephalon, pons and medulla as suggested by a 5-HT:5-HIAA ratio higher in trained cats (2.61 ± .12) than in control cats (1.09 ± .14).In the DR situation, the low 5-HT turnover rate in the brainstem was circumscribed to the pons and medulla without raphe nuclei accompanied by a higher 5-HT:5-HIAA ratio in trained animals (2.02 ± .23) than in controls (1.17 ± .27). In these two learning situations, the tryptophan hydroxylase activity was significantly increased in all raphe nuclei. Moreover the 5-HT metabolism was also modified in other cerebral structures such as neostriatum, hippocampus, amygdala or frontal cortex in two ways: either by increasing both 5-HT and 5-HIAA content (DR and SD) or decreasing only the 5-HIAA content (go-nogo VD) thus suggesting different mechanisms. The NA metabolism was largely affected by the SD situation. In fact, a significant increase in the NA content was observed in the amygdala, thalamus, frontal cortex, neostriatum whereas a lower NA content was found in the amygdala and the frontal cortex. In DR and go-nogo VD tasks, the NA content was decreased in either the frontal cortex and amygdala or in the pons and hypothalamus.

These findings indicate that learning affected the 5-HT metabolism differently according to the degree of difficulty inherent to the performance of the task. (Supported by M.R.C. of Canada MA-6950)

40.4 EFFECTS OF PERIPHERAL STIMULI ON NEURONAL ACTIVITY IN THE VENTRAL TEGMENTAL AREA, SUBSTANTIA NIGRA AND MIDBRAIN RETICULAR FORMATION IN RATS. H. Maeda* and G. J. Mogenson (SPON: J. J. Seguin). Department of Physiology, University of Western Ontario, London, Ontario, Canada N6A 5C1.

Extracellular single unit recordings were obtained from the ventral tegmental area (VTA), substantia nigra, pars compacta (SNC) and pars reticulata (SNR), midbrain reticular formation (RF), and adjacent areas of urethane- or chloral hydrate-anesthetized adult female rats. Units from the VTA and SNC were divided into two groups based on their electrophysiological characteristics: Type A neurons had rather long-duration action potentials (> 2.6 ms) and slow firing rates (< 6 Hz), and type B neurons had shorter-duration action potentials and wider range of firing rates. The responsiveness of the two types of VTA and SNC neurons to peripheral stimuli such as cervical probing with a glass rod, foot pinching with bare forceps and tail pinching with padded sponge forceps was investigated. Prior to recording experiments, behavioral responses to cervical probing and tail pinching were observed and vaginal smears were taken to determine the estrous state of each rat.

Type A neurons, located exclusively in the VTA and SNC and considered to be dopaminergic, were mainly suppressed by foot pinching. With cervical probing three-quarters of Type A neurons were suppressed and one-quarter were activated. Equal numbers of Type B neurons were activated or suppressed by foot pinching. With cervical probing two-thirds of Type B neurons were suppressed and one-third were activated. For approximately 80% of Type B neurons the direction of effect was the same. Most neurons in RF and SNR were activated by the two peripheral stimuli. Responses to tail pinching were usually similar to those to foot pinching, although the magnitude tended to be less. There was no significant difference in responsiveness during the various estrous states.

The two stimuli, cervical probing and foot pinching, have quite different behavioral effects. In response to cervical probing the female rat assumes the lordosis posture and is relatively immobile, whereas foot pinching is a nocioceptive stimulus resulting in escape and other forms of behavioral activation. Thus when neurons respond in the same way to these two peripheral stimuli it is likely the result of a non-specific reaction. This is the case for RF and SNR neurons and for a majority of VTA and SNC neurons. In contrast the response of the remaining VTA and SNC neurons was variable and depended on the nature of the stimulus. It seems likely that these VTA and SNC neurons are related to specific response patterns to peripheral stimuli and do not merely reflect changes in arousal.

(Supported by NSERC of Canada)

40.5 L-DOPA, NASAL MARKING AND AROUSAL IN THE SQUIRREL MONKEY (SAIMIRI SCIUREUS). Sherry L. Berg*, Daniel L. Jones and Russell E. Dill. Dept. of Microscopic Anatomy, Baylor College of Dentistry, Dallas, TX. 75246

Videographic samples were taken and an analysis of the behavioral topography was made from squirrel monkeys treated systemically with 50 mg/kg L-DOPA plus 20 mg/kg carbidopa and 4 mg/kg ascorbic acid injected i.p. Primates were confined in a 30 x 28 x 60 cm plexiglass test chamber and photographed in the presence of the experimenter. Incidences of two types of nasal marking, sneezing and nasal rubbing, were documented as early as 10 min into the test and occurred in filmed behavioral samples taken one hour later.

Observations of nasal marking are particularly relevant to the inference that L-DOPA induces a heightened state of arousal as experimental and naturalistic reports on this behavior (Schwartz, G.G. & Rosenblum, L.A., Behav. & Neural Biol., 28:116-122, 1980) suggest an excitatory state as well as implications for olfactory communication and thermal regulation. (Supported by the Huntington's Chorea Foundation and NIH Grant NS-15020)

40.6 FAILURE TO ALTER ANXIETY OR THE ANXIOLYTIC PROPERTIES OF CHLORDIAZEPOXIDE AND ETHANOL BY DESTRUCTION OF THE DORSAL NORADRENERGIC SYSTEM. G.F. Koob, R.E. Strecker*, D.C.S. Roberts and F.E. Bloom. A.V. Davis Ctr. for Behavioral Neurobiology, The Salk Institute, San Diego, California 92138.

It has been hypothesized that the noradrenergic projection from the locus coeruleus plays a role in the manifestation of anxiety. It was therefore of interest to examine the effect of lesions to the forebrain projection from this nucleus on a behavioral task in which "anxiety" or conflict influences performance. The effect of these lesions on the behavioral effects of two anxiolytic drugs (alcohol and chlordiazepoxide) was also examined.

Rats were trained on a Geller-Seifter conflict test modified for incremental shock (Pollard and Howard, Psychopharm. 62, 117-121 (1979)) and consisting of three components: reward, time-out and conflict. Responses during the reward component were reinforced on a random interval (RI) schedule--30 sec; responses during the time out component were never reinforced and responses during the conflict component were continuously reinforced with food and foot shock (scrambled constant current, biphasic, square wave produced by a SGS-003 stimulator-BRS/LVE). However, the current increased by 0.1 mA with each successive shock during the conflict component until reaching 2.2 mA where it remained for the duration of the conflict period. A session consisted of two cycles each consisting of a 5 min reward period, 2 min time out and 2 min conflict period presented in succession. This procedure yielded a total testing time of 18 min.

Unoperated rats averaged 21 total shocks (incrementing to 1.0 MA) during each daily session without drug. Chloridiazepoxide (5 mg/kg) produced a significant release of punished responding during the conflict period and a small increase in responding during the reward component. Alcohol produced a significant release of punished responding during the conflict component. Virtual total destruction (98% depletion in hippocampus) of the dorsal noradrenergic innervation of the forebrain by 6-hydroxydopamine lesion to the central tegmental tract failed to significantly alter baseline conflict responding or the release of punished responding to chlordiazepoxide (5 mg/kg) or ethanol (0.5, 0.75 and 1.00 g/kg). These results do not support the hypothesis that the locus coeruleus forebrain projections have a role in anxiety or in the anxiety reducing properties of chlordiazepoxide or alcohol (supported by AA 03504).

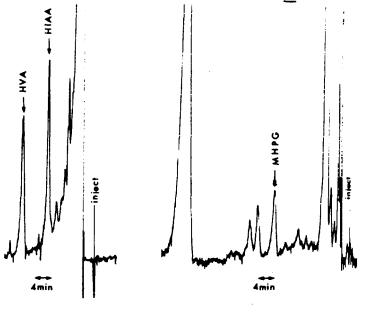
40.7 TRANSIENT OR PERMANENT HYPERACTIVITY FOLLOWING NEONATAL 6-HYDROXYDOPAMINE: A FUNCTION OF BRAIN DOPAMINE DEPLETION. Thomas Heffner Frederick Miller*, Connie Kotake*, Alfred Heller and Lewis Seiden. University of Chicago, Chicago, IL 60637.

Selective destruction of brain dopamine (DA) neurons by the neurotoxin 6-hydroxydopamine (6-HDA) results in locomotor hyperactivity in neonatal rats but not in adults (Shaywitz et al. Sci. 191:305,1976; Erinoff et al. Br. Res. 164:195,1979). Whereas Shaywitz et al. reported that the hyperactivity decreases with maturation, Erinoff et al. reported that hyperactivity can persist into adulthood. We sought to determine if the duration of 6-HDA-induced hyperactivity is related to the extent of destruction of central DA neurons. Neonatal male rats were given ivt. injections of 6-HDA (25,35,50 or 100 µg) or the vehicle following desmethylimipramine (20 mg/kg,s.c.) at 3 and 6 days of age. Adult male rats received a single ivt. injection of 6-HDA (125,200, or 275 µg) or the vehicle following pargyline (50 mg/kg,i.p.) and desmethylimipramine (25 mg/kg,i.p.) at 48 days of age. From day 16 to 32 of life, locomotor activity in all groups of rats treated neonatally with 6-HDA was significantly increased compared to controls. During this period, rats given either 2 x 50 µg or 2 x 100 µg of 6-HDA displayed consistently high levels of locomotion (150 counts/hr compared with 30 counts/hr in controls). In contrast, the hyperactivity seen in neonatal rats given 2 x 25 µg or 2 x 35 µg of 6-HDA decreased from 150 counts/hr at day 18 of life to 75 counts/hr at day 32 of life. When neonatally-lesioned rats were tested as adults (days 55-66 of life) only those given 2 x 100 µg of 6-HDA displayed higher levels of locomotion than controls (4.5 times the control level). None of the rats treated with 6-HDA as adults displayed increased locomotion. Analysis of the effects of 6-HDA on the regional levels of DA in brain revealed dose-dependent selective depletion of DA in both neonatally-treated and adult-treated rats. Rats given the 2 x 100 µg 6-HDA dose showed the greatest DA depletions of any neonatally-treated group. The extent of DA depletion in 8 nerve terminal fields was comparable in neonatal and adult rats. These results support previous findings that locomotor hyperactivity induced by 6-HDA depends upon the stage of development at the time of 6-HDA treatment. The present results further indicate that the duration of locomotor hyperactivity in neonates is related to the extent of DA neuron destruction. Near-total loss of central DA neurons in neonates is followed by hyperactivity that is sustained throughout neonatal as well as adult life. However, smaller losses of central DA neurons is accompanied by locomotor hyperactivity in neonatal but not adult life. (Supported by USPHS NS-12324; MH-10562; MH-14274).

40.8 DETERMINATION OF BIOGENIC AMINE METABOLITES IN HUMAN LUMBAR CEREBROSPINAL FLUID BY HPLC WITH EC DETECTION. L. Tune, R. Zaczek* and J.T. Coyle. Dept. of Pharmacol. and Expt. Therapeut., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Simple, sensitive and inexpensive methods for measuring biogenic amine metabolites in human cerebrospinal fluid (CSF) havebeen developed in which the compounds are separated by HPLC and quantified by electrochemical detection. For measurement of 5-hydroxyindole acetic acid (HIAA) and homovanillic acid (HVA), 20 1 of CSF was injected directly with the following chromatographic parameters: a HIBAR II, LiChrosorb RP 18 5.0 m column (VWR) with a mobile phase of 50 mM sodium acetate buffer, pH 2.8, containing 0.01% (w/v) disodium EDTA and 3.5% (v/v) N-propanol at a flow rate of 1.0 ml/min; the potentiometer (BAS-Model LC-4 with glassy carbon electrode) setting was 0.72V. HIAA eluted at 9.0 min with a response of 13.0 nAMP/ng and HVA eluted at 14.0 min with a response of 5.2 nAmp/ng. 3-Methoxy-4-hydroxy phenylglycol (MHPG) was first separated from CSF by acidifying 200 1 CSF with 20 1 of 4 N formic acid and then extracting twice with 1.0 ml of ethyl acetate with an 60% recovery. The organic phases were combined and dried under a gentle vacuum at 50°C. The concentrate was then dissolved in 200 1 of the mobile phase buffer (50 mM sodium acetate, pH 2.8 containing 0.01% (w/v) disodium EDTA); 100 1 was run over the same column as above with a flow rate of 1.0 ml/min. MHPG eluted at 20.4 min with a response of 7.8 nAmp/ng.

Lumbar CSF samples were obtained from 40 patients undergoing mylograms who ranged in age from 17-86 and had a variety of neurologic diagnoses; the CSF was frozen on dry ice within 15 min of lumbar puncture. HVA levels for the 35 patients ranged from 7.7 to 75.2 ng/ml with a mean of 38.4 ± 3.0 ng/ml (± S.E.M.); the range for HIAA was 10.5 to 39.5 ng/ml with a mean 22.1 ± 2.1 ng/ml. Analysis of the effects of freeze-thawing (5 times) of the CSF did not reveal any alterations in HVA or HIAA values. MHPG levels from 15 patients averaged 4.9 ± 0.8 ng/lf. The results obtained with this technique compare favorably with those reported by others using gas chromatographic - mass spectrometry or fluormetric assay methods.



41.1 EXCITATORY DRIVE FROM THE MESENCEPHALIC CORE TO MEDIAL AND INTRALAMINAR THALAMIC NEURONS PROJECTING TO THE CAUDATE NUCLEUS AND NEOCORTEX. Loyd L. Glenn and Mircea Steriade. Lab. Neurophysiol., Fac. Med., Laval Univ., Québec, Canada G1K 7P4.

The medial and intralaminar thalamic nuclei are major rostral targets of neurons in the midbrain reticular formation (MRF). To elucidate the electrophysiological properties of pathways underlying cortical activation processes, we made extracellular recordings in the ventralis medialis (VM) and centralis lateralis-paracentralis (CL-Pc) thalamic nuclei. Some cats were prepared acutely and others chronically. Recording sessions were conducted 7 to 20 days after lesioning the ipsilateral pontine tegmentum just caudal to the MRF stimulating electrodes. The anterograde degeneration that followed ensured that MRF-evoked responses did not result from the stimulation of fibers of passage.

Two hundred CL-Pc and VM neurons were identified by antidromic invasion from multielectrode arrays inserted in cortical areas 4 & 6, anterior suprasylvian areas 5 & 7, and the caudate nucleus. Ninety-nine percent were backfired selectively from one of these three targets. As a rule, neurons recorded in CL-Pc projected to areas 4,6, or 5, while VM neurons restricted their projection to the precruciate areas. Our disclosure of CL-Pc and VM neurons projecting to the anterior neocortex is the electrophysiological counterpart of data obtained by others using retrograde and anterograde tracing techniques. In CL-Pc, about 40% of tested neurons were antidromically activated from the precruciate gyrus and 30% from the anterior suprasylvian gyrus. In VM, about 25% were activated from the precruciate gyrus. The median conduction velocity of CL or Pc → precruciate axons was significantly higher (≈10 m/sec) than that of VM axons (≈6.5 m/sec), reflecting in all likelihood the larger size of cells in the dorsolateral CL.

MRF-evoked monosynaptic excitation, as inferred from latencies below 1.5 msec and the ability to follow high-rate stimuli, was obtained in 70 CL-Pc and VM neurons, 27 of which projected to caudate or cortical areas. The facilitatory nature of the MRF influence was demonstrated by (1) increased probability of antidromically elicited discharges in CL-Pc neurons to cortical or caudate volleys and (2) transformation of their abortive or broken spikes into full spikes following a conditioning MRF stimulation.

A direct excitatory MRF → thalamic pathway has thus been demonstrated, providing a rapid route for transferring activating MRF influences to anterior neocortical areas.

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41.2 EXCITABILITY OF MEDIAL AND INTRALAMINAR THALAMIC NEURONS DURING WAKING AND SLEEP STATES. Mircea Steriade and Loyd L. Glenn, Lab. Neurophysiol., Fac. Med., Laval Univ., Québec, Canada G1K 7P4.

The characteristics of the reticular afference and telencephalic efference of centralis lateralis-paracentralis (CL-Pc) and ventralis medialis (VM) neurons are described in a companion abstract (Glenn and Steriade, this volume). The spontaneous discharge rate and probability of antidromic and/or synaptic activation in a group of 22 neurons located in CL-Pc and the subjacent VM territory were analyzed during the natural sleep-waking cycle in unanesthetized, behaving cats. Chronic lesions of the ipsilateral pontine tegmentum, with consequent anterograde degeneration of ascending fibers coursing through the midbrain reticular formation (MRF), ensured that MRF-evoked synaptic responses did not result from the stimulation of fibers of passage.

During synchronized sleep (S), CL-Pc and VM units discharged in high-frequency, protracted bursts separated by long periods of silence. Burst frequencies paralleled the range for cortical spindle waves (7 to 14/sec); this relation could be established for either single neurons or neuronal pairs. In wakefulness (W) and desynchronized sleep (D), the firing rate increased and the burst-silence sequence during S either decreased in duration or was completely blocked, resulting in a single spike background of regular discharge.

The stimulation current for antidromic and synaptic responses was adjusted to straddle the threshold, so that fluctuations in excitability across behavioral states were detectable. In all instances, the probability of antidromic invasion increased by >100% during W and D as compared to S. In one CL neuron that projected to area 5, spikes with an initial segment-somadendritic break during S developed into unbroken discharges in W. Interestingly, this enhanced antidromic responsiveness was detected 10 sec prior to cortical desynchronization of W or D, when the EEG was in full synchrony.

Neurons discharging singly to MRF stimulation at monosynaptic latencies had a strikingly increased response probability in W and D. A group of cortically-projecting neurons in CL responded to MRF stimuli with high-frequency (>600/sec), long bursts. In W and D, both the latency and duration of the burst decreased. This phenomenon was concurrent with enhanced antidromic conduction during W and D. The diminished number of spikes within the synaptically-evoked burst may be ascribed to a reduction in the latency of postexcitatory inhibition during cortical activation.

All evidence is consistent with the hypothesis that CL and VM neurons, with their wide cortical projections, may mediate activating influences over the neocortex in W and D states.

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41.3 LOCALIZATION OF BRAINSTEM NUCLEI AND THEIR ASCENDING PATHWAYS THAT CONTROL THE HIPPOCAMPAL EEG. Robert P. Vertes. Dept. Physiol., Univ. of Mich. Med. Sch., Ann Arbor, MI 48109.

In a previous report in the anesthetized rat we mapped the entire brainstem with stimulation to determine its effect on hippocampal slow-wave activity. The results of that study (Vertes EEG, in press) showed that: (1) The most effective brainstem synchronizing sites were those within the reticular nucleus pontis oralis (RPO) and those in and around the medial longitudinal fasciculus (MLF) at the pontine level. We suggested that the nucleus pontis oralis was the brainstem source for hippocampal theta generation and that its fibers primarily exit the brainstem via the MLF. (2) The median raphe nucleus (MR) was the only brainstem site at which stimulation produced hippocampal desynchronization. Desynchronization at the MR was accompanied by a sharp reduction in the amplitude of hippocampal EEG activity.

In the present investigation, also in the anesthetized rat, the rostral brainstem was re-stimulated and we confirmed the existence of the RPO and the MR as strong synchronizing and desynchronizing sites, respectively. In addition, the entire midbrain was systematically stimulated in an attempt to follow the synchronizing (RPO) and desynchronizing (MR) systems rostrally. The results showed that three separate synchronizing pathways and one desynchronizing pathway could be followed through the midbrain into the caudal diencephalon. The three synchronizing pathways were: (1) MLF - A very effective synchronizing system could be followed along the entire length of the MLF as it traversed the midbrain. (2) Medial Forebrain Bundle (MFB) - A synchronizing system lying ventral to nucleus cuneiformis was evident throughout the midbrain. This pathway moved progressively ventral as it ascended and appeared to join the MFB at the level of the diencephalon. (3) Central Tegmental Tract (CTT) - A third system that was weaker than the other two was found dorsally lateral to the midbrain central grey and corresponding to the position of the CTT. This pathway could be followed as far rostral as the thalamic intralaminar nuclei. The sole desynchronizing system traveled forward from the MR along the midline and progressively maintained a more ventral position as it ascended. Throughout approximately the caudal two-thirds of the midbrain this tract ran parallel to and about 1mm below the MLF synchronizing system. At the level of the interpeduncular nucleus this system turned lateral and continued forward in a region corresponding to the ventral medial part of the MFB. These results suggest that specific brainstem nuclei are directly involved in controlling hippocampal EEG activity and that their axons ascend to the forebrain in discrete fiber tracts.

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41.4 L-PROLINE BLOCKS HIPPOCAMPAL EXCITATORY SYNAPTIC ACTIVITY. A. Van Harreveld and F. Strumwasser. California Institute of Technology, Pasadena, CA 91125.

Field potentials, elicited by stimulation of the Schaffer collaterals, were led off from the stratum radiatum of CA1 in rat hippocampal slices suspended on a nylon net in a chamber perfused with a physiological salt solution. The slices were completely submerged. L-proline in suitable concentrations was applied in the salt solution. The responses to the stimulus consisted of a negative synaptic potential and one or more population spikes preceded by a small spike ascribed to conduction in the Schaffer collaterals. In a concentration of 2 mM, L-proline had no noticeable effect, but 3 mM enhanced the response, the population spikes becoming larger and more numerous. At 5 mM or more the synaptic responses were reversibly abolished but the presynaptic spike was not affected. This effect was counteracted by simultaneously applied 4-amino pyridine (0.5 mM), which prolonged the presynaptic spike and was postulated to increase the release of the transmitter, believed to be L-glutamate and L-aspartate (see e.g., Nadler et al., Nature 260, 538, 1976). This can be expected to favor the binding of the transmitter to the postsynaptic membrane in its competition with L-proline for glutamate receptors. These observations support the postulate that L-proline is a glutamate antagonist, as has been suggested for instance by its blocking effect on the responses of hippocampal neurons to applied glutamate (Segal, Br. J. Pharmacol. 58, 341, 1976), and on the crustacean neuromuscular junction, which is believed to be glutamate mediated (Van Harreveld, J. Neurobiol., in press).

It has been suggested that L-proline binding with extrasynaptic glutamate receptors causes a moderate increase in Na^+ permeability of the dendritic membrane and a release of K^+ (Van Harreveld, J. Neurobiol. 10, 355, 1979). The enhancement of the synaptic response to Schaffer collateral stimulation in 3 mM proline may be due to this K^+ release, which was supported by the observation of a similar enhancement by an increase in the K^+ concentration in the perfusion fluid. However, for the time being the possibility that the enhancement is due to an initial agonistic effect of L-proline (Shank and Freeman, J. Neurobiol. 7, 23, 1976) cannot be excluded. D-proline even in 10 mM concentration had no effect on the responses to collateral stimulation. (Supported by NS 07071 and the Pew Foundation.)

41.5 ELECTROPHYSIOLOGICAL GUIDANCE OF HIPPOCAMPAL ELECTRODES FOR INTRACRANIAL SELF-STIMULATION (ICSS) IN ANGULAR BUNDLE, PERFORANT PATH AND DENTATE GYRUS.
T.J. Collier*, J.S. Miller* and A. Routtenberg. Cresap Neuroscience Laboratory, Northwestern University, Evanston, ILL 60201.

The entorhinal cortex (EC) provides a massive projection to the molecular layer of the dentate gyrus and supports stable ICSS responding (Collier, et al., 1977, Br. Res., 137:188). The present study investigates ICSS in the EC-dentate projection system. If ICSS derived from EC is mediated by activation of the EC projection to the dentate gyrus, then ICSS should be observed in the perforant path and the dentate gyrus.

Male albino rats were chronically implanted with bipolar stimulation electrodes using electrophysiological guidance. Probes were guided into the angular bundle/perforant path system by stimulating at 50 μm steps during electrode penetration, while recording evoked granule cell population responses from the dorsal dentate. Reversal of potential in the molecular layer confirmed the local nature of the stimulation effect, as did silver impregnation studies following lesions at these stimulation sites. ICSS electrodes aimed at the dentate gyrus were guided into position by recording, in sequence, CA1 and granule cell spontaneous activity during electrode penetration.

Low to moderate ICSS rates (50-150 responses/15 min) were derived from dorsal angular bundle and dentate gyrus. In comparison to hypothalamic sites, onset (in days) to begin ICSS responding was slower in the EC-dentate system, and peak rates were substantially less than those derived from hypothalamic sites. However, ICSS in dentate gyrus, like that derived from hypothalamus, was not decreased by administration of an inebriating dose of ethanol prior to testing.

The use of this electrophysiological guidance procedure enables: (a) fine resolution (< 50 μm) of electrode location, (b) relative homogeneity of implanted groups, (c) explicit placement and confirmation that electrodes are activating axonal pathways, and (d) postponement of death of the animal necessitated by histological analysis. Supported by N.I.M.H. 25281 and N.S.F. 20630 to A.R.

41.6 PARAMETRIC VARIATIONS IN GROUND POTENTIALS DO NOT AFFECT INTRACRANIAL SELF STIMULATION RATES.
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A long standing notion has been that intracranial self-stimulation (ICSS) behavior is delineated by the path of the stimulating current and its intensity. Many authors have contended that for this reason no arguement could be made for the site specific effects of ICSS since the path of the electrical current with respect to ground varied throughout the course of an experimental session. Thus, this study investigates this problem of the existence of ground potentials with respect to the stimulating electrode during ICSS.

Eight Sprague-Dawley rats were stereotaxically implanted with bi-polar electrodes aimed at various diencephalic self-stimulation sites. After recovery from surgery, all rats were shaped to respond for 700 msec. trains of .1 msec. monophasic rectangular pulse pairs of intracranial stimulation. The first pulse in a pair (C-conditioning pulse) was followed by a second (T-test pulse) which was parametrically varied in time.

Animals were placed in a paradigm in which they received C-T stimulation from either a capacitance coupled or from an optically isolated constant current stimulator. The capacitance coupled stimulator was constructed from several BRS/LVE 200 series DIGIBIT logic modules and had a reference impedance which was 120 ohms relative to ground. The constant current stimulator was designed with two GE 4N26 optoisolators and had a reference impedance of two megaohms with respect to ground.

All animals tested demonstrated no significant differences between C-T response rate functions obtained on the capacitance coupled vs. the opto-isolated stimulation units. Thus, this study indicates that direct manipulation of ground potentials between 120 ohms to 2 megaohms do not significantly alter ICSS responding. Furthermore, since the locus of ground with respect to the stimulating current plays no significant role in ICSS, stimulation's effects can be more fully understood from the vantage point of site specific neural ICSS loci rather than from one advocating current spread or any such non-specificity hypothesis.

41.7 RELATIVE PREFERENCE AS A MEASURE OF THE REINFORCING VALUE OF ELECTRICAL BRAIN STIMULATION.
P.J. Fray* (SPON: L.T. Rutledge). Neurosci. Lab., Univ. Michigan, Ann Arbor, Mich. 48109.

Attempts to establish the neurochemical systems involved in the rewarding effects of electrical brain stimulation have been frustrated by the lack of a consistent and sensitive measure of reinforcement value. Although rate of responding, in relation to current intensity, has been widely employed, its reliability has been questioned by findings that rats will often choose current intensities for which they respond at a low rate over current intensities for which they respond at a high rate. Choice provides the only unambiguous way of determining a preference. However, animals given simultaneous access to two alternatives will usually choose one almost exclusively. Thus, a small difference between the alternatives leads to as great a preference as a large difference. Nevertheless, Herrnstein and others have demonstrated that if two alternative probabilities of food reinforcement are made available on independent, concurrent variable interval schedules, the distribution of responses and time between the alternatives is proportional to relative reinforcement frequency. If a relationship can be established between relative reinforcement and relative responding and time allocation, the relationship between two unknown levels of reinforcement can be assessed by measuring relative preference in this paradigm. Five rats were trained to work on such schedules for electrical stimulation of the lateral hypothalamus. Relative responding and time allocation on the two alternative schedules were found to vary reliably as a function of relative reinforcement frequency, and to be independent of the total amount of reinforcement delivered. With relative probability of reinforcement held constant, responding and time allocation were found to be functions of relative current intensity and relative number of stimulation trains earned per reinforcement opportunity. The technique provides a sensitive measure of reinforcement value that is independent of absolute rate of responding and reinforcement, and should prove useful in the investigation of the neurochemical basis of reward.

41.8 AN ASSESSMENT OF THE REINFORCING PROPERTIES OF FOODS FOLLOWING AMYGDALOID LESIONS IN THE MONKEY.
J. P. AGGLETON* AND R. PASSINGHAM* (SPON: B. RICHMOND). Dept. of Exp. Psychol., Oxford University, United Kingdom.

One of the most striking effects of amygdalectomy in monkeys are dietary changes: the ingesting of previously rejected items such as meat and even faeces. To characterise this abnormality further comparisons were made of the food preferences in fifteen rhesus monkeys (Macaca mulatta) before and after a variety of amygdaloid lesions. The surgical targets in the experimental groups were: (a) lateral amygdala (LAT n=4), (b) basolateral amygdala (n=4), (c) dorsal amygdala (n=2), (d) the white matter of the temporal albal stalk adjacent to the amygdala (n=2) and (e) total amygdala (AMX n=3). All animals received bilateral radiofrequency lesions centered in the appropriate nuclei.

Relative food preferences were estimated from the frequency with which a particular food was chosen when paired with another A profile of such preferences was thus established. None of the monkeys with subtotal amygdaloid lesions displayed changes in food preferences after operation, though all of the AMX monkeys would now eat raw meat and would occasionally choose meat rather than a previously accepted food. The remainder of the AMX group's food choices appeared normal.

In an attempt to obtain an absolute measure of preference for various foods the LAT and AMX groups were trained to press a panel on a Progressive Ratio (PR) schedule. This schedule consists of a sliding "fixed ratio" such that each reward causes the number of panel-presses required for the next reward to be increased by five. The PR score represents the ratio at which the animal stopped responding for an arbitrary criterion period (120 secs). The PR scores were sensitive to alterations in the size and type of food reward and the degree of deprivation. However, neither AMX nor LAT groups showed any changes from their preoperative response patterns on these PR schedules.

This study thus found no evidence of a specific lesion site within the amygdala sufficient to bring about dietary changes, nor evidence of any alterations in food preferences other than meat-eating following total amygdalectomy. These results suggest that amygdalectomy may result in a normal appreciation of food rewards and that only the readiness to sample and eat "unpleasant" foods is changed.

42.1 THE EFFECTS OF PRIOR TRAINING AND DRUG PRETREATMENT ON PIMOZIDE-INDUCED AVOIDANCE DEFICITS. R.J. Beninger, A.G. Phillips and H.C. Fibiger. Depts. Psychology and Psychiatry, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

There are numerous reports of the deleterious effects of neuroleptic drugs on one way avoidance behavior. Observing the rate of acquisition, neuroleptic-injected animals were retarded in a dose-related manner when compared to vehicle-treated controls. Some controversy surrounds the effects of prior training. Using a small number of drug sessions, some authors have suggested that prior training attenuates the effects of neuroleptics whereas others used more extensive drug testing and reported that avoidance responding was disrupted. In the present experiment the amount of training prior to the initiation of treatment with pimozide was varied. Four groups of rats received either 2 or 10 training sessions (10 trials/session; intertrial interval = 30 sec; CS = 10-sec tone; US = 2.4 mA footshock). Drug treatments began with i.p. injections of pimozide (0.5 or 1.0 mg/kg) occuring 90 min prior to each testing session. Four additional groups received the same prior training and drug treatment but pimozide injections were given in the home cage on the 3 days preceding the first drug session. These groups were included to determine any possible cumulative effects of repeated injections. The results revealed that the number of avoidance responses per session of all drugged groups deteriorated over sessions in a dose related manner. There was no effect of prior training on the rate of decline in responding of the 0.5 mg/kg groups but the group receiving 10 prior training sessions was more resistant to the effects of the higher dose than the group receiving 2 prior training sessions. Pretreated groups showed the same effect as the nonpretreated groups except that the effect of prior training in the groups receiving the higher dose no longer was observed. Thus, 10 sessions of prior training attenuated the effect of 1.0 mg/kg pimozide. This effect of prior training was not observed following pretreatment with pimozide.

42.2 EVALUATING CYCLIC AMP MEDIATION OF MEMORY. Douglas L. Chute, John W. Villiger*, and Mike Dragunow*. Department of Psychology, University of Otago, Dunedin, New Zealand.

A number of authors have hypothesized that postsynaptic cyclic adenosine 3',5' monophosphate, by its action on protein kinases, may be an important mediator of memory formation. One consequent prediction would be that increased intracellular cAMP ought to facilitate memory. In a series of experiments involving latent learning, maze learning, and passive avoidance tasks, a performance facilitation was observed in several varieties of mice. They had received either a phosphodiesterase inhibitor, papaverine or RO-20-1276/1, or dubutyryl cAMP raising intracellular levels of cAMP. The injection was given immediately after acquisition which, in conjunction with other experimental controls, tends to preclude such non-specific factors as arousal, attention, fear, motivation and motor performance as possible explanations of the observed performance facilitation. Increased intracellular cAMP has a prophylactic effect on catecholamine depletion amnesia.

We speculate that the behavioural evidence of a performance facilitation, which can be interpreted as memory facilitation, supports hypotheses of cAMP mediation of memory.

42.3 LONG TERM CHANGES IN EMOTIONAL REACTIONS AND NOT IN LEARNING AFTER INTRACEREBRAL INJECTIONS OF CYCLOHEXIMIDE OR GLUTAMATE IN NEONATAL CHICKS. Paul R. Sanberg, Ian J. Faulks*, Judith M. Anson* and Richard F. Mark* Department of Behavioural Biology, Research School of Biological Sciences, Australian National University, Canberra, ACT, 2600, Australia.

It has previously been demonstrated that an injection of cycloheximide (CXM) into the forebrain of newly-hatched chickens can cause permanent slowing of learning in later life[1]. Cycloheximide has been shown to increase the concentration in the brain of glutamate (GLU) in neonatal chicks, and injection of this amino acid can also cause slow learning similar to that seen following CXM. It has been suggested that an accumulation of glutamate may underlie the slow learning[2] induced by cycloheximide. Slow learning has been measured mainly with a pebble floor task.

We have assessed the effects of bilateral intracerebral injections of CXM (20µg in 25µl saline) or GLU (210µg in 25µl saline) in 2 day-old chicks on the subsequent performance, in operant, open-field and tonic immobility behaviors in order to find out the nature of the behavioral deficits. Birds were tested between 4 and 8 weeks after injection.

There were no significant differences between birds injected with saline and birds injected with CXM or GLU on acquisition, performance or extinction of a continuous reinforced pecking response as measured in modified pigeon operant boxes. In the open-field test birds injected with either CXM or GLU were less active, as reflected by longer latencies to leave the first square, reduced ambulation, preening episodes and pecks at the floor. They defecated more too. Tonic immobility was increased in duration. All effects were greater in birds treated with CXM.

The failure to find any differences in the operant experiment does not support the conclusion that either treatment causes a primary impairment in the ability to form memory traces. Instead the results of experiments in the open field and measurements of tonic immobility suggest that injections of CXM or GLU in neonatal chicks enhance emotional reactions and may increase the fear of novelty. The learning deficit previously reported is most likely to be a consequence of disordered emotional responses.

1 Rogers, L.J., Drennen, H.D. and Mark, R.F., *Brain Research* 79: 1974, 213-233.

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42.4 IMPAIRED BEHAVIORAL SENSITIZATION TO COCAINE IN VASOPRESSIN DEFICIENT RATS. R.M. Post, N.R. Contel,* P. Gold.* NIMH, Bethesda, Maryland 20205.

Vasopressin (VP) exerts effects on a variety of learning and memory paradigms in animals and man. In addition, VP has been shown to influence the development of tolerance to drugs, which has been conceptualized as a form of cellular learning (de Wied, 1978). We postulate that reverse tolerance or behavioral sensitization, in which there are long-lasting increases in stimulant responsivity based on previous drug exposure and environmental context, could also represent a model of learning and memory. In order to elucidate a possible role of VP in the process of behavioral sensitization to psychomotor stimulants, rats with a genetic deficiency in VP were administered repeated injections of cocaine and effects on motor activity and stereotypy examined. Twelve male Brattleboro homozygotes who show absolute VP deficiency resulting in diabetes insipidus (D.I.) were studied in parallel with 10 litter mate heterozygote controls (H.C.). All were housed in groups with ad lib food and water on a 7-7 light-dark schedule. Rats were injected with cocaine (10 mg/kg, i.p.) once daily for 10 days, rechallenged with cocaine 3 weeks later (Challenge I), given daily cocaine again for 15 days, and rechallenged after an interval of 16 weeks (Challenge II) and 21 weeks (Challenge III) to test for persistence of differential response. Horizontal and vertical motor activity were measured and stereotypy rated for two 10-minute baseline periods prior to each injection and 40 minutes following cocaine.

Over the first 10 days of cocaine H.C., but not D.I. rats, showed significant cocaine sensitization, as evidenced by increases in vertical hyperactivity and stereotypy ($p < .01$). D.I. rats eventually showed evidence of sensitization, but it was slower in onset and less robust than in H.C. Moreover, on all cocaine rechallenges (I-III) at intervals of 3 to 21 weeks following cocaine, H.C. rats showed greater cocaine-induced hyperactivity than D.I. animals ($p < .01$). Although H.C. weighed more (454 ± 9 gms) than D.I. animals (377 ± 17 gms), this would not appear to account for the differential response to cocaine as cocaine-induced hyperactivity did not differ on day 1 and baseline exploratory activity 20 and 10 minutes prior to each of the cocaine challenges did not differ between groups.

The decreased responsivity in VP-deficient rats compared to H.C. suggests that the neuropeptide VP is involved in the development and persistence of behavioral sensitization to repeated cocaine. Vasopressin thus appears to be involved in the long-term coding of cocaine-induced motor activity and stereotypy and related behaviors of psychiatric relevance. This extends previous reports of VP's role in enhancing learning and memory to the model of pharmacologically-induced behavioral sensitization.

42.5 AMPHETAMINE COUNTERACTS DECREMENTS OF AGGRESSION IN MICE. James T. Winslow* and Klaus A. Miczek. Dept. Psychology, Tufts Univ., Medford, MA 02155.

The decline in aggressive behavior as a result of repeated episodes of fighting may represent a habituation phenomenon. At present, the stimulus conditions determining this decremental process and the underlying mechanisms have been primarily investigated in fish, but little is known about the habituation of aggression in mammals. The objectives of our experiments were (1) to investigate the features of the declining rate of attack during the course of consecutive episodes of fighting between two male mice, and (2) to intervene pharmacologically at different phases of the habituation process with the catecholamine-releasing drug d-amphetamine. The effects of d-amphetamine were studied on the initial high rates of attacks and threats as well as on later phases of the decremental process when aggressive behaviors reached stable low frequencies. Aggressive behavior was generated in a male mouse, housed with a female, by introducing an unfamiliar group-housed male mouse into the homecage (n=10 resident males). Initial experiments investigated the parameters of intruder presentation and found that ten consecutive resident-intruder confrontations, each 5 min long, every 10 min resulted in an orderly decline of attack and threat behavior toward the intruder. The rate of decrement in attacks and threats by resident mice over the 10 trials was reliably reproduced in consecutive sessions, each 7 days apart. In 3 additional trials with a new intruder, the rate of attacks and threats recovered to about 50% of the original level suggesting fatigue as well as habituation contributing to the decrement in aggression. Further experiments examined the effects of d-amphetamine (0.63-5.0 mg/kg, i.p.) on the declining rate of aggressive behavior as well as on non-agonistic locomotion. Administration of d-amphetamine, 5 min before the first trial, counteracted the decrement of attacks and threats. Initial high rates of attacks were unaffected, whereas the low rates of attacks in the late phase were greatly increased, 1.78 mg/kg being the most effective dose. By contrast, d-amphetamine increased locomotion in a dose-dependent monotonic manner, the largest increase occurring in the initial trials with the highest dose (5 mg/kg). d-Amphetamine produced no reliable effects on behavior in the 3 dishabituation trials. Further experiments investigate the possible different mechanisms controlling initial and late phases of habituation of aggression. The selective effects of d-amphetamine on attacks and threats when these behaviors have declined to a low level suggest an excitatory role for catecholamines in the habituation process.

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42.6 NEUROTRANSMITTER INTERACTIONS IN CHEMICAL KINDLING. C.G. Wasterlain, V. Jonec, Epilepsy Research Laboratory, VA Medical Center, Sepulveda, CA 91343, Dept of Neurology and Brain Research Institute, UCLA School of Medicine.

Injection of subconvulsive amounts of muscarinic agonists into the amygdaloid complex of Holzman rats resulted in the progresive kindling of epileptic seizures. This kindling was dependent on dose and on interstimulus interval, long-lasting, induced by 3 different muscarinic agonists, completely blocked by 3 different blockers of muscarinic receptors, stereospecific, potentiated by cholinesterase inhibitors, and the kindling potencies of muscarinic ligands in vivo were closely related to their affinities for muscarinic receptors in vitro.

Amygdaloid injection through stereotaxically implanted chemitrodes of other agonists and antagonists of putative transmitters modified kindled seizures. Nanomole amounts of dopamine and norepinephrine, injected in a 1 µl sterile isotonic solution inhibited seizures compared to their antagonists, propranolol and haloperidol. Naloxone, glutamate and nicotinic blockers had no effect. Muscimol (3 nmoles), GABA(50-150 nmoles) were profoundly inhibitory.

Diazepam and phenobarbital were very effective, valproate moderately effective and dilantin poorly effective in blocking seizures in kindled animals. While seizure suppression was of short duration for most pharmacological agents intraamgdaloid muscimol produced a lasting loss of excitability.

These result suggest that GABAergic and catecholaminergic systems exert an inhibitory influence on seizures induced by excitation of muscarinic receptors in or around the rat amygdala.

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42.7 ENHANCEMENT OF AGED RODENT RETENTION VIA PHARMACOLOGICAL MODULATION OF CHOLINE AVAILABILITY. R.T. Bartus, R.L. Dean, K. Sherman, and E. Friedman. Med.Res.Div. of Amer. Cyanamid, Pearl River, NY and N.Y.U. School of Medicine, New York, NY.

Loss of memory and other cognitive functions is commonly recognized to be one of the most serious neurobehavioral dysfunctions associated with old age. Evidence from many neuroscience disciplines suggests that dysfunctions in central cholinergic mechanisms may play a critical role in these impairments. Recently, in vitro neurochemical assays indicate that increasing the availability of choline should stimulate cholinergic activity. However, no clear evidence of improved performance on geriatric cognitive tasks has yet been reported. One possibility for this apparent paradox may be that the very cholinergic dysfunction which contributes to the age-related memory deficit may prevent choline from being effectively converted to acetylcholine in the aged brain. For example, choline uptake, choline actyltransferase and oxidative metabolism have all been reported to be decreased in aged brain. These deficiencies may not allow choline to be incorporated into acetylcholine as easily as occurs in younger brain. One manner of attempting to compensate for this possibility might be to improve ATP synthesis, while simultaneously increasing choline availability.

We studied this possibility in aged Fisher 344 rats (20 to 28 mo. old). All animals were administered either choline, piracetam (a cyclic derivation of GABA purported to enhance ATP synthesis), or both agents simultaneously for a period of 1 week. All rats were trained on a passive avoidance task and tested for retention 24 hr. later. Aged F344 rats had previously been shown to suffer severe retention impairments on this task. Those rats administered only choline exhibited retention scores only slightly higher than control rats given saline ($p < .1$). Although the rats administered piracetam exhibited better retention than the saline and choline groups ($p < .02$), the rats administered the choline/piracetam mixture exhibited retention scores several times better than those rats given piracetam alone ($p < .0001$). These data, therefore, provide preliminary evidence that the effects of cholinergic manipulation, particularly increased precursor availability, may be greatly enhanced by the simultaneous administration of a pharmacological agent purported to enhance oxidative metabolism. Further tests are currently in progress to determine the generality of these behavioral effects and to help establish what neurochemical changes in the brain may have been induced by the combined treatment.

43.1 CONNECTIONS BETWEEN THE CEREBRAL CORTEX AND AMYGDALA IN THE RAT. B. TURNER and J. ZIMMER*. Dept. Anat., Howard Univ. Med. Sch., Washington, D.C. 20059, and Inst. Anat. B, Aarhus Univ., Aarhus, Denmark.

Anterograde and retrograde tracing techniques were used to determine the presence and extent of connections between the cerebral cortex and the amygdala of the rat. Caustic heat lesions of varying size and cortical location were made in 25 rats, their brains processed by the Fink-Heimer and cupric silver techniques, and the amygdalae examined for degenerating afferents. In another 5 rats, the possibility of a reciprocating amygdalo-cortical relationship was explored by injecting small quantities of retrogradely transported fluorescent dyes into the cortical areas shown to project to the amygdala. To assist in defining both the subdivisions of neocortex where lesions or injections were made, and the nuclei within the amygdala shown to be connected with the cortex, topographical maps of the cerebral cortex and amygdala were constructed on the basis of cell, fiber, Timm's, and AChE staining patterns in normal brains. Results showed that no part of the neocortex of the rat projects directly to the amygdala. However, all sensory cortices send topographically arranged efferents to the periallocortex lying along the rhinal fissure, i.e., areas 35, 36, 13, and 14. Of these, the supra-rhinal parts of areas 13 and 35 project heavily to the amygdala. Anteriorly, area 13 sends efferents to the central nucleus, and to the anterior half of area X and the lateral and basal nuclei; this cortex receives afferents from the lateral and basal nuclei, and from the prepiriform and periamygdaloid cortices. Caudally, area 13 projects to the anterior part of the basal nucleus, and to the entire extent of area X and the lateral nucleus. Area 35 projects lightly to the caudal part of area X, and heavily to the posterior two-thirds of the lateral nucleus; a restricted portion of the lateral nucleus projects back to area 35.

43.2 DOUBLE AND TRIPLE LABELING OF RAPHE NEURONS FOLLOWING INJECTIONS OF FLUORESCENT SUBSTANCES INTO THREE SEPARATE LIMBIC FOREBRAIN STRUCTURES. L. Heimer and J. de Olmos. Inst. of Anatomy, Univ. of Aarhus, Denmark, and Inst. "M. y M. Ferreyra", Argentina.

A retrograde labeling procedure utilizing fluorescent substances (van der Kooy et al. 1978) was used to establish the presence of branching axons in the ascending raphe system of young rats. The three fluorescent substances, propidium iodide (PI), granular blue (GB) and nuclear yellow (NY), which were dissolved in 2% DMSO or water, were injected in the amount of 0.1-0.2 µl each by the aid of a hydraulic pressure injection system using glass pipettes with tip diameter of 100-150 µm. 5% GB and 3% PI were injected into the medial thalamus and the septum 3 days before sacrifice whereas 1% NY was injected into the olfactory bulb or olfactory cortex on the same side 18-20 hours before the time of sacrifice.

Whereas the number of double labeled cells in various combinations was relatively large in both the dorsal and median raphe nuclei, triple labeled cells occurred more rarely, and usually in a restricted part of the dorsal raphe nucleus. Generally, the intensity of the fluorescence decreased with the number of fluorescent substances incorporated by the neuron, and it was in several instances almost impossible to decide if a neuron was triple or even double labeled. The number of clearly double and triple labeled neurons, therefore, is probably smaller than the number of cells that actually project to two or three of the injected limbic structures. Nevertheless, the fact that each class of neurons, i.e. single, double and triple labeled neurons, were shown to have a predominant distribution within specific parts of the raphe nucleus seems to indicate that double and triple labeling with fluorescent substances is a useful technique for unraveling functionally important subdivisions within a nuclear complex. (Supported by NIH and the Danish Medical Research Council).

43.3 BIOCHEMICAL AND PHYSIOLOGICAL PHENOMENA INDICATIVE OF HIPPOCAMPAL - HYPOTHALAMIC - PITUITARY - THYROID AXIS INTERACTIONS. H. M. Murphy, C. H. Wideman and T. S. Brown *. John Carroll Univ., Cleveland, OH 44118 and DePaul Univ., Chicago, ILL 60614.

At the last meeting of the Society for Neuroscience data were presented indicating that rats with hippocampal lesions had disturbances in the hypothalamic - pituitary - thyroid axis. Animals with hippocampal lesions had significantly lower levels of TSH (thyroid stimulating hormone), total T_4 (thyroxine), and free T_4. These findings would lead one to suspect that such animals might be hypothyroid. The present study examined several parameters indicative of the hypothyroid state. The parameters studied were: blood urea nitrogen levels in the plasma, SGOT (serum glutamic oxaloacetic transaminase or aspartate aminotransferase) levels in the plasma, body temperature at room temperature, body temperature after being in a warming chamber, and arterial blood pressure after being in a warming chamber.

The results strengthened the hypothesis of hypothyroidism in rats with hippocampal lesions. Animals with such lesions had significantly higher blood urea nitrogen levels than control animals ($p = .002$). A positive nitrogen balance is associated with hypothyroidism. Animals with hippocampal lesions had significantly higher SGOT levels than control animals ($p = .02$). Elevated SGOT levels are associated with the hypothyroid state. At room temperature animals with hippocampal lesions did not differ from controls in body temperature. However, after 15 min. in a warming chamber, animals with hippocampal lesions had a significantly lower body temperature than did controls ($p = .002$). This phenomenon has also been observed in hypothyroid animals. Arterial blood pressure was significantly lower in animals with hippocampal lesions than control animals ($p = .002$). This may be due to the fact that in order to obtain blood pressure readings, animals had to be placed in a warming chamber before the blood pressure was recorded. Rats with hippocampal lesions were quite docile in this situation and readily entered the heated restraining chamber for blood pressure recording. Control rats were quite active and somewhat reluctant to enter the chamber.

This study indicates that animals with hippocampal lesions are mildly hypothyroid. It is postulated that the hippocampus has an excitatory effect on the hypothalamic - pituitary - thyroid axis via neuronal connections between the hippocampus and the tubero-infundibular neuron which in turn ends on a median eminence portal capillary. The median eminence is one of the areas of the brain that releases TRH (thyrotropic releasing hormone).

43.4 SUB-THRESHOLD STIMULI ALTER IPSILATERAL/CONTRALATERAL AFTERDISCHARGE DURATIONS. H. Scott Swartzwelder, D.A. Mobray* and R.S. Dyer. The American Univ. and U.S. Environmental Protection Agency, Research Triangle Park, N.C.

Electrically induced hippocampal (HPC) afterdischarges (ADs) and their post-ictal sequelae vary as a functinn of the relationship of the eliciting stimulus intensity to the threshold for AD production. Thus it is clear that AD threshold determination is a methodological necessity when such variables are under consideration. Two AD threshold determination methods have most often been employed (Pinel et al.,1976; Racine, 1972). These methods involve dramatically different procedures. Method A (adopted from Pinel, et al.) involves presentation of a series of ascending subthreshold stimulus intensities, at one min intervals, until an AD is induced. Typically, 15-25 unsuccessful stimulations are presented prior to AD induction. Method B (adopted from Racine) involves zeroing in on the AD threshold through a series of successful and unsuccessful stimulations presented at 48h intervals. Usuially 4-5 unsuccessful stimulations and 4-5 successful stimulations are presented prior to threshold localization. Recent evidence has demonstrated that these methods yield different AD threshold values. Furthermore, AD thresholds determined under method A were lower following a 45-day interval, while repeated threshold assessments using method B showed no such fluctuations.

The present report describes yet another aspect of the HPC AD which is influenced by the procedural differences of these two methods. Rats implanted with bilateral HPC electrodes had AD thresholds determined using methods A and B (in that order). Threshold level ADs produced under method B were longer in the HPC contralateral (contra) than in the HPC ipsilateral (ipsi) to the stimulated side. No such variations in the ratio of ipsi to contra AD durations were observed using method A. Furthermore, similar differences in the ratio of ipsi to contra AD durations were observed when ADs were induced with stimuli at 115% and 400% of AD threshold when thresholds were determined by method B, prior to the initiation of method A procedures. Thus the ipsi/contra AD duration ratios are not related to the tendency of method B to produce slightly lower AD thresholds, or to the order in which the methods are employed.

It has been shown that stimuli below the threshold for motor seizure (MS) production but above AD threshold produce alterations in MS thresholds (kindling). The present data indicate that stimuli below AD threshold alter the properties of subsequently elicited ADs.

43.5 THALAMOCORTICAL CONNECTIONS OF CINGULATE CORTEX AND THE PRESUBICULUM IN THE MONKEY. Douglas L. Rosene, Brent A. Vogt and Deepak N. Pandya. Dept. of Anatomy, Boston University School of Medicine, Boston, MA 02118 and Veterans Administration Medical Center, Bedford,MA 01730.

Injections of horseradish peroxidase into the cingulate cortex or the parahippocampal gyrus have demonstrated the major thalamic nuclei which send afferents to these limbic cortical areas. As previously reported (Sci. 204: 205, 1979), injections in the anterior cingulate cortex (area 24) result in labelling of neurons in the midline and intralaminar thalamic nuclei as well as some labelling in the medial dorsal nucleus, but injections in the posterior cingulate cortex (areas 23 and 29) result mainly in labelling of the anterior thalamic nuclei and some labelling in the medial dorsal and lateral dorsal nuclei. In addition, HRP injections into either the hippocampus or the parahippocampal gyrus that involve the presubiculum resulted in retrograde labelling of neurons in the lateral dorsal thalamic nucleus.

While these HRP results establish a definite topographic pattern of thalamocortical projections to these limbic areas, the exact distribution of thalamocortical termination in these areas can only be determined by examining the anterograde termination of these afferents. In order to accomplish this, injections of radioactively labelled amino acids (RLAA) have been placed in some of these nuclei. An injection involving both the midline nuclei and the anteromedial nucleus resulted in termination in areas 24a, 24b, 23a and 23b on the medial surface of the cingulate gyrus. An injection limited to the anteroventral nucleus without any involvement of the midline or intralaminar nuclei produced anterograde termination in area 29b of the retrosplenial granular cortex, but not in areas 23 or 24. In contrast, an injection into the medial dorsal thalamic nucleus resulted in anterograde termination in areas 24c and 23c within the depths of the cingulate sulcus. Finally, an injection into the lateral dorsal thalamic nucleus resulted in heavy termination throughout the presubiculum as well as significant termination in the adjacent area 29a, the part of granular retrosplenial cortex which extends below the corpus callosum. In this case there was no evidence of termination in area 23 or any other subdivision of area 29. These findings clearly demonstrate that the thalamocortical connections to the limbic cortex in the rhesus monkey are not homogeneous but display a topographic specificity.

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43.6 MULTIPLE NEUROTRANSMITTER STUDIES IN THE ISLANDS OF CALLEJA COMPLEX OF THE BASAL FOREBRAIN: I. LIGHT MICROSCOPY. S. Isaacs*, J.H. Fallon and C.E. Ribak. (SPON: M. Poo)Department of Anatomy, University of California, School of Medicine, Irvine, CA 92717.

A series of combined light and electron microscopic studies in the basal forebrain has again directed our attention to the eight intriguing clusters of granule (7-9μm) cells in the deep olfactory tubercle called the Islands of Calleja. Our previous studies (J. Comp. Neur. 181: 375-396, 1978) examined the anatomy and some connections of the cells with nearby allocortical structures. We have extended these studies of the small cells and associated larger cells, defined here collectively as the Islands of Calleja Complex (ICC), to experiments aimed at the localization and interrelationships of neurotransmitters within the ICC of the albino rat. At the light microscopic level, acetylcholinesterase (AChE) staining of cell bodies (DFP pretreatment technique) reveals that the large cells located within the core of an ICC, or immediately adjacent to an ICC, stain intensely for AChE. Immunohistochemical staining for GAD indicates a very strong staining in the ICC region, especially in the core and neuropil surrounding an ICC. It appears as if GABA terminals are contacting large somatic and dendritic processes, perhaps of the large AChE cells. Dopamine fibers (DA), originating in the SN-VTA, are also present within the core and neuropil adjacent to an ICC. This intense and multiple neurotransmitter-specific staining suggests a strong relationship between the ACh, GABA and DA systems in the ICC of the basal forebrain. A second abstract in this volume will detail some of the features of the ICC using electron microscopic techniques. A third abstract will focus on some of the connections of the large cells in the ICC and will also attempt to correlate the neurotransmitter histochemistry and pathways that are associated with the Islands of Calleja Complex of the basal forebrain.

Supported by NIH grants NS 15321, NS 16017 and NS 15669.

43.7 MULTIPLE NEUROTRANSMITTER STUDIES IN THE ISLANDS OF CALLEJA COMPLEX OF THE BASAL FOREBRAIN. II ELECTRON MICROSCOPY. L. Richman*, C.E. Ribak, S. Isaacs*, C.R. Houser and J.H. Fallon (SPON: R.A. Giolli) Dept. of Anatomy, Univ. of Calif., Irvine, CA 92717 and Div. of Neurosci., City of Hope Res. Ctr., Duarte, CA 91010

An initial analysis of the cells and their processes within the Islands of Calleja Complex (ICC) was first made in normal ultrastructural preparations to determine the synaptic relationships of the various neurotransmitter elements found within this brain region. As indicated by our previous light microscopic description, the ICC consists of two cell types, small granule cells and large multipolar cells. In electron microscopic preparations, the somata of granule cells are grouped together, and each is apposed to one another. Specialized junctions (4-6nm wide) which occur at these sites of somal apposition suggest ephaptic coupling of granule cells. The granule cell somata have nuclei that contain clumps of heterochromatin adjacent to smooth nuclear envelopes. The perikaryal cytoplasm of these cells consists of a relatively thin rim containing mitochondria, microtubules, cisternae of Golgi complex and many free ribosomes. Spinous dendrites of small diameter are occasionally found in continuity with these cells. Axon terminals rarely form synapses with the somata of granule cells, but are more frequently found to synapse on their dendrites and dendritic spines.

The somata of the large cells are found either within the core or around the perimeter of an ICC. The large cells have infolded nuclei and an abundant perikaryal cytoplasm that contains many cisternae of granular endoplasmic reticulum and Golgi complex, relatively few free ribosomes, many mitochondria, and occasional microtubules and neurofilaments. Large diameter dendritic shafts emanate in many directions from these somata. Axon terminals cover nearly the entire surface of these somata and dendrites where they commonly form symmetric synaptic junctions. An immunocytochemical localization of glutamic acid decarboxylase in the ICC shows specific reaction product for GABA within many of these axon terminals.

In sections of the ICC from rats injected with DFP, acetylcholinesterase was localized using a modification of standard histochemical techniques. The reaction product that is specific for this enzyme was located in only the large cells within the inner surfaces of their cisternae of granular endoplasmic reticulum and Golgi complex, and within their nuclear envelopes. In these preparations, granule cells lacked this specific reaction product. These results indicate that the large cells of the ICC may use acetylcholine as a neurotransmitter. The synaptic pattern of axon terminals with these cells suggests a strong GABAergic-cholinergic interaction in this part of the basal forebrain. (Support from NIH Grants NS 15669, NS 12116, NS 15321, and NS 16017.)

43.8 MULTIPLE NEUROTRANSMITTER STUDIES IN THE ISLANDS OF CALLEJA COMPLEX OF THE BASAL FOREBRAIN: III. Connections, Correlations and Reservations (C.C.+ R's). J.H. Fallon and C.E. Ribak. Department of Anatomy, University of California, Irvine, CA 92717

This is the third abstract in a series on the Islands of Calleja Complex (ICC) in the basal forebrain of the albino rat. In this study we attempted to determine some connections of the ICC, especially of the large AChE-positive cells within the core and the surrounding neuropil. Injections of HRP were made into various forebrain structures, including the ICC, and retrograde and anterograde transport of HRP was traced with the TMB technique. Fluorescent retrograde tracers, such as Nuclear Yellow and True Blue, were also used. The efferents of the large cells of the ICC were found to project to the lateral and supramammillary region of the hypothalamus, including the nuclei gemini, and the midline and adjacent dorsomedial nuclei of the thalamus. Labeled fibers were also observed in the ipsilateral and contralateral stria medullaris. Reciprocal projections were observed from these same hypothalamic and thalamic regions. Additional afferent connections from the medial septum, amygdala, piriform cortex and periamygdaloid cortex confirm previous studies on connections of the ICC (Fallon et al, J.Comp. Neur. 181: 375-396, 1978). We had suggested earlier that the ICC may be involved in the central neural control of reproduction. To explore this possibility further, we used the ^{3}H-estradiol binding technique in conjunction with the AChE-technique. The results clearly show that the large AChE-positive cells in the ICC form part of the basal forebrain system of ^{3}H-estradiol-binding cells. Together with a recent report (Phillips et al, Anat Rec. 196: 148A, 1980)of LHRH positive fibers projecting to the ICC, these results make a strong case for the participation of this brain region in reproductive function. In addition, the high density of GABA-ergic terminals on AChE-positive cells in the ICC are reminiscent of the pattern observed in the globus pallidus. The afferent connections from surrounding allocortical-associated ventral striatal structures, and efferents to the diencephalon generally support the notion that the ICC environs form a ventral-pallidal component of the basal forebrain. In this sense, topographical limbic cortico-striatal-pallidal-subcortical loops could be surmised (e.g.,Superficial piriform-olfactory-amygdaloid-septal "cortex"→Deep piriform-olfactory-amygdaloid-septal "striatum"→ICC "pallidum"→Lateral mammillary-supramammillary area (→hippocampus) + medial-midline thalamus (→ventral striatal structures). These loops would, therefore, be parallel to analagous loops defined for the dorsal forebrain. One functional correlate of this segment of ventral pallidum (ICC) and its limbic and olfactory inputs appears to be the neural control of reproduction. (Supported by NIH grants NS 15321, NS 16017 and NS 15669.)

44.1 CATEGORIES OF AXONS IN BLADDER NERVES. C.E. Hulsebosch and R.E. Coggeshall. Depts. of Anatomy and of Physiology and Biophysics and the Marine Biomedical Institute. The University of Texas Medical Branch, Galveston, Texas 77550.

There are three main nerves which innervate the bladder: the pelvic nerve (parasympathetic), the hypogastric nerve (sympathetic) and the pudendal nerve (somatic). The number and types of fibers in these nerves are obviously important in considerations of bladder function. Previous light microscopic studies counted the number of myelinated axons (MY) but unmyelinated axons (UN) could not be resolved by these techniques. The present ultrastructural study determined the number of unmyelinated axons as well as myelinated axons. The number of axons in these bladder nerves are shown in the following table:

	PUDENDAL NERVE		PELVIC NERVE		HYPOGASTRIC NERVE	
	MY	UN	MY	UN	MY	UN
1	1682	3845	913	3758	152	940
2	1664	4660	994	4678	171	1105
3	2306	4458	1177	5641	245	1476
4	2348	4087	1297	6343	271	1221
5	1580	3298	843	2667	437	1763
6	1534	3755	924	3647	433	1991
7	1430	2974	860	3346	117	1295
8	1275	3506	1072	4359	114	1092
9	1836	3500	821	3462	163	1339
10	--	--	886	3440	74	1366
Mean	1737	3786	979	4134	250	1513

Note that the hypogastric nerve has the fewest number of axons and that the pelvic and pudendal nerves have approximately equal numbers of axons. Also note that there are three times as many unmyelinated axons as myelinated axons in these nerves.

Selective surgical procedures to determine the origin of the various fibers which comprise these nerves are underway. The results of these experiments will be discussed. Supported by grants NS 06246, Ns 10161, NS 11255 and NS 07377.

44.2 RETROGRADE TRANSPORT OF HRP BY PERIVASCULAR AFFERENTS AND EFFERENTS AROUND CEREBRAL ARTERIES. M.A. Moskowitz, M. Mayberg* and R.S. Langer*. Laboratory of Neural and Endocrine Regulation, MIT, Cambridge, MA 02139; Department of Neurosurgery, Mass. General Hospital, Boston, MA 02114; Section of Neurology, Peter Bent Brigham Hospital, Harvard Medical School, Boston, MA 02115.

The proximal segments of large cerebral arteries are one of the few structures within the cranium which when stimulated give rise to the sensation of pain. Afferent nerve fibers which convey this information have not been identified, although the area to which pain is referred (e.g., the forehead) implicates the trigeminal nerve. We have recently become interested in the existence of perivascular pathways which arise from the trigeminal system as a possible neuroanatomical explanation for the sharply circumscribed unilateral head pains associated with certain vascular syndromes. We have therefore initiated neuroanatomical studies in cats to identify the existence of perivascular nerve endings and to localize their cell bodies within trigeminal and other ganglia.

HRP was applied to the middle cerebral artery in seven cats. To prevent the spread of peroxidase from around the blood vessel, HRP was impregnated into a polymer and applied as a viscous solution to the proximal segment of the cat middle cerebral artery. The treated polymer was coated and surrounded by either untreated polymer, or a second polymer. Spread of the marker enzyme was limited to less than 7 mm lateral to the vessel and 5 mm beneath the adjacent cerebral cortex. (In vitro experiments have established that 70% of the protein was liberated from impregnated polymer within 24 hours and that during this period, there was no decrease in the specific activity of the peroxidase enzyme.) After 72 hours, animals were perfused with 2.5% glutaraldehyde in 0.1 M phosphate buffer. Both trigeminal ganglia were serially sectioned (30 µM) and prepared for HRP histochemistry using the chromogen, tetramethyl benzidine. In all seven cats, peroxidase-containing cells were found in the ipsilateral trigeminal ganglia. Cell bodies containing HRP were found among clusters of cells that project via the supraorbital nerve to innervate the forehead. Peroxidase-containing cell bodies were also found in the ipsilateral superior cervical ganglia.

The above studies provide useful information about perivascular afferent and efferent neurons within sensory and autonomic ganglia. The existence of perivascular sensory pathways provides an important explanation for the hemicranial distribution of headaches associated with strokes and migraine.

44.3 SYMPATHETIC INNERVATION OF MURINE THYMUS: RELATION OF ADRENERGIC FIBERS TO MAST CELLS. J.M. Williams* and D.L. Felten (SPON: P.A. Shea). Dept. of Anatomy, Indiana Univ. School of Med., Indianapolis, IN 46223

Sympathetic innervation of vascular and parenchymal elements in the normal murine thymus has been implicated in the modulation of lymphocyte development and activity (Williams, J.M. and D.L. Felten, Anat. Rec. 196:205A, 1980). This study was undertaken to further examine the relationship of sympathetic fibers to cellular elements of the murine thymus. Autofluorescent yellow, granular mononuclear cells were observed in the C3H mouse thymus. These cells demonstrated several characteristics of mast cells, including metachromasia with toluidine blue staining and fluorescence spectra characteristic of mast cells. These fluorescent cells were found adjacent to noradrenergic fiber plexuses in perivascular and subcapsular locations, and frequently clustered around the terminal arrays of noradrenergic varicosities in the thymic parenchyma. Some single mast cells were also scattered within the thymic parenchyma. NZB mice, which characteristically develop spontaneous hemolytic anemia, exhibited a greater number of varicosities and linear profiles of noradrenergic fibers in the thymic parenchyma, and also showed a many-fold increase in the number of mast cells associated with these fibers compared with the control C3H mice. Catecholamines have been shown to inhibit mast cell degranulation, and histamine has been shown to have an in vitro role in both lymphocyte differentiation and the activity of T-cell subpopulations. The noradrenergic fibers and their associated mast cells, described in the present study, may represent the substrate for a modulatory role of sympathetic nerve fibers over lymphocyte development and responsiveness, either directly through the release of norepinephrine within lymphoid organs, or indirectly by alteration of histamine release from mast cells. The increased number of noradrenergic varicosities and mast cells within the NZB murine thymus may be a part of the abnormal thymic microenvironment which has been proposed as a causative factor in their spontaneous autoimmunity. Supported by an Alfred P. Sloan Foundation Fellowship (D.L.F.).

44.4 THE SEGMENTAL ORIGIN OF SYMPATHETIC PREGANGLIONIC AXONS IN THE UPPER THORACIC SPINAL CORD OF THE CAT. B.J. Oldfield* and Elspeth M. McLachlan*, (SPON: L.P. Renaud) Neuropharmacology Group, Dept. Physiol. Monash Univ. 3168 Australia.

It has been conventionally thought that the axons of sympathetic preganglionic neurons pass from the spinal cord via the ventral roots of the segment in which their cells of origin lie. However, recent anatomical evidence has been interpreted as indicating that preganglionic axons may follow an intraspinal pathway traversing several segments before entering a ventral root. (Faden, A.I. and Petras, J.M., Brain Res., 144:358, 1978; Chung, K. et al., J. Comp. Neurol., 185:23, 1979). We have investigated this problem by applying horseradish peroxidase (HRP) to the cut ends of individual white rami.

In separate experiments on adult cats (either sex, 2.6-3.5 kg) HRP was applied selectively to the cut end of the T_1, T_2 or T_3 white ramus. HRP was completely isolated from the surrounding tissue by either enclosing the sectioned end of the ramus within a perspex bath (T_1 and T_2) or within a length of polyvinyl chloride tubing (T_3). Generally the tissue underlying the application, including the cut end of the descending sympathetic chain was further protected with a thin sheet of plastic film. After 24-28 hrs the animals were perfused with a buffered aldehyde solution and sections of spinal segments C_8 to T_5 (or T_7 in one case) were incubated using either diaminobenzidine or tetramethylbenzidine for the demonstration of reaction product.

In all cases HRP was localised within ipsilateral sympathetic preganglionic neurons. These were restricted in their longitudinal extent to the length of only one spinal segment adjacent to the treated ramus; however, the rostral and caudal limits of this population of labelled cells did not correspond exactly to the boundaries of the segment as defined by the position of dorsal rootlets. All axons in the T_1 ramus which were labelled were derived from cells in the rostral 2/3 of T_1 but cells containing HRP after T_2 or T_3 application were always found in the rostral 2/3 of the segment corresponding to the treated ramus and in the 1/3 of the segment immediately rostral to it. Labelled neurons were found within each of the autonomic subdivisions in the thoracic spinal cord. Neurons projecting axons in the T_1 ramus were widely distributed between the lateral funiculus and the medial autonomic nuclei, whereas the majority of neurons (74-98% in different experiments) with axons in the T_2 and T_3 rami were restricted to the principal part of the intermediolateral column. These data are therefore not consistent with the concept of an intraspinal pathway for axons leaving the spinal cord in the upper thoracic rami but rather suggest that these preganglionic axons are segmentally derived. (Supported by grants to E. McL. from the NHMRC and NHF of Australia).

44.5 LOCALIZATION OF CELL BODIES OF ORIGIN OF THE CERVICAL AND SUBDIAPHRAGMATIC PORTIONS OF THE VAGUS NERVE. S.J. Dennison*, B.L. O'Connor*, M.H. Aprison, V.E. Merritt* and D.L. Felten (SPON: C. Loullis). Depts. of Anatomy and Biochemistry, and the Inst. of Psychiatric Research, Indiana Univ. Sch. of Medicine, Indianapolis, IN 46223.

Efferents of the cervical and subdiaphragmatic branches of the vagus nerve were traced to their cell bodies of origin in the brain stem utilizing the retrograde horseradish peroxidase (HRP) transport method. Cell bodies in these two areas occupy a continuous longitudinal, spindle-shaped column, thinner caudally than rostrally. This column was present rostral to the classically described dorsal motor nucleus of the vagus (DNV), was present throughout the DNV itself, extended caudally into the nucleus commissuralis, where it became attenuated, and further extended into the spinal cord in the midline and paramedian portion of the dorsal commissural gray of the upper six segments of cervical spinal cord. This entire longitudinal cell column is called the DNV cell column, and extends both rostrally and caudally beyond the boundaries of the classically described DNV. In addition, cell bodies contributing axons to the cervical vagus, but not the subdiaphragmatic vagus, are also found in the nucleus ambiguus.

Cells of the cervical vagus were found diffusely throughout the ipsilateral DNV and nucleus ambiguus. Cells of the subdiaphragmatic portion of the vagus established a viscerotopic localization, and were found in the lateral-most portion of the rostral region, the anterolateral portion of the middle region, and the anterior one-half of the caudal region of the clasically described DNV. Fibers from the anterior subdiaphragmatic vagus had their cell bodies located primarily in the left DNV cell column, while the posterior subdiaphragmatic vagus derived from bilaterally situated cells, with 60% of the cells found in the right column and 40% in the left column. These findings suggest that the subdiaphragmatic vagus derives from cells of the DNV which are viscerotopically organized, and further suggest that the cells of origin of the vagus nerve are more extensive both rostrally and caudally than has previously been reported.

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44.6 THE CENTRAL DISTRIBUTION WITHIN LISSAUER'S TRACT AND THE SPINAL GRAY MATTER OF PRIMARY AFFERENTS FROM THE PELVIC NERVE AND PUDENDAL NERVE OF THE CAT DEMONSTRATED BY TRANSGANGLIONIC TRANSPORT OF HRP. Charles Morgan, William C. de Groat, Irving Nadelhaft. Dept. Pharmacol., Univ. of Pittsburgh Sch. of Med. and V.A. Hospital, Pittsburgh, PA 15261

Previous studies identified a prominent visceral primary afferent (PA) projection in the cat sacral spinal cord in Lamina I on the lateral side of the dorsal horn (DH) extending into the sacral parasympathetic nucleus (SPN). Present experiments show a substantial medial pathway and an extensive intersegmental distribution of sacral PA.

HRP was applied to the central cut ends of the pelvic n. or its branches to colon or bladder and to the pudendal n. HRP in central PA axons was demonstrated with benzidine or TMB processing. After pelvic n. labelling HRP reaction product (RP) was detected in Lissauer's tract (Ltr) from L_5 to C_{X2}. The heaviest RP was found in $S_{1,2,3}$ from the dorsal root entry to the lateral edge of the DH but considerable RP was also present in medial Ltr. In lumbar and coccygeal segments RP occupied a decreasing area in mid-lateral Ltr. Smaller amounts of RP were noted in adjacent dorsal columns and dorsolateral funiculus.

In S_2, axons from Ltr entered the dorsal surface of Lamina I extending medially and laterally around the DH into Laminae V, VI, VII & X. The prominent lateral projection was traced into lateral Lamina V (the dorsal band and an interneuronal area of the SPN) and divided into 4 groups: one reached the lower 1/3 of the dorsal commissure; a second continued to the intermediomedial nucleus; a third turned dorsad to the lateral part of the internal basal nucleus (IBN); the fourth entered Lamina VII (lateral band of the SPN). From the medial DH axons reached three areas: the ipsi-and contralateral IBN (upper 2/3 of the dorsal commissure) and the lateral IBN. Rostral and caudal to the SPN PA decreased in frequency but had many of these same patterns. The other nerves labelled could not be traced so extensively. Colon and bladder n. contribute to medial and lateral Ltr while pudendal n. tends to the medial Ltr. All three enter the gray on both sides of the DH but bladder PA are stronger on the lateral side, pudendal PA are stronger medially.

In conclusion it appears that the central projections of sacral PA exhibit a striking correspondence with the soma and dendritic distribution of preganglionic neurons and interneurons in the SPN. Sacral visceral PA also send projections via LTr to lumbar and coccygeal segments of the cord. These projections may be involved in the coordination of somatic mechanisms during defecation and micturition.

44.7 ANATOMIC EVIDENCE OF RECIPROCAL CONNECTIONS BETWEEN THE AMBIGUUS NUCLEI OF THE CAT. John T. Paul, Betty L. Hamilton and Richard A. Gillis. Depts. of Anatomy and Pharmacology, Georgetown Univ. Schs. of Med. & Dent., Washington, D.C. 20007.

Numerous studies have demonstrated that the nucleus ambiguus (NA) is an important central nervous system site for the control of a cardio-inhibitory mechanism. The data from Gunn et al. (1968) and Thomas and Calaresu (1974) suggested that in addition to its projections via the ipsilateral vagus nerve, NA projected decussating fibers to the contralateral NA and/or vagus nerve. These studies were based solely on electrophysiologic evidence, but our laboratory (Hamilton et al. 1979) demonstrated these crossing fibers using silver degeneration techniques following kainic acid lesions of NA.

By employing the horseradish peroxidase method we have now verified the presence of these reciprocal fibers between the two ambiguus nuclei in the brainstem of the cat. HRP was injected into the rostral pole of NA in three cats, and labeled soma were observed in the contralateral NA of each animal. Injections in more caudal portions of NA, to date, have failed to demonstrate these reciprocal connections, although this area is very active in cardiac control. This apparent difference in the rostral and caudal NA projection systems and the data from Ciriello and Calaresu's (1980) study suggesting a ventrolateral-dorsomedial organization of the NA indicate that this is a complex nuclear area that will require careful and systematic study in terms of physiological, biochemical, and anatomical mapping.

44.8 CONNECTIONS OF VISCERAL AFFERENT CORTEX IN THE RAT. C.B. Saper and A.D. Loewy. Dept. of Neurology, New York Hospital-Cornell Medical Center, New York, NY 10021 and Dept. of Anatomy/Neurobiology, Washington University School of Med., St. Louis, MO 63110.

We have recently delineated a system of highly interconnected cell groups in the mammalian brainstem and basal forebrain, which appear to play an important role in central autonomic control (Saper and Loewy, Efferent connections of the parabrachial nucleus in the rat, Brain Research, in press) and identified a direct projection from the medial parabrachial nucleus to the granular insular cortex, a region which has been found to receive taste and other visceral afferent information. We now report the results of a horseradish peroxidase (HRP) study of the afferents and efferents of the granular insular cortex.

Small (20-30 nl) injections of a 10% solution of HRP were placed in the granular insular cortex in adult albino rats. The brains were processed by standard histochemical methods using tetramethylbenzidine as a chromogen. Retrogradely labeled neurons were seen in layers III and V of the contralateral homologous cortex, in the lateral and basolateral amygdaloid nuclei and in the lateral hypothalamic area and zona incerta. In the thalamus retrogradely labeled neurons were seen in the reticular, ventroanterior, ventrobasal, ventromedial basal (VPMpc), reuniens, centromedial, paracentral, centrolateral and parafascicular nuclei. In the pons a collection of labeled neurons was seen in the ventromedial part of the parabrachial nucleus bilaterally. Retrograde labeling in the subthalamic nucleus, the substantia nigra and the pedunculopontine nucleus seemed to depend primarily upon the extent of spread of HRP from the injection site into the underlying caudate-putamen.

Anterograde labeling could also be traced in these experiments but the apparent transport of HRP into collaterals of axons with terminals at the injection site makes interpretation somewhat hazardous. Nevertheless, there appeared to be anterograde transport to layers II, III and V of the contralateral homologous cortex and to each of thalamic cell groups which contained retrograde labeling, as well as in the medial part of the dorsomedial nucleus. Anterograde labeling was also seen ipsilaterally in the central amygdaloid nucleus, the lateral hypothalamic area, the zona incerta, the medial part of the parabrachial nucleus and, on the contralateral side of the brain in the dorsomedial spinal trigeminal nucleus and adjacent lateral and anteromedial parts of the nucleus of the solitary tract.

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45.1 CIRCADIAN RHYTHMS OF RETINAL SEROTONIN N-ACETYLTRANSFERASE ACTIVITY AND MELATONIN CONTENT IN THE CHICKEN. H. E. Hamm* and M. Menaker, Zoology Dept. Univ. of Texas, Austin Tx 78712.

The chicken retina appears to be another active site, with the pineal gland, of melatonin synthesis. There is a large amplitude circadian rhythm of serotonin N-acetyltransferase (NAT) activity and radioimmunoassayable (RIA) melatonin content in the chicken retina-pigment epithelium, with 15-fold higher levels at night than during the day in LD12:12. The rhythm persists in constant darkness (DD); there is a 4-fold increase in NAT activity and and RIA melatonin during the subjective night after 2 days in DD. NAT activity and melatonin levels are sensitive to light; one hour of light (1400-1500 lux) given at midnight causes NAT activity and melatonin to fall to daytime levels ($t_{1/2}$ = 10 min for NAT activity).

The characteristics of retinal indoleamine metabolism are similar under several conditions to those of the pineal gland. The pineal is not responsible for controlling these rhythms, since the circadian rhythm of NAT activity in the retina persists in pinealectomized chicks. Localization studies show that rhythmic NAT activity and RIA melatonin are found predominantly in the retina; very low levels were found in the pigment epithelium.

To investigate whether retinal melatonin contributes to circulating melatonin levels, serum melatonin levels were measured at mid-light and mid-dark in intact, sham pinealectomized, pinealectomized (pin-x), blinded, or blind-pinealectomized chicks. There is a 2.5 to 3 fold day-night difference in serum melatonin levels in intact, sham-pin-x, and blinded chicks ($p<.01$). In pin-x and blind-pin-x chicks, the day-night change is lost (pin-x, 1.25-fold, $p>.05$; blind-pin-x, 1.1-fold, $p>.05$); however, daytime levels ($\sim$0.5 ng RIA melatonin/ml serum) are still present in both groups. Thus it appears that retinal melatonin is not a major contributor to the circulating melatonin rhythm; rather, it may play a local role. Because of the circadian rhythm in retinal melatonin, and since melatonin is known to affect circadian parameters in some systems, it could function as a circadian signal to regulate and synchronize the many functions in the retina, and particularly in the photoreceptors, that show rhythmic fluctuations.

45.2 CIRCADIAN RHYTHM OF NEUROSECRETORY ACTIVITY IN THE ISOLATED EYESTALK OF CRAYFISH. Hugo Aréchiga, Teresa de la Vega* and Victor Anaya. Dept. Fisiol. Ctr. Estud. Avanzados, I.P.N. México 14, D.F.

The eyestalk of crustaceans is known to produce several neuropeptides and to participate in the regulation of circadian rhythmicity. However, its role as an endogenous oscillator has been a matter of controversy. With the aim of deciding whether it is capable of sustaining a circadian rhythm of neurosecretion, the activity of neurodepressing hormone (NDH) (Aréchiga, H. Cabrera-Peralta, C. and Huberman, A. J. Neurobiol. 10: 409, 1979) was determined at different times in isolated eyestalks maintained in organ culture for several days. NDH activity was quantified by determining the reduction in firing rate of isolated abdominal stretch receptors, kept under constant tension. The eyestalks were incubated in culture medium (MEM, Gibco) at constant temperature (17°C) and NDH was obtained by extracting in chloroform and acetone and then successively passing through G-25 and G-15 Sephadex columns (see Huberman, A. et al. Europ. J. Biochem. 99: 203, 1979). Four groups of eyestalks were studied. a) eyestalks excised from animals entrained to a normal L:D cycle, and cultured in continuous darkness. b) eyestalks from animals in normal L:D cycle and cultured in continuous illumination. c) eyestalks from animals kept under a reversed L:D cycle for one week and cultured in continuous darkness, and d) eyestalks from animals kept in reversed L:D cycle for one week and then cultured in continuous light. In groups a) and b), NDH activity fluctuates in a circadian manner from 1-5 units during the subjective night, to 30-40 units during the subjective day. The rhythmicity was followed for up to one week without dampening. In groups c) and d), NDH cycle is reversed, being the secretion higher at night than at day time. In eyestalks excised from animals kept in either normal or reversed L:D cycles, and entrained "in vitro" to a different light schedule, a corresponding phase-shift was obtained. These results suggest that the eyestalk is capable of sustaining an endogenous, circadian rhythm of neurosecretion, and that it contains the photoreceptive mechanism for entrainment.

45.3 ROLE OF THE SUPRACHIASMATIC NUCLEI (SCN) IN THE SHORT-DAY-INDUCED ATTENUATED CASTRATION RESPONSE. F. Turek, S. Losee*and G. Ellis*. Dept. of Biol. Sci., Northwestern Univ., Evanston, IL 60201.

Castration of hamsters that have regressed testes due to their maintenance on short days (e.g., LD 6:18) results in only a slight increase in serum gonadotropins. If castrated hamsters are transferred from short to long days (e.g., LD 14:10), serum gonadotropin levels increase to normal high post-castration values. The neural components involved in mediating the effects of light on this steroid-independent response to the photoperiod have not been identified, and in the present investigation we examined the potential role of the SCN in this response.

Ten-week-old hamsters that had been maintained on LD 14:10 since birth were transferred to LD 6:18. Nine weeks later blood was collected via cardiac puncture under light ether anesthesia from a subsample of 10 animals. The animals were then castrated and divided into 4 treatment groups (11-16 animals/group): 1) Sham- or non-lesioned and maintained on LD 6:18, 2) Sham- or non-lesioned and immediately transferred to LD 14:10, 3) SCN-lesioned and maintained on LD 6:18, 4) SCN-lesioned and immediately transferred to LD 14:10. Blood was collected from each animal on days 3, 10, 20, and 35 after surgery. Prior to castration serum follicle stimulating hormone (FSH) levels were 100 ± 21 ng/ml. Mean (±SE) serum FSH levels (ng/ml) over the 35-day period following castration are shown below.

		Days			
Lesion	Photoperiod	3	10	20	35
None	6L → 6L	451±130	177± 17	199± 22	386±103
None	6L → 14L	508± 94	856±121	2490±195	2748±225
SCN	6L → 6L	1006±212	1061±201	1823±228	2303±349
SCN	6L → 14L	857±134	989±250	1749±253	2341±277

Non-lesioned animals that were castrated and maintained on LD 6:18 showed the attenuated castration response and mean serum FSH levels never rose above 500 ng/ml. In contrast, transfer to LD 14:10 induced an increase in serum FSH levels in the non-lesioned animals. Similarly, lesioning of the SCN resulted in a rapid increase in serum FSH levels, and this response appeared to be independent of the photoperiod. On days 10, 20 and 35, serum FSH titres in the non-lesioned animals transferred to LD 14:10 and in the SCN-lesioned animals exposed to either photoperiod were similar and significantly greater ($p < 0.01$) than those observed in the non-lesioned animals maintained on LD 6:18. These results demonstrate that ablation of the SCN abolishes the short-day-induced attenuated castration response, suggesting that the SCN mediate the steroid-independent effect of short photoperiods on pituitary gonadotropin release. (Supported by USPHS Grants HD-12622, HD-09885, and HD-00249.)

45.4 PINEAL MELATONIN RHYTHMS IN THE SYRIAN HAMSTER MAINTAINED IN ENVIRONMENTAL AND LABORATORY CONDITIONS. G.C. Brainard*, L.J. Petterborg*, and R.J. Reiter. Department of Anatomy, University of Texas Health Science Center, San Antonio, TX 78284.

The objective of the following study was to compare the pineal melatonin rhythms of hamsters maintained in outdoor versus laboratory conditions. One hundred and forty-four adult male hamsters were employed in this experiment. On November 29, half the animals were caged under controlled laboratory conditions: LD cycle of 10:14 (lights on 0700 hours), 22°C, and 50% humidity. The remainder of the animals were maintained under outdoor conditions in a well ventilated, three-sided shelter open to the southeast. At this time of year, the photoperiod is short (approximating LD 10:14) and is decreasing in length. During the experiment, mean sunrise time was 0700 hours and mean sunset time was 1612 hours. Though protected from the rain, these animals were exposed to natural temperature fluctuations ranging from -4°C to 27°C, and humidity changes from 15% to 100%. Animals in both groups were kept 5 per cage and given food and water ad libitum. On December 20th and 21st, eight animals from each group were sacrificed at the following times: 0800, 1200, 1700, 2000, 2200, 2400, 0200, 0400 and 0600 hours. Pineal glands were immediately dissected out, placed in a 1 ml of phosphate buffered saline, disrupted by sonication and stored at 4°C until ready for assay. Radioimmunoassay (Rollag and Niswender, Endocr. 98:482, 1978) was used to determine melatonin content of pineal glands. The significance of the results was assessed by analysis of variance and Student's t test.

Both groups of animals displayed a distinct circadian pattern of pineal melatonin content. Peak nighttime levels of melatonin (between 0200 and 0600 hours) were five to ten fold greater than daytime levels in both groups of animals. At 0200 and 0600 hours, the pineal melatonin content of animals maintained outdoors was higher than that of animals kept in the laboratory $P<.05$ and $P<.005$, respectively.

Two implications may be discerned from the above results. First, animals maintained outdoors witness a graded and changing photoperiod as well as dramatic changes in temperature and humidity. These and/or other factors present in the winter environment but not in the laboratory appear to cause the pineal to produce more melatonin. Second, caution should be used in generalizing results produced in the laboratory to animals living in the wild.

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45.5 INHIBITION OF MELATONIN-INDUCED GONADAL ATROPHY BY TWICE DAILY MELATONIN INJECTIONS. P. K. Rudeen, S. K. Symmes* and W. O. McKinley*. Department of Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

A daily injection of melatonin for 9 weeks (25 µg) will induce gonadal atrophy in golden hamsters, if the injection is given within a three-hour period prior to the onset of darkness when animals are maintained in a long photoperiod. A subcutaneous implant of melatonin (1 mg) will block the antigonadal effects of afternoon injections of melatonin. It is unknown if the inhibitory effects of continual melatonin administration is mediated due to the constant presence of melatonin. Eight groups of adult hamsters were given two daily subcutaneous injections. Hamsters received either ethanolic saline, or melatonin dissolved in ethanolic saline (25 µg) late in the photoperiod, combined with a subcutaneous injection of ethanolic saline, or various concentrations of melatonin (1 µg, 5 µg, 25 µg, 100 µg or 500 µg) early in the photoperiod. Animals were treated 6 days per week for 9 weeks after which testicular and accessory sex organ weights were measured. Hypothalamic LHRH content, serum LH and serum testosterone were measured by RIA. Severe testicular and accessory sex organ atrophy occurred in animals subjected to morning injections of either ethanolic saline, 1 µg or 5 µg of melatonin combined with late afternoon injections of melatonin. Serum LH levels and serum testosterone levels were lowered in these animals when compared with animals that received saline in the morning and afternoon. There was a tendency for hypothalamic LHRH content to be increased in the animals subjected to low doses of melatonin in the morning combined with melatonin given in the afternoon when compared with saline injected animals. Injections of 25 µg or 100 µg of melatonin in the morning partially blocked the effects of the afternoon melatonin injection, whereas a 500 µg injection of melatonin was capable of completely blocking the effects of the afternoon injection of melatonin. The results are consistent with the hypothesis that melatonin present at times other than a "sensitive" period will result in a "down regulation" of melatonin receptors inhibiting gonad atrophy when melatonin is presented during the sensitive phase. Inhibition of gonadal atrophy by a single injection of melatonin at another period of time during the day suggests that melatonin may not need to be continuously present to inhibit melatonin-induced gonadal atrophy.

45.6 CONTRIBUTION OF WATER AND ANGIOTENSIN TO CIRCADIAN CHANGES IN VENTRICULAR WEIGHT TO BODY WEIGHT RATIO IN RATS. Maurice S. Holder*, Estrellita Howard*, Willie Pennick* and Charles A. Walker. Florida A&M University, School of Pharmacy, Tallahassee, FL 32307.

Ventricular hypertrophy in rats have been assessed by ventricular weight to body weight ratios (VW/BW). These measurements are generally taken at different times in the normal light dark cycles of animals. At the same time, tissue water levels and hormonal levels are thought to influence these results depending on when in the cycle readings are made. The circadian pattern of water and Angiotensin and their relationship to VW/BW was investigated in male Sprague Dawley rats that were acclimatized in an environmental chamber and exposed to a fixed 12 hour light - 12 hour dark cycle. After a period of adjustment, animals were sacrificed at three hour intervals. Blood was collected; hearts were weighed, and 1 mg samples were taken for Angiotensin I (AI) and II (AII) using radioimmunoassay. Ventricles were weighed, dried in a constant temperature oven and re-weighed to determine water content. AI and AII levels in the heart demonstrated circadian patterns that were similar. Peak concentrations occurred at 0300 hours of the dark phase while the troughs occurred some 12 hours later (0300) hours light phase). Water content of the ventricle remained essentially unchanged throughout the 24 hour cycle. (Average = 76.9%; high = 78.2%; low = 75,7%). VW/BW was highest during the dark phase with the peak occurring between 2300 hours and 300 hours. The lowest VW/BW recorded was during 1100 and 1600 hours of the light cycle after which gradual increases were obtained up to the peak response. These results demonstrate that VW/BW is significantly different during various periods of the light dark cycle and that the level of Angiotensin II in the tissue parallel this finding. The results support the idea that AII levels may influence protein synthesis in the myocardium and thereby the elevation in ventricular mass. (Supported by a grant from the National Aeronautics and Space Administration).

45.7 ALTERED CNS-PITUITARY FUNCTION IN ZUCKER (fa/fa) FATTY RATS: ABSENT CIRCADIAN PERIODICITY OF ACTIVITY, FEEDING, AND PLASMA CORTICOSTERONE (B) CONCENTRATIONS AND ELEVATED BRAIN AND PITUITARY NEUROINTERMEDIATE (NI) LOBE BETA-ENDORPHIN (B-Ep) CONCENTRATIONS. Marie J. Gibson and Dorothy T. Krieger, Division of Endocrinology, Mount Sinai Medical Center, New York, New York, 10029.

Recent data have indicated interrelationships between feeding and B rhythmicity and with pituitary B-Ep concentrations. Genetically obese animals (ob/ob mice, fa/fa rats) are reported to have normal or absent circadian periodicity of plasma B, elevated or normal plasma ACTH and/or B concentrations, and elevated pituitary B-Ep concentrations. The present studies were designed to further delineate these parameters in the (fa/fa).

Studies were performed on seven (fa/fa) and seven lean (Fa/?) female rats, two months of age. The circadian periodicity of plasma B was determined by sequential sampling, q. 4 h over a 24-h period. Such periodicity was absent in (fa/fa) and present in (Fa/?). Mean plasma B concentrations were: (fa/fa): 20.3 ± 2.0 µg/100 ml; (Fa/?): 18.4 ± 2.7 µg/100 ml. Circadian rhythmicity of feeding and drinking behavior and of activity was absent in (fa/fa) and present in (Fa/?). Total food consumption was 23.3 ± 1.1 gm (fa/fa) vs. 17.1 ± 1.0 gm (Fa/?), $p<0.01$, and water consumption was 34.8 ± 1.8 ml (fa/fa) vs. 28.4 ± 2.1 ml (Fa/?), $p<0.05$. Body temperature patterns were similar in both groups, although the overall mean temperature of (fa/fa) animals (36.4° ± 0.1) was less than (Fa/?) animals (36.8° ± 0.1), $p<0.05$. Plasma B following 30 minutes of immobilization stress did not differ between the groups.

Immunoreactive B-Ep and ACTH concentrations were assayed in anterior and NI pituitary lobes and in brains of 10-month-old animals. Animals were sacrificed by decapitation. Brain and pituitary lobes were homogenized in 0.2 N HCl and frozen until assay.

	Body Wt (gm)	Brain Wt (mg)	Pituitary Wt (mg) Ant	NI	B-Ep Ant	NI	Brain
fa/fa	524±21	1438±39	10.9±1.2	2.2±.3	.12±.02	.98±.18*	35.3±7.3*
Fa/?	259±10†	1528±15	11.2±1.0	2.2±.2	.09±.01	.39±.15	17.8±3.0
					(µg/mg wet wt)		(pg/mg wt)

*$p<0.05$ †$p<0.01$

There were no differences between groups in concentrations of ACTH in either pituitary lobe or in the brain. Sephadex gel characterization of these peptides is in progress.

These studies indicate significant neuroendocrine disturbances with regard to circadian periodicity in (fa/fa) rats which are not present in their lean littermates. This may be related to reported abnormalities in neurotransmitter concentrations and/or their periodicity. The present findings also indicate an increase in both brain and pituitary B-Ep concentrations, with the latter specific to the NI lobe.

45.8 EPENDYMAL TANYCYTES ON THE FLOOR OF THE THIRD VENTRICLE OF EWES EXHIBIT SUBTLE ALTERATIONS IN SURFACE FEATURES DURING THE ESTROUS CYCLE. Penelope W. Coates and Steven L. Davis*. Dept. of Anat. Texas Tech Univ. Health Sci. Center., Lubbock, TX 79430 and Dept. of Animal Sci., Univ. Idaho, Moscow, ID 83843

Ultrastructural surface features of ependymal tanycytes on the floor of the third ventricle in a group of normally cycling mature female sheep (ewes) were evaluated by scanning and transmission electron microscopy (SEM and TEM). Three ewes each in luteal and follicular phases and one estrual ewe were analyzed. Blood was taken from the same animals at the time of sacrifice to determine luteinizing hormone (LH) concentration by radioimmunoassay (RIA). (Cycle stages were confirmed by LH concentrations of 43.5 ng/ml in the estrual ewe as compared to 126.0 pg/ml ± 16.2 for the luteal group and 141.4 pg/ml ± 51.5 for the follicular group of ewes). With SEM, tanycytes of follicular phase ewes demonstrated a relatively bare surface with fewer microvilli compared with estrual and luteal phase ewes. Miniblebs were present. At estrus, SEM revealed a predominantly microvillous covered tanycyte surface. Luteal phase ewes exhibited many microvilli on the luminal aspect of tanycytes, compared to follicular phase ewes. However, since some tanycytes were not completely covered with microvilli, a patchy appearance resulted. Miniblebs were also evident in luteal phase ewes. TEM showed that tanycytes in follicular ewes possessed few microvilli at their surfaces, reinforcing the data from SEM. TEM of tanycytes in the estrual ewe revealed a proliferation of luxuriant, often branched microvilli. This continued into the luteal phase where both microvilli and miniblebs were evident with TEM. Miniblebs contained cell cytoplasm as well as some organelles. The SEM appearance of tanycytes at estrus and in the luteal phase is similar to our previous findings for estrous ewes compared with estrogen-progesterone treated anestrous ewes respectively. (Coates & Davis, Biol. Reprod. 17:567-573, '77). The onset of appearance of a fully developed microvillous coat on tanycytes in the normally cycling ewe third ventricle appears to coincide with estrus and a peak LH concentration, while the continued maintenance of the microvillous state coincides with levels of estradiol that are known to fluctuate and an elevated progesterone level. These data, in combination with previous studies, showing that estrogen can cause proliferation of certain features, may suggest that there is a functional relationship in ewes between the development of microvilli in tanycytes and the preovulatory surge of estrogen. (Supported by grant no. HD12833 from the NIH and a grant from the Inst. Biomed. Res., TTUSM).

46.1 FREE AND CONJUGATED SEROTONIN, CATECHOLAMINES AND NORMETANEPHRINE IN HUMAN PLATELETS. G.B.Picotti+, C.Ravazzani+, A.M.Cesura+ and M.Da Prada+° (SPON: L.Valzelli) Inst. Pharmac.Univ. of Milan,Italy and °Pharm.Res.Dept., Hoffmann-La Roche & Co., CH-4002, Basle, Switzerland.

Platelets of all mammalian species so far investigated contain variable amounts of free (unconjugated) amines, e.g. 5-hydroxytryptamine (serotonin 5-HT), histamine, catecholamines (CA) and octopamine, which are prevalently accumulated in storage vesicles, i.e. the so called "5-HT organelles". A recently developed radioenzymatic assay for normetanephrine (NMN) has permitted to establish the presence of this noradrenaline (NA) methoxyderivative in platelets of various species, including man (M.Da Prada et al., in "Platelets:...", J.Wiley & Sons Ltd., 1980 in press).

The enzyme phenol sulfotransferase has been recently demonstrated in human platelets (Hart et al., Life Sci. 24, 125, 1979). In order to assess whether platelets contain sulfoconjugated amines, free and total (acid hydrolysis at 100°C) 5-HT, CA and NMN were measured radioenzymatically in human and animal platelet extracts. Apparently following acid hydrolysis only sulfoconjugated derivatives are hydrolyzed, glucuronoconjugated being acid-resistant. In human platelets (six healthy subjects) conjugated 5-HT, dopamine, adrenaline, NA and NMN corresponded to about 10, 93, 69, 33 and 76%, respectively. Only trace amounts (< 5%) of these amines were found in conjugated form in rabbit, rat and guinea-pig platelets, suggesting that, among the different mammalian platelets investigated, only human platelets contain high sulfotransferase activity.

Accordingly, in vitro studies showed that human, but not rat, rabbit or guinea-pig platelets are able to sulfoconjugate the ^{14}C-5HT, -NA or -NMN (1 uM) taken up by platelets on incubation in plasma (37° for 30 min). In these experiments most (over 80%) of ^{14}C-NMN accumulated by human platelets was found to be sulfoconjugated, whereas ^{14}C-5HT and -NA were conjugated to a lesser extent (10 and 35% of the accumulated radioactivity, for ^{14}C-5HT and -NA respectively). Reserpine (10 uM) effectively released free but not conjugated radioactive amines from preloaded platelets(G. B.Picotti et al., Br.J.Pharmac., 1980 in press).

From these and other results it appears that human platelets differ substantially from animal platelets, since they apparently have an extragranular cytoplasmic pool of sulfoconjugated CA, NMN and 5HT. Presumably the conjugated amines are in dynamic equilibrium with an additional pool of free amines within the cytoplasmic compartment and with the bulk of free amines stored in the 5-HT organelles.

Partly supported by CNR grant 79.01090.83

46.2 COMPARISON OF SEROTONIN BINDING PROTEINS OF NEURONAL AND MESENCHYMAL (MAST CELL + BASOPHIL) ORIGIN. H. Tamir, P.W. Askenase*, T.C. Theoharides and M.D. Gershon. N.Y. State Psych. Inst.; Dept. of Psychiatry and Anatomy, Columbia University and Dept. of Internal Medicine, Yale Univ.

Serotonergic neurons of both brain and gut contain a specific serotonin binding protein (SBP) that is probably a component of the amine storage mechanism. Serotonin (5-HT) is also stored in mast cells (mouse and rat), platelets and enterochromaffin cells. The properties of neuronal SBP differ from those of the platelet proteins that bind 5-HT. The present study was done to determine if 5-HT binding proteins are present in rat mast cells and rat basophil leukemia (RBL) cells and if so, to compare them with the SBP of neurons. RBL cells were maintained in culture and harvested at log phase. Rat mast cells were obtained by peritoneal lavage and purified on BSA gradients. The 5-HT binding capacity of non-membranous macromolecules of disrupted cells (100.000g supernatant) was measured using [^{3}H]-5-HT (0.2 uM) and molecular sieve chromatography. 5-HT binding macromolecules were detected in both cell types; however, since RBL cells could be obtained in greater abundance, the properties of the macromolecules from RBL cells were studied more extensively. Binding was specific (inhibited by excess of non-radioactive 5-HT but not by an excess of histamine) and linear up to 100 ug protein/ml (7pmole 5-HT/mg Protein). Neuronal SBP and the RBL macromolecule that bound serotonin showed similarity in trypsin sensitivity; in enhancement of binding by Fe^{+2} and in inhibition of binding by high salt media (Krebs-Ringer) and ATP. They differed in the following properties: 1) Only one dissociation constant was observed for RBL protein (K_D=3.9x10^{-7}M), the neuronal high affinity binding was lacking. 2) Ca^{+2} (10^{-3}M) did not inhibit the RBL protein. 3) The RBL protein showed partial heat lability (45% decrease of binding; 80°C; 10 min). 4) When RBL cells were disrupted by gentle homogenization, the binding capacity for 5-HT was only partially inhibited by reserpine (50%; 5 x 10^{-5}M) but when extensively homogenized (Polytron) the binding capacity was increased three-fold and was more sensitive to reserpine (>80% inhibition; 5 x 10^{-5}M). We suggest that vigorous homogenization causes release from intracellular granules of a second 5-HT binding protein which is reserpine sensitive. The reserpine insensitive protein may originate from an extra granular compartment. It is concluded that neuronal SBP is different from the 5-HT binding proteins of mast cells and RBL cells.

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46.3 MG·ATP AND BICARBONATE DEPENDENT STIMULATION OF ACETYLCHOLINE UPTAKE BY TORPEDO SYNAPTIC VESICLES. Stanley M. Parsons, Robert Koenigsberger,* and Joan E. Rothlein. Department of Chemistry, University of California, Santa Barbara, CA 93106.

Uptake and storage of [^{3}H]acetylcholine (ACh) by cholinergic synaptic vesicles isolated from Torpedo californica electric organ is being studied. [^{3}H]ACh was taken up passively with a half-life of 8 min by [^{14}C]mannitol equilibrated vesicles, to give an osmotically labile concentration inside equal to that outside as determined by ^3H to ^{14}C ratios. Passive uptake was directly proportional to the external [^{3}H]ACh concentration up to 50μM. In the presence of MgATP, uptake of [^{3}H]Ach was inhibited about 2-fold. Addition of HCO_3^- stimulated uptake 6-fold, so that a 3-fold concentrative uptake resulted. The effects were not dependent on Na^+, K^+, Cl^-, or ionic strength and HCO_3^- stimulation required MgATP. Incorporated [^{3}H]ACh migrated with synaptic vesicles in equilibrium ultracentrifugation in a glycerol density gradient. [^{14}C]Choline behaved identically with [^{3}H]ACh in passive and suppressed uptake, but its uptake was not stimulated by HCO_3^-. Stimulated uptake of [^{3}H]ACh was inhibited at low temperature, and stimulated uptake was dependent on the MgATP and HCO_3^- concentration in a saturable way. CaATP and HCO_3^- also stimulated uptake. A Ca^{2+}/Mg^{2+}-ATPase associated with vesicles similarly is stimulated by HCO_3^-. It seems likely that acetylcholine uptake by synaptic vesicles is linked to a bicarbonate stimulated Ca^{2+}/Mg^{2+}-ATPase. (Supported by the Muscular Dystrophy Association and NINCDS).

46.4 TRANSMEMBRANE POTENTIAL AND Na^+ GRADIENT IN ISOLATED SYNAPTIC VESICLES: A POSSIBLE MODEL FOR ENERGIZATION OF VESICULAR UPTAKE OF ACETYLCHOLINE. J. B. Suszkiw and M. O'Leary*. Physiol.Section, Biol.Sci. Group, Univ. of Conn. Storrs, CT 06268.

Synaptic vesicles isolated from the electromotor nerve terminals of Narcine and purified in iso-osmotic NaCl-sucrose gradients accumulate the lipophylic cation [^{3}H]methyltriphenylphosphonium (MTPP) as well as [^{3}H] choline (Ch) or [^{3}H]acetylcholine (ACh) but exclude the lipophylic anion thio[^{14}C]cyanate. Membrane potential of isolated vesicles, estimated from the steady-state distribution of MTPP ranges from 50 mV to 150 mV, inside negative. The accumulation of Ch or ACh by the vesicles parallels the accumulation of MTPP and ranges from 8x to over 80x the medium concentration of these cations.

Using the potential-sensing fluorescent probe 3,3'diethylthiadicarbocyanine, it can be demonstrated that gramicidin D hyperpolarizes vesicles and stimulates an electrogenic influx of Ch (ACh) but valinomycin is ineffective in either respect, confirming the observation by Carpenter&Parsons (J. Biol. Chem.(1978) 253: 325). However, vesicles purified in KCl-sucrose gradients can be hyperpolarized by valinomycin,but in this case subsequent electrogenic influx of Ch (ACh) is not observed.

The foregoing results are interpreted to mean that concentrative translocation of ACh into the synaptic vesicles is a function of vesicle membrane potential and is stimulated by imposition of a suitable ΔNa^+ (in>out) across the vesicle membrane. It is suggested that in situ, exocytotic release of ACh and simultaneous generation of ΔNa^+ due to rapid equilibration between the intravesicular and the extracellular medium may be prerequisite for energization of ACh uptake by the reformed vesicles, and might explain preferential charging of the mobilized but not the reserve vesicles with the newly synthesized transmitter.

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46.5 REGULATION OF ACETYLCHOLINE BIOSYNTHESIS DURING DEVELOPMENT AND AGING. M. Marchi*, D.W. Hoffman*, E. Giacobini and R. Volle. (SPON: W.A. Wilson). Lab. of Neuropsychopharmacology, Dept. of Biobehavioral Sciences, Univ. of Connecticut, Storrs, CT 06268, and Dept. of Pharmacology, Univ. of Connecticut Health Center, Farmington, CT 06032.

The relationship among biochemical parameters of cholinergic metabolism in autonomic nerve terminals in the chick iris were determined throughout the lifespan of the animal. Choline (Ch) uptake, acetylcholine (ACh) content and ACh turnover increase steadily up to the adult stage (3 months - 1 year) and then decline in aged animals (5 years). These changes appear to reflect the activity and the state of the cholinergic innervation of the iris. Other changes which occur with age include a steady decline in the ratio Ch:ACh, and a decrease in the ratio of endogenous Ch concentration relative to exogenous (^{3}H)Ch taken up from the medium. However, the ratio of (^{3}H)Ch to (^{3}H)ACh does not vary significantly with age from 10 days of incubation (d.i.) up to 5 years of age.

Experiments were also performed to examine the influence of age on the effects of drugs, temperature, and Na^+, on uptake and metabolism of (^{3}H)Ch, and on the mechanism of inhibition of uptake by cholinesterase inhibitors. Uptake of (^{3}H)Ch and synthesis of (^{3}H)ACh are both inhibited by these treatments, and cholinesterase inhibitors appear to inhibit uptake by increasing intracellular ACh, confirming previous reports.

Age related changes in the nervous system appear to be more evident in pre- rather than postsynaptic elements. Our reseach has lead us to the view that peripheral nerve terminals are both a major locus for aging in the nervous system, and a valuable model for studying these changes.

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46.6 DIRECT ACTIONS OF CHOLINOMIMETICS ON RABBIT SYMPATHETIC NEURONS. A.E. Cole and P. Shinnick-Gallagher, Dept. of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

In a previous report (Br. Res. 187, 1980), we suggested that the slow inhibitory postsynaptic potential (S-IPSP) in sympathetic ganglia may be caused by a direct action of acetylcholine (ACh). To test this possibility, we applied ACh and the muscarinic agonists methacholine (MCh) and muscarine (MUS) to the ganglia to mimic the nerve-evoked response.

We recorded in vitro from rabbit superior cervical ganglia (RSCG) using an extracellular, sucrose-gap technique. All drugs were added to the physiological solution superfusing the preparation. Hexamethonium (5×10^{-4}M) was added routinely to all solutions to block the nicotinic response. Control and drug responses were compared in the same ganglion.

ACh (10^{-3}M) and muscarine (10^{-5}M) produced a biphasic response in the ganglia that was similar to the S-IPSP and the slow excitatory postsynaptic potential (S-EPSP), i.e. a hyperpolarization ($.1 - .44$ mV) followed by a depolarization ($.08 - .4$ mV). MCh (10^{-3}M) had a similar effect.

The response of the ganglion to the cholinomimetics diminished with repeated application. However, application at one hour intervals avoided this phenomenon and elicited consistent reproducible responses.

When synaptic transmission was blocked by a zero Ca^{++}/high Mg^{++}, EGTA (1 mM) solution, as evidenced by elimination of the evoked slow potentials, ACh, MCh and MUS still hyperpolarized and then depolarized the ganglion membrane. The biphasic response was also maintained in the presence of tetrodotoxin (TTX) (10^{-6}M). Both the evoked slow potentials and the drug-induced responses were blocked by atropine (10^{-7}M).

These data suggest that the nerve-evoked slow potentials, possible modulators of ganglionic transmission, are generated by a direct action of ACh on postganglionic muscarinic receptors. Our results question the existence of a disynaptic pathway and the involvement of catecholamines in the mediation of the S-IPSP.

46.7 ALPHA-BUNGAROTOXIN BINDING SITES IN SENSORY GANGLIA. G. Polz-Tejera*, A. Reiner and J. Schmidt. Department of Biochemistry, State Univ. of New York, Stony Brook, N.Y. 11794.

Sensory fibers are known to be sensitive to acetylcholine and nicotinic-cholinergic ligands (Paintal, A.S., Pharmacol. Rev., 16:341, 1964). We therefore undertook to analyze sensory ganglia of rat for alphabungarotoxin (αBuTX) binding.

For biochemical analysis, freshly dissected trigeminal ganglia were homogenized and binding of ^{125}I-αBuTX (10^6Ci/mole) to particulate matter was measured with a centrifugation assay. Toxin receptor concentrations of approximately 1 femtomole per mg wet tissue and a K_D of 2×10^{-11}M were found. Inhibition constants for nicotine, d-tubocurarine and atropine were determined to be 1.2×10^{-6}, 4×10^{-6} and 1.0×10^{-4}M, respectively, indicating the nicotinic nature of the binding sites.

Autoradiography was carried out to identify the location of binding sites. It revealed labeling of large neuronal cell bodies. No binding over glia or neuropil was detectable.

High levels of toxin binding sites have been reported in the substantia gelatinosa of rat spinal cord (Hunt S. and Schmidt J., Brain Res., 157:213, 1978). Is it possible that toxin receptors are synthesized extrasynaptically in the perikaryon of ganglia cells and then transported to a presynaptic location at the central sensory fiber terminal in the spinal cord? To test this possibility, several consecutive lumbosacral dorsal roots were sectioned on one side. However, no effect on toxin binding in the substantia gelatinosa was observed 6-8 days after rhizotomy, although substand P levels, determined immunochemically, did decrease at the site of the sectioned roots. These findings indicate that cholinergic fibers are most probably restricted to the peripheral process of their sensory neuron. The modality of the toxin-positive sensory fibers is currently being investigated. An autoradiographic survey of the mesencephalic trigeminal nucleus revealed no label, indicating that proprioceptive fibers may not carry αBuTX binding sites.

46.8 POOR CORRELATION BETWEEN [^{3}H] QNB BINDING AND A MUSCARINIC CHOLINERGIC RESPONSE IN DISPERSED RAT PAROTID CELLS. Edward Olender* and James N. Davis, (SPON: E. Busse). Department of Medicine (Neurology) and Pharmacology, Duke University Medical Center and Veterans Administration Medical Center, Durham, N. C. 27705.

The radioligand [^{3}H] quinuclidinyl benzilate (QNB) has been used to study muscarinic cholinergic binding sites in many tissues. We used a model system, dispersed rat parotid cells to correlate [^{3}H] QNB binding to a muscarinic cholinergic response, K^+ release. [^{3}H] QNB bound to a homogeneous population of sites on dispersed cells and membranes from cells (18 fmole [^{3}H] QNB bound/mg protein). Computer modeling of the ability of agonists to compete with [^{3}H] QNB for binding was also consistent with one class of sites on these cells. However the potency of a small series of agonists and antagonists in eliciting or blocking K^+ release differed from their ability to compete for binding. The two antagonists studied, QNB and atropine, were equipotent in competing for [^{3}H] QNB binding but QNB was 20 times more potent than atropine in blocking carbachol-induced K^+ release from cells. Agonists competed for [^{3}H] QNB binding with the relative potency oxotremorine > carbachol > acetylcholine. By contrast acetylcholine was more potent than carbachol in eliciting K^+ release. Although difficult to measure, oxotremorine appeared to be a more potent partial agonist than carbachol or acetylcholine. Oxotremorine produced only a slight release of K^+ at maximum concentrations compared to the K^+ release elicited by maximum concentrations of carbachol. The EC_{50} values for K^+ release for carbachol and acetylcholine suggests that only 10% of the [^{3}H] QNB binding sites participate in K^+ release. Since parotid [^{3}H] QNB binding appears similar to binding described in other tissues, our results suggest caution must be employed before equating [^{3}H] QNB binding with the muscarinic cholinergic receptor.

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47.1 RETINAL PROJECTIONS IN A FRUGIVOROUS MICROCHIROPTERAN AND AN ECHOLOCATING MEGACHIROPTERAN. M.R. Braford, Jr. and R.A. Suthers. Department of Anatomy, Georgetown University, Washington, D.C. 20007 and Physiology Section, Medical Sciences Program, Indiana Univ. School of Med., Bloomington, IN 47401.

Among bats, detailed studies of the retinal projections have been reported for several insectivorous microchiroptera of the genus Myotis, which depend heavily on audition for echolocation, and two frugivorous megachiroptera of the genus Pteropus, which depend heavily on vision. These two groups probably represent the two extremes among the Chiroptera in terms of the development of the visual system. We have examined, with autoradiographic methods, the retinal projections in two species which might be expected to be intermediate between Myotis and Pteropus in this respect: a frugivorous microchiropteran, Phyllostomus hastatus, and an echolocating megachiropteran, Rousettus aegyptiacus. In both species the majority of the retinal fibers cross in the chiasm and distribute to the contralateral diencephalon and mesencephalon; the ipsilateral components are, however, substantially larger in Rousettus than in Phyllostomus. In Phyllostomus the retina projects contralaterally to the following cell groups: suprachiasmatic nucleus; ventral lateral geniculate nucleus; dorsal lateral geniculate nucleus; nucleus of the optic tract; the olivary and posterior pretectal nuclei; the medial, lateral and dorsal terminal nuclei of the accessory optic tract; and the superior colliculus. Ipsilateral terminations are present in the suprachiasmatic nucleus, ventral lateral geniculate nucleus, nucleus of the optic tract, olivary pretectal nucleus, and possibly very sparsely in the dorsal lateral geniculate nucleus. In Rousettus the retina projects contralaterally to all of the targets seen in Phyllostomus and, in addition, to the lateral posterior nucleus of the thalamus and the anterior pretectal nucleus. Ipsilateral input in Rousettus is distributed to the suprachiasmatic nucleus, ventral lateral geniculate nucleus, dorsal lateral geniculate nucleus, lateral posterior nucleus, nucleus of the optic tract, the olivary and anterior pretectal nuclei, and restricted portions of the superior colliculus. The dorsal lateral geniculate nucleus is small and relatively simply organized in Phyllostomus, whereas in Rousettus it is large and consists of three major cellular laminae and five major terminal laminae. The retinal input in Phyllostomus is more extensive than that in Myotis and that in Rousettus appears to be similar to but slightly less extensive than that in Pteropus. (Supported by NSF Grant BNS 76-01716 to RAS.)

47.2 NEW OBSERVATIONS ON THE RETINAL PROJECTIONS TO THE HYPOTHALAMUS AND ACCESSORY OPTIC NUCLEI IN THE GOLDEN HAMSTER AS DEMONSTRATED BY ANTEROGRADE HRP. G.E. Pickard and A.J. Silverman, Department of Anatomy, Columbia University, P&S, New York, NY 10032.

The available anatomical data on the central organization of the optic projections in the hamster is limited and often conflicting. Controversy has centered on the existence of an inferior accessory optic fasciculus (Eichler and Moore, Acta Anat., 89:359, 1974; Lin et al., Anat. Rec., 186:451, 1976) and the route taken by retinal fibers destined for the hypothalamus (Printz and Hall, Anat. Rec., 179:57, 1974; Eichler and Moore, 1974). The central projections of the retinal ganglion cells of the golden hamster were re-examined in this study using horseradish peroxidase (HRP) as the anterograde tracer molecule.

Monocular injections of four ul of a 30% HRP (Sigma,type VI) solution (0.05 M Tris, pH 7.6) were made into the vitreous. Following survival times of 6, 12, 24, 36 or 48 hrs, the animals were perfused with 0.9% saline followed by 2% glutaraldehyde. Following overnight fixation in a 1.5% glutaraldehyde solution containing 5% sucrose, the brains were sectioned in the coronal plane at 60 um. HRP in optic fibers and terminals was demonstrated histochemically using the tetramethylbenzidine (TMB) procedure (Mesulam, J. Histochem. Cytochem., 26:106, 1978).

The sensitivity of the TMB procedure allowed the clear demonstration of previously controversial projections, clearer definition of established projections and the discovery of a new retinofugal pathway. An inferior accessory optic system was shown to be unequivocally present in this species and to consist of both crossed and uncrossed components. A projection to the suprachiasmatic nucleus (SCN) of the hypothalamus was confirmed but the distribution of terminals as seen by this procedure was substantially different than previously reported; both rostral-caudal and medial-lateral asymmetries in the distribution of label between the ipsi- and contralateral SCN were observed. Substantial differences in the retinal projection to the SCN in the hamster and the rat were also noted. It is suggested that these differences may reflect the different effects light has on the neuroendocrine-gonadal axis in these two species. Finally, labeled retinal axons were followed leaving the optic tract and coursing anteriorly through the plexiform layer of the piriform cortex; other labeled fibers were seen to enter the septal region. The physiological significance of these previously undescribed retinal projections is not known.

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47.3 DIRECTION-SELECTIVE NEURONS IN THE FROG'S VISUAL SYSTEM. S.L. Cochran, W. Precht*, and N. Dieringer*. Neurobiol. Abt., Max-Planck-Inst. für Hirnforschung, 6 Frankfurt/Main 71, FRG.

Two midbrain nuclei have been found to respond selectively to optokinetic stimulation. Both are innervated by retinal ganglion cells from the contralateral eye. One of these nuclei, the basal optic nucleus, is composed of neurons which respond preferentially to large targets (striped or random dot patterns) moved vertically. These cells have no resting discharge. Some are activated exclusively by upward movement, while others are only activated by downward movement. A proportion of these cells also respond to horizontal, naso-temporal optokinetic stimulation, but none of these cells respond to temporo-nasal movement. A nucleus distinct from the basal optic nucleus is sensitive to horizontal movement. Its neurons are spontaneously active. A temporo-nasal movement of a large pattern (presented to the contralateral eye--optimally moving less than 10°/sec) results in a brisk increase in the firing frequency of these cells, while such stimulation in the naso-temporal direction results in a reduction in frequency and in a silencing of these cells. These neurons vary in their sensitivity to vertical movement, but all are predominantly sensitive to horizontal movement, and all are excited by temporo-nasal movement. Ejection of horseradish peroxidase from the recording microelectrode and subsequent histological processing reveals the location of these cells to be approximately 1 mm ventral to the rostral tectal surface, dorsolateral to the oculomotor and basal optic nuclei. This region may thus correspond to the pretectal nucleus of the frog (see Scalia, 1976) and may be homologous to the nucleus of the optic tract and/or the dorsal terminal nucleus of higher vertebrates. The basal optic nucleus is likely homologous to the ectomammillary nucleus of the bird and the medial and ventral terminal nuclei of the mammal. Electrolytic lesions, confined to the region of these horizontally-sensitive neurons result in a marked diminution in movement of the head to the lesioned side with temporo-nasal stimulation of the contralateral eye. Similar lesions of basal optic neurons result in no impairment of horizontal following of the head. It would thus appear that these horizontally-sensitive neurons are the first necessary, central link for the nervous system's ability to mediate head, and possibly eye, movements causal from and compensatory to horizontal movement of the visual surround.

47.4 BASAL OPTIC PROJECTION IN THE FROG (Rana pipiens). Edward R. Gruberg and Keith L. Grasse*. Res. Lab. of Electronics, Mass. Institute of Technology, Cambridge, Mass. 02139 and Psychology Dept., Dalhousie Univ., Halifax, Nova Scotia.

We have investigated the physiology and anatomy of the basal optic projection (BOP) using single-unit extracellular recording and HRP injections.

With the animal on its back and using a ventral penetration through the upper mouth, we have been able to record routinely single units in the BOP which are driven primarily by the contralateral eye. We have found 3 classes of units: 1) those responsive to stimuli moving in a vertical direction, 2) those responsive to stimuli moving in a horizontal direction, 3) those responsive to changes in ambient light but not to moving stimuli. All directional units have significant maintained activity. Vertical units increase firing to either slow upward movement or slow downward movement; stimulus motion in the opposite direction gives a reduced response. Horizontal units increase firing either to slow naso-temporal movement or to slow temporo-nasal movement. They, too, give reduced responses in the opposite direction. All these units yield broad tuning curves of response vs direction. A wide variety of sizes and shapes of stimuli elicit responses. The best response is obtained for most units when stimuli are moved with angular velocity in the range of 0.1° to 1°/sec. Those units which did not respond to moving stimuli had maintained activity that was greater in the dark than in the light.

For HRP injections, electrophysiological recordings were first made to ascertain location and depth of the BOP in each animal. Since the target region is very small, we injected HRP solution in volumes of 1/2 nl or less. We have followed efferent fibers from the BOP through the posterior commissure to the opposite side. We have also followed a set of stained fibers which course caudally through the ipsilateral ventral medulla. We have not been able to trace the terminal fields of either set of fibers. We have found ipsilateral afferent projections to the BOP from 3 principal areas: the posterolateral tegmental field, the posterior nucleus of the thalamus, and a wide extent of the ventral thalamus particularly in the anterior part. HRP-filled cells in the retina are primarily found in the ganglion cell layer; however, in keeping with Montgomery et al (1979), some large displaced ganglion cells are also stained.

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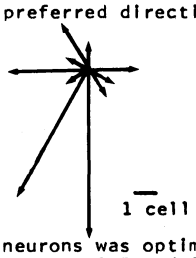
47.5 DIRECTIONAL SELECTIVITY IN THE MEDIAL TERMINAL NUCLEUS OF THE CAT. Keith L. Grasse* and M. S. Cynader (SPON: J. Rutherford). Psychol. Dept., Dalhousie University, Halifax, Nova Scotia, Canada.

Single unit recordings were made from 30 cells in the medial terminal nucleus (MTN) of the accessory optic system in anesthetized, paralyzed cats. Receptive field sizes were very large and located primarily in the contralateral, and to a lesser extent, ipsilateral visual fields. Most cells were found to be contralaterally driven and responsive to chiasmatic stimulation. In response to a 40° x 40° square wave grating pattern (period 8°) moving in a direction normal to the stripe orientation, the majority of cells exhibited marked directional selectivity. Typically, a unit's response consisted of an excitation exceeding the resting discharge by 2-3 times for movement along one axis, coupled with an inhibition which could fall to 1/2 to 1/3 of the maintained rate for motion in another direction. The general axes of preferred directions were:



The activity of MTN neurons was optimally modulated at stimulus velocities between 0.5 and 10°/sec. Local targets such as small light bars proved to be ineffective stimuli for these cells. A variety of diffuse light sensitivities were seen for the directional units ranging from 'on' or 'off' to 'on-off'. An additional but much smaller population of cells was also found (8 cells) which were responsive to chiasmal shock and some aspect of diffuse light stimulation, yet showed no directional properties. Taken together, the large receptive fields, slow velocity specificity and marked directional selectivity are reminiscent of visual units reported in the NOT of the cat. Unlike those units, however, the cells of the MTN mostly prefer the vertical down direction.

47.6 BRAINSTEM PROJECTIONS TO THE CAT'S LATERAL POSTERIOR-PULVINAR THALAMIC COMPLEX. M.Rodrigo-Angulo* and F. Reinoso-Suárez. Departamento de Morfología, Facultad de Medicina, Universidad Autónoma, Madrid-34, Spain.

The lateral posterior-pulvinar thalamic complex (LP-Pu) has been considered as a nodal link in the extrageniculate pathways of the visual system toward the posterior association cortex. It is known that the LP-Pu receives fiber projections from the superior colliculus, pretectum and cerebral cortex. Other roles attributed to this complex have been the following: 1) a nucleus of the so-called 'dorsal spindle system', 2) a station in the most important path mediating the thalamocortical volleys which trigger the penicillin epileptic burst and 3) a participant in the integration of some motor activities. These functions suggest the existence of a broad spectrum of brainstem projections to the LP-Pu. With the aim of studying these brainstem afferents we have made stereotaxic injections of 0.04-0.10 ul of a solution of HRP in the various parts of the LP-Pu in adult cats. After a survival time of 40-48 hr. animals were perfused and brains processed according to Mesulam (1978) technique to reveal HRP brainstem neurons. The injected HRP was always confined to the LP-Pu. Fairly large numbers of labeled neurons were found in some or all of the following brainstem and cerebellar formations: 1) the pretectum, 2) the superficial and/or the deep layers of the superior colliculus, 3) the nucleus tegmenti pedunculo ponti, 4) the locus coeruleus complex, 5) the lateral cerebellar nucleus. Likewise scattered labelled neurons can be found in 6) the central gray matter, 7) the oculomotor nucleus, 8) the mesencephalic raphe nuclei, 9) the dorsal oral pontine tegmentum, 10) the dorsal tegmental nucleus, 11) the nucleus incertus and 12) the nucleus praepositus hypoglossi. The topographical selectivity of these findings depended both on the size and the location of the injection. Control injections were made in the neighbouring thalamic nuclei. These findings of a widespread origin of brainstem projections to the LP-Pu, and specifically those coming from the ponto-mesencephalic tegmentum, can provide a morphological substrate for the functions described above that have attributed to the LP-Pu.

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47.7 VISUAL CORTICAL AND SUBCORTICAL CONNECTIONS OF THE CENTRAL LATERAL NUCLEUS IN THE CAT. Elin F. Spring and Alan C. Rosenquist. Dept. Anat., School of Medicine, University of Pennsylvania, Philadelphia, Pa. 19104

Using the horseradish peroxidase (HRP) retrograde tracing technique, we have studied the connectivity of the central lateral nucleus (CL) of the intralaminar thalamic group with subcortical structures and electrophysiologically defined visual cortical areas in the cat (Tusa et.al., 1978). In a first set of experiments, we mapped visual receptive field locations using a micropipette filled with an HRP/tritiated leucine mixture. This recording technique was used to guide the placement of 0.05-0.10 ul pressure injections into visual cortical areas 17, 18, 19, 20a, 20b, 21a, AMLS, ALLS, PMLS, PLLS, DLS, and VLS. In a second series of experiments, we made similar injections stereotaxically which were confined primarily but not exclusively to CL. The tissue was processed for HRP histochemistry using the chromagen o-dianisidine.

We observed HRP-labelled neurons in the ipsilateral CL after injections into each of the above visual cortical areas except 17. We found no evidence for any retinotopic organization in the CL projections to visual cortex. After injections into the CL, a number of subcortical structures were found to contain HRP-labelled neurons. These included the stratum griseum intermedium and stratum griseum profundum of the superior colliculus, the nucleus of the optic tract of the pretectum, the periaqueductal grey of the midbrain, the periventricular grey of the pons, and the pontine tegmentum.

We conclude that CL projects to all visual cortical areas that we have studied except area 17 and that these projections are not retinotopically organized. Further, it is likely that cells in CL which project to visual cortex convey information from subcortical structures such as the pretectum and superior colliculus. This may represent another parallel pathway for visual and non-visual information to reach visual cortex. (Supported by 5T32 GM07517 and 1R01 EY02654).

47.8 SUPERIOR COLLICULUS NEURONS WHICH PROJECT TO THREE THALAMIC NUCLEI IN THE CAT. J.V. Harrell*, R.B. Caldwell, L.H. Reed*, and R.R. Mize. Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163

We have quantitatively examined the cells of the cat superior colliculus which project to the ventral lateral geniculate (VLG), dorsal lateral geniculate (DLG), and lateral posterior (LP) nuclei of the thalamus. Cells were labeled following HRP injections made 24 hours prior to sacrifice. Four parameters were measured. Topographic distribution was studied by plotting cell location on projection drawings. Cell depth was measured with an eyepiece reticule. Cell area was measured using a computer controlled digitizer. Estimates of cell type were made from camera lucida drawings based on criteria used in Golgi studies.

Our results show that superior colliculus neurons which project to all three thalamic nuclei vary dramatically in depth, size, and morphology. On the other hand, differences between the three groups can be demonstrated quantitatively for these three parameters. Depth: 248 cells labeled after VLG injections ranged in depth from 51-1472 μm (mean=375 μm). After DLG injections, 364 labeled cells were 67-2247 μm deep (mean=502 μm). 107 cells labeled after LP injections ranged in depth from 120-2141 μm (mean=627 μm). Area: Cells projecting to VLG had a mean area of 142 μm^2. Cells projecting to DLG had a mean area of 165 μm^2. Cells projecting to LP had a mean area of 243 μm^2. Morphology: Four morphologies were distinguished. The number of labeled vertical and horizontal cells was relatively constant in all three groups. By contrast, the VLG group had many labeled granule cells and few labeled stellate cells. The DLG group had fewer labeled granule and more labeled stellates. The LP group had the highest percentage of labeled stellates and the lowest percentage of labeled granule cells.

Three conclusions can be drawn from these results. 1) There is no strict laminar segregation of cells projecting to the three thalamic nuclei as reported in other species. Cells throughout the superficial gray and optic layers project to all three nuclei. A few projection cells lie even deeper. 2) No single cell class projects exclusively to any one of these thalamic nuclei. All three nuclei receive colliculus efferents from cells with several differing morphologies. 3) There are clear differences in the weighting of the inputs to the three nuclei. VLG projection cells are on average the smallest and most superficial, and many have a granule morphology. LP projection cells are on average the largest, deepest, and many have a stellate morphology. The DLG projection group is intermediate in size, depth, and percentage of cell types. These gradients must reflect differences in the functional input to the three nuclei (Supported by EY-02973).

47.9 REGIONAL DIFFERENCES IN THE RETINORECEPTIVE LAYERS OF PIGEON OPTIC TECTUM. T. A. Duff, G. Scott* and R. Mai*. Depts. of Surgery (Neurosurgery) and Anat., Univ. Wisc., Madison, WI 53792.

We previously described a regional variation in the size of pigeon retinotectal axons, characterized by a population of small fibers arising from temporal retina and projecting to anteroventral tectum (avT) and a more heterogeneous collection arising from nasal retina and projecting to posterodorsal tectum (pdT). Because of these findings, we undertook an ultrastructural investigation of the retinoreceptive layers (2-7) in avT and pdT and have found a number of regional differences in the morphology of optic terminations and of their post-synaptic elements.

The most obvious ultrastructural difference between avT and pdT is the presence of large, primarily horizontally-oriented dendrites in the latter region. These dendrites are most conspicuous from the upper boundary of layer 2 to the superficial aspect of layer 4. In order to quantitate the difference in dendritic size, tracings were made of the perimeters of all dendrites, excluding those clearly cross-sectioned in a tangential manner, within 1000 μm^2 sample areas of layers 2-3; mean dendritic diameter in pdT was 0.77 μm and in avT was 0.43 μm. Measurements were also made of all dendrites with which one or more optic terminals formed synaptic junctions, defined by the presence of a post-synaptic density. The mean diameter of such dendrites in pdT was 0.75 μm and in avT was 0.51 μm.

In order to quantitate possible differences in bouton size, tracings were made of each sectioned optic terminal, or when grouped, of an entire islet, within 1000 μm^2 areas from the midportion of each of the retinoreceptive layers. No clear differences in these size measurements was seen among layers within either tectal region, but mean diameter values in pdT were uniformly larger than those in avT: (1.14 μm vs. 0.78 μm).

A more detailed analysis of synaptic organization was made within layer 5. Specifically, the number of post-synaptic elements contacted by each terminal or, when grouped, by the entire islet, was counted within 1000 μm^2 areas, and the mean values for avT and pdT were 1.12 and 1.87 respectively.

The regional differences in optic terminations and in tectal dendritic size show interesting parallels to the variation in retinal fiber input, providing evidence that anatomical, and presumably functional, differences extend beyond the mere transmission of information from the eye to the optic tectum. The architecture of pdT suggests a circuitry which imposes relatively greater dispersion of retinal input at least at the initial synaptic level and which may be designed to enhance the detection of movement stimuli.

47.10 SENSORY REGIONS OF THE CAT'S CLAUSTRUM: AN ANATOMICAL AND PHYSIOLOGICAL STUDY. C.R. Olson* and A.M. Graybiel (SPONS: W.J.H. Nauta) Dept. Psychology, Mass. Inst. of Technology, Cambridge, MA 02139.

In axon transport experiments, we have identified regions of the claustrum related to the somatic sensory and visual cortex. We explored these regions in electrophysiological mapping experiments. The findings suggest that specific subregions of the cat's claustrum are specialized for processing visual and somesthetic information within the context of topographic maps.

Primary visual cortex projects densely to a small dorsocaudal region of the claustrum. Neurons recorded in this division responded exclusively to visual stimulation, were binocular (except in the monocular crescent) and had receptive fields ranging from a few to a few hundred deg^2. The region was organized retinotopically. During downward traverses through its center, receptive fields progressed from the extreme contralateral periphery to the midline. Lower fields were plotted in more rostral tracks. Regions mapping the upper and lower peripheries were not found but could exist in compressed form. We used the distinguishable retrograde (and anterograde) tracers nuclear yellow (NY*) and horseradish peroxidase (HRP) and also ^{3}H-amino acids (^{3}H-AA) to study the connections of this region. 1) ^{3}H-AA deposits in claustrum labeled all layers of ipsilateral area 17 and peristriate cortex, with dense label in layer I; they also weakly labeled visual cortex contralaterally. 2) Claustral projections to areas 17, 18 and 19 appeared to arise from neurons organized retinotopically and in register: tracers injected into different parts of area 17 labeled nonoverlapping slabs in claustrum, whereas largely overlapping uptake occurred when retinotopically matched regions of two cortical areas were injected. 3) NY deposits in claustrum labeled neurons in layer VI of area 17; HRP injected simultaneously into the LGN_d labeled an almost totally distinct set of layer VI cells. 4) Injections of HRP or ^{3}H-AA in area 17 labeled the part of contralateral claustrum representing the visual-field midline.

In parallel tracer experiments we found that primary somatic sensory cortex is reciprocally connected with a rostral zone of the claustrum. Double-label findings showed that the claustrocortical projection is somatotopic at least along the body's longitudinal axis. Neurons recorded in somatosensory claustrum responded to moderate pressure applied to restricted areas of the contralateral body surface. Somatotopic ordering was present, with hindparts dorsal, foreparts and face ventral.

We have not detected HRP in the claustrum following injections in primary auditory cortex (AI).

*We thank Drs. H. Loewe and O. Dann for fluorescent tracers. Supported by NIH R01EY02866-02, EY05316-01 and NSF 5T32GM07045.

48.1 AFFERENT INPUTS TO THE CAT'S MOTOR CORTEX FROM AREA 5: A PHYSIOLOGICAL STUDY. O. Favorov*, R. S. Waters, A. Mori* and H. Asanuma. The Rockefeller University, New York, N. Y. 10021.

In this study we examined the physiological characteristics of neurons lying along the caudal bank of the ansate sulcus (area 5) that were activated from sites around the pericruciate region of the motor cortex (area 4γ). Under halothane anesthesia the motor and parietal cortices were exposed and a double-barreled closed chamber was installed over the skull. Through the anterior chamber an array of 8 microelectrodes, 1.5 mm apart, was driven into the motor cortex and fixed at a depth of 1.2 mm. Receptive field and muscle response information was obtained for each electrode in the array. Through the posterior chamber, a single microelectrode was driven down the caudal bank of the ansate sulcus while intra-cortical microstimulation (ICMS) was delivered through the motor cortex electrodes. Whenever an activated neuron was encountered within the area 5 the responsible stimulating electrode was identified and its receptive field was examined and compared with the receptive fields of neurons around the stimulating electrode in the motor cortex.

Altogether, 99 neurons were activated in area 5. Of these neurons, 65% were antidromically activated, 7% were orthodromically activated, and 28% were unclear. Of the antidromically activated neurons, 32% had clear receptive fields. In the majority of these cases the receptive fields of neurons projecting to the forelimb area of the motor cortex were confined to only a part of that limb, while 26% had much broader receptive fields. The projection of neurons in area 5 was restricted to a small region of the motor cortex, since no cases were found in which the same area 5 neuron could be activated from more than one stimulating site. There were small clusters of neurons of similar receptive fields around the ansate sulcus, which were activated from two or more sites in the motor cortex.

In 18 cases receptive field information was obtained from both motor cortex neurons around the stimulating electrode and area 5 neurons. They were classified into three categories: (a) overlapping-identical (44%), (b) contiguous (28%), and (c) non-contiguous (28%). The projections to motor cortex from antidromically activated neurons along the caudal bank of the ansate sulcus appear to be organized in the following manner: neurons in the deep part of the medial ansate gyrus and neurons at all depths in the lateral ansate gyrus project to the paw and forearm region of the motor cortex, while those in the upper and middle bank of the medial ansate gyrus project more diffusely throughout the entire motor cortex.

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48.2 PHYSIOLOGICAL PROPERTIES OF CORTICO-CORTICAL CONNECTIONS FROM AREA 2 TO AREA 4γ IN THE CAT.
R. S. Waters and H. Asanuma. The Rockefeller University, New York, N. Y. 10021.

Anatomical studies have shown that HRP injected into the forelimb region of motor cortex is retrogradely transported to a number of cortical sites including somatosensory area 2. In the present study, we examined the physiological properties of the cortico-cortical connections between area 2 and area 4γ. Under halothane anesthesia, a double-barreled closed chamber was installed over the sensory and motor cortices. An electrode holder housing 8 microelectrodes was placed on the anterior chamber and each electrode was separately inserted to a depth of 1.2 mm below the surface of the cortex. Receptive fields of neurons and muscle responses to intra-cortical microstimulation (ICMS) were examined through each electrode. Through the posterior chamber, a single recording electrode was inserted along the entire bank of the ansate sulcus while delivering ICMS from the 8 electrodes in the motor cortex. Upon isolation of antidromic responses of the sensory neurons the effective stimulating electrode for each neuron was determined and examination was made of the receptive field of the neuron. This receptive field was then compared with that of the neurons around the stimulating electrode. The results could be classified in one of three categories: a) identical receptive fields, b) contiguous receptive fields, and c) non-contiguous receptive fields. In addition, the receptive field was routinely examined throughout each penetration.

Altogether, 157 neurons in 9 cats were antidromically activated by stimulation through the 4γ electrodes. From these neurons, the receptive fields of 44 neurons could be compared to those of cortical neurons located near the responsible electrodes. Of these pairs, 52% had identical receptive fields, 25% had contiguous receptive fields, and 23% had non-contiguous receptive fields. In addition, the ansate region could be subdivided into a medial and lateral division. Projections to motor cortex from the lateral division were generally localized to the forelimb region of the motor cortex, whereas projections from the medial division were more diffuse. In the largest number of cases, but not without exception, area 2 neurons simultaneously recorded from a given depth could only be activated from a single motor cortex electrode. These results will be discussed in relation to our previous report concerning the strength of the connection of the input to the motor cortex from sensory area 3a.

(Supported by the NIH Grant NS-10705)

48.3 SOMATOSENSORY INPUTS TO CORTICO-CORTICAL AND PYRAMIDAL TRACT NEURONS OF CORTICAL AREAS 1 AND 2. P. Zarzecki and D. Wiggin*. Dept. Physiol., Queen's Univ., Kingston, Ont., Canada K7L 3N6.

Neurons of somatosensory cortex may be involved in motor control by way of their projections upon the motor cortex or directly upon the spinal cord. For the purpose of assessing the information which might be carried by these projections, we have investigated cortico-cortical and PT neurons of cortical areas 1 and 2 for inputs from cutaneous and muscle afferents in the Nembutal-anesthetized cat.

Extracellular recordings were made from neurons in areas 1 and 2 which were antidromically activated by microstimulation of the motor cortex (area 4γ) or from the medullary pyramid. Such identified cortico-cortical or PT neurons were tested for influences originating from the following nerves of the contralateral forelimb: deep radial (muscle), superficial radial (cutaneous), median (mixed) and the dorsal cutaneous and palmar (muscle) branches of the ulnar. Nerve stimuli were graded and controls made for stimulus spread between nerves using volleys recorded from proximal nerve trunks. Collision-extinction techniques and nerve-evoked alterations of antidromic spike shape verified that the peripheral inputs influenced the same neurons which projected to the motor cortex or through the pyramidal tract. With these techniques supra- and subthreshold excitatory inputs could be detected.

Most cortico-cortical neurons of areas 1 and 2 were not modality specific. Of 29 cortico-cortical neurons, 27 (93%) showed evidence of excitatory inputs from more than one peripheral nerve; 26 (90%) from both cutaneous and muscle nerves. In addition, most received excitation from dorsal plus ventral (80%), and distal plus proximal (86%) aspects of the forearm.

Pyramidal tract neurons of areas 1 and 2 also receive convergent somatosensory inputs. Excitatory inputs from both cutaneous and muscle nerves reached 13 of 19 PT neurons (68%). Inputs from more than one region of the forearm were evident for 12 (63%).

This extensive convergence is similar to that which we have previously reported for cortico-cortical and PT neurons of area 3a (Neurosci. Abstr. 5, 1979). Whatever their effects upon the motor cortex or the spinal cord, the cells of origin of cortico-cortical and PT projections from somatosensory cortex are potential sites for the integration of information from several somatosensory modalities.

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48.4 NEURONAL TYPES IN THE MOTOR CORTEX OF CATS AS REVEALED WITH INTRACELLULAR HRP-INJECTIONS AND GOLGI METHODS. C.E. Ribak and C.D. Woody. Department of Anatomy, Univ. of Calif., Irvine, CA 92717 and Departments of Anatomy and Psychiatry, Univ. of Calif., Los Angeles, CA 90024.

The pericruciate cortex of cats was studied in Golgi-stained preparations in order to confirm the neuronal types observed following intracellular recording and labelling with horseradish peroxidase (HRP). Pyramidal and non-pyramidal cells were prevalent in both of these preparations. In the Golgi-stained sections, small pyramidal cells were observed in supragranular layers while larger pyramidal cells, including Betz cells, were found in the deeper cortical layers. These pyramidal cells had apical and basal dendrites that displayed numerous spines. Axons arose from the base of these cells, and then descended into the white matter after giving rise to a few collaterals. Some large pyramidal cells in layer V possessed a unique apical dendrite in that one of its thick dendritic branches curved down toward the cell body and then turned upwards. A few dendrites arose from this bowed dendrite, and they ascended toward the pial surface along with the main apical dendrite. In addition, inverted pyramidal cells were observed in the deep cortical layers, and these cells had their apical dendrite directed toward the white matter. In contrast to other pyramidal cells, the inverted cells were largely aspinous. Non-pyramidal cells that were found in all cortical layers were characterized by either spinous dendrites or beaded dendrites with smooth surfaces. The somata of these cells were either round or fusiform-shaped, and their locally-distributed axons arose from dendrites or somata.

Preliminary electron microscopic studies have confirmed the light microscopic appearance of spinous and beaded dendrites in both Golgi-stained and HRP-labelled cortical neurons. In addition, axon terminals were observed to form synaptic junctions with both of these kinds of dendrites. The electrophysiological properties of non-pyramidal cells were compared using intracellular recording methods to determine if different dendritic morphologies correlated with different functional properties. Preliminary data indicate no significant differences in the electrophysiological properties of these cells. In conclusion, the use of both intracellular HRP-injections and Golgi methods has been helpful in identifying the various types of neurons in the motor cortex of cats.

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48.5 DISCHARGE OF NEURONS IN CAT MOTOR CORTEX DURING VOLUNTARY MUSCLE CONTRACTION. D. Vicario, J. Martin* and C. Ghez. Div. of Neurobiol. & Behav, Depts. of Physiology & Neurology, Columbia P&S & Rockefeller University, New York, N.Y. 10032.

We have shown that accurate responses with reaction times of 60-70 msec are commonly observed in cats performing an isometric tracking task (Exp. Brain Res. 33:173, 1978). The present study was undertaken to determine if neurons in the motor cortex could contribute to the initiation of such rapid responses to exteroceptive cues.

Cats were trained to apply force isometrically with their forearms to a strain gauge to match a target level which was stepped at random times. They were provided with a display of their force error and responded to target shifts by rapidly adjusting the force applied. Neurons were recorded within areas of motor cortex where microstimulation produced contraction of muscles active during task performance. Projection neurons were identified antidromically from electrodes in the cerebral peduncles; receptive fields and the effects of microstimulation (under 20 μa) at recording sites were routinely determined.

Task related units recorded in the pericruciate cortex of three cats could be divided into two groups. The majority (85%) modulated their activity after response initiation. Most of these neurons had clear superficial or deep receptive fields in the forelimb, to which their activation could be attributed. The remainder showed changes in activity preceding (mean: 30 msec) the contraction of agonist muscles (lead cells). Such neurons either were not clearly driven by peripheral stimuli, or had receptive fields with complex features (directional specificity, temporal lability, multiple foci etc). Neurons with these properties were characteristically located at anterior sites in the pericruciate cortex, suggesting functional specialization.

Eighty percent of lead cells were antidromically driven from the cerebral peduncles. Their task-related discharge reflected both the direction and magnitude of force exerted. Equal numbers of cells varied their activity with the level of force, its first derivative, or both. This distribution is similar to that reported for motor cortex neurons in the monkey and differs from the predominantly phasic properties previously shown to characterize red nucleus neurons in the cat. In short reaction time movements (60-70 msec), task related activity of lead cells occurred 30-40 msec after the change in target location. The latency of this activity is comparable to that of the "intended response" of neurons in the primate motor cortex to proprioceptive inputs (Evarts & Tanji, J. Neurophysiol. 39:1069, 1976) and supports the notion that similar mechanisms underlie voluntary responses with the most rapid reaction times, irrespective of the stimulus modality (Exp. Brain Res. 33:173, 1978).

48.6 INTRACORTICAL SEIZURE PROPAGATION IN EXPERIMENTAL FOCAL EPILEPSY. M. David Fairchild*, Patricia R. Callahan* and John A. Kusske. Division of Neurological Surgery, University of California, Irvine, CA 92717

To study the patterns of seizure propagation within vertical cortical columns neuronal activity has been recorded from the midportion of the suprasylvian gyrus of cats subsequent to the placement of four tungsten microelectrodes into the cortex; the tip of each successive microelectrode positioned 350 micra deeper than the one above it. Recordings were obtained from a column extending from the most superficial to the deepest layers of the cortex. Histological controls were utilized to confirm electrode position. Periodic, recurring of afterdischarge was produced by electrical stimulation of the homotopic, contralateral cortex utilizing bipolar silverball electrodes. Both multineuronal unit activity and slow waves were recorded on magnetic tape; the time course of seizure activity was studied by transcribing the magnetic tape records on paper by means of a Honeywell Visicorder. Triggered correlations of unit activity and field potentials recorded by each electrode were performed utilizing a PDP 11/20 computer. The multineuronal unit activity was passed through voltage discriminators so that two populations of units from each channel were analyzed. In preseizure recordings it was unusual to detect spontaneous unit activity in all electrodes simultaneously; usually activity was recorded on one or two electrodes of the four and in most instances it was unit activity of low amplitude. Seizures were characterized by the spread of ictal activity from the deeper layers of the cortex to the most superficial; usually burst firing was detected in the deepest layers first with subsequent involvement of the more superficial layers. Latencies in the range of 5-8 msec were also observed between bursts and their accompanying field potentials recorded in deeper cortical layers as compared to similar activity recorded in superficial layers. Units, with large potentials, were recorded subsequent to the onset of the seizure while the units of lower amplitude often stopped discharging following the appearance of the afterdischarge. As each seizure progressed in time the latencies observed between activity recorded in deeper layers and superficial layers disappeared and a marked degree of synchronous discharge was noted at all cortical levels.

This preparation serves a useful role in the study of the spread of ictal activity through the cortex and allows for a quantitative description of the nature of neuronal activity at various cortical depths during the spread of a seizure into otherwise normal cortex.

48.7 A REMOTE EFFECT TO A FOCAL INJURY OF THE CORTEX. W.G. Dail, D.M. Feeney, H.M. Murray, R.T. Linn and M.G. Boyeson. Depts. of Anatomy and Psychology, Univ. of New Mexico, Albuquerque, NM 87131.

The morphological reaction of the brain to injury has been well-documented. Typically, attention has been focused on the fate of cells in the area of necrosis and in the reaction of neurons and glia immediately adjacent to the area of focal necrosis. Less is known of the reaction to neurons and glia which are distant to the site of primary injury. As a part of a broader study of the reaction of the brain to injury, we report here an interesting loss of the activity of an enzyme in areas quite remote from the site of direct injury. Craniotomies were performed over the hindpaw area of the motor cortex of rats. Weights which produced a known force were dropped on the cortex to produce a contusion injury while the cortex was undercut to produce a laceration injury. Both types of injury produced a well-defined and reproducible area of necrosis in the underlying cortex. The most notable feature of the injury site was the gradual appearance of a cavity bordered by cells reactive for acid phosphatase. At 36 hrs following the injury, a peculiar loss of staining for the enzyme alpha-glycerophosphate dehydrogenase (α-GDPH) was noted. α-GDPH activity was markedly depressed in cortical layers II and III on the side of the injury. Although the cavitation site was in the hindpaw area of the motor cortex, loss of staining occurred throughout the hemisphere. The depression of α-GDPH activity extended far laterally across the rhinal fissure into the pyriform cortex. A similar loss was seen in the anterior-posterior extent of the hemisphere. The decrease in α-GDPH staining was prominent four days after the injury; however, the staining pattern had returned to normal at nine days. To determine if the response was peculiar to an injury of the motor cortex, additional animals were lesioned in the occipital cortex. The reaction in these animals paralleled that seen in animals with a lesion in the motor cortex. Further studies were performed to determine if an area of the cortex which had received an earlier lesion would show a specific loss of α-GDPH activity after a second lesion. Animals which had received an undercut lesion in the motor cortex 56 days earlier were contused in the occipital cortex. The old injury site presented the same sequelae of changes as seen in other lesioned animals. Additionally, a suction ablation injury involving only a small part of motor cortex resulted in the same widespread reduction of staining for α-GDPH in layers II and III. The derangement in energy metabolism suggests that cells in layers II and III of the cerebral cortex may be particularly vulnerable to perturbations induced by cortical trauma. These findings may be related to the diffuse and transient functional losses observed in head injury in man. (Supported by NIH Grant NS13684-02.)

48.8 COOPERATIVITY IN BRAIN FUNCTION: ASSEMBLIES OF 30 NEURONS. Gordon L. Shaw, Physics Dept., Univ. of California, Irvine, CA. 92717, Erich Harth, Physics Dept., Syracuse, Univ. Syracuse, N. Y. 13210 and Arnold B. Scheibel, Anatomy Dept., Univ. of California, Los Angeles, CA. 90024.

One of the most intriguing features of higher brain function is the occurrance of considerable fluctuations in spike train responses of individual neurons to a repeated, specific "meaningful" stimulus in situations where there is a reliable behavioral response. We propose that the brain deals with these neuronal fluctuations in the cortex by the cooperative interaction of assemblies having a minimum of roughly 30 pyramidal cells. These small assemblies then form the fundamental subunits of neural networks (as in the organizational scheme of Mountcastle, V. B., Neurosc. Fourth Study Program, 1979, p. 31) and provide the basis for a) statistical reliability, b) safety factor against local damage and c) maintainance of firing activity for ~sec in the network. Our suggested minimum of ~30 neurons is roughly an order of magnitude smaller than previous estimates of assembly size (e.g. ,Hebb, D. O., The Organization of Behavior, 1949). We present six, separate arguments to support this minimum number of ~30: 1) Number of repeated stimuli presentations needed to obtain reproducible post stimulus histograms. 2) Number of pyramidal neurons in an orientation column. 3) Number of apical dendrites in a dendritic bundle (Roney, K. J., A. B. Scheibel and G. L. Shaw, Brain Res. Rev. 1, 225 (1979)). 4) Analytical calculation of assembly size from a memory storage model (Shaw, G. L. and Roney, K. J. Phys. Lett. 74A 146 (1979)). 5) Computer calculation on maintainance of firing activity in the network versus assembly size (Harth, E., N. S. Lewis and T. J. Csermely, J. theor. Biol. 55, 201 (1975)). 5) Reliability calculation comparison showing size for which a stochastic system dominates over a deterministic system of neurons (Lewis, N. S., Ph.D. Thesis, Syracuse Univ., 1974). We discuss the experimental and theoretical significance in brain function of these small assemblies. Finally, we speculate on the "purpose" of the neuronal fluctuations as related to fast switching from one sustained firing response to another.

48.9 ENTROPY CORRELATIONS WITH DRUG-INDUCED CHANGES IN SPECIFIED PATTERNS OF NERVE IMPULSES: EVIDENCE FOR "BYTE" PROCESSING IN THE NERVOUS SYSTEM. C. J. Sherry and W. R. Klemm. Dept. of Biology, Texas A & M Univ., College Station, TX 77843.

Entropy, a major information theory descriptor, was calculated on the basis of relative interval coding techniques that encode the serial relationships of 2-5 neuronal action potential intervals as a series of symbols (+, 0, -). Entropy was used to determine the relative amount of 'information' associated with each symbol or set of symbols. It was also used to describe the relative amount of 'information' associated with the relative positive of a specific symbol in a string of symbols.

Average entropy, percent total entropy, and the variability of entropy (standard deviation, coefficient of variation) all correlated with the number of patterns (symbol groups) whose probability of occurrence changed in a statistically significant way in response to ethanol injections. Thus, the overall 'message', or at least the change in the message, induced by ethanol, seems to be encoded by the relative changes in the probability of occurrence of certain quadgrams (i.e. the grouping of four symbols based on the serial relationships of 5 intervals). The ethanol-induced 'message' may be based on the combination of certain quadgrams that increase in incidence and those that decrease in incidence (i.e. the quadgrams whose incidence changes in opposite directions may be 'linked' in some way). This suggests some higher order pattern analysis or 'byte' processing by neurons in the nervous system.

The fact that the 'message' or at least the change in the 'message' is involved in the complex interactions of a series of 'linked' trigrams (three symbols based on the serial relationships of 4 intervals) or quadgrams, might explain why it has been difficult to extract the signal from the noise and, in fact, why some, particularily those who use non-sequential analysis techniques, believe that inter-spike intervals are all noise.

48.10 A THEORETICAL STUDY OF SPATIAL-TEMPORAL INTEGRATION IN BRANCHING DENDRITIC SYSTEMS. Barry Horwitz, Physics Department, Texas Woman's University, Denton, Texas 76204.

There have been a large number of theoretical studies which have investigated the ways by which single neurons integrate their synaptic inputs to produce a neuronal output. In most of these studies either the spatial distribution of the synapses has been ignored, or else the temporal differences in the firing of these synapses have been neglected, although a few computer models have dealt with both (e.g., Pellionisz and Llinás, 1977). In general however, there have been few efforts to study spatial-temporal integration analytically, especially in neuronal models in which the dendritic branching pattern can be varied.

I have developed a theoretical method which enables one to calculate analytical expressions for voltage transients at specific locations in neuronal systems with branching dendritic trees. These time-varying voltages are in response to current inputs at one or more other locations in the dendritic trees. Model systems are studied in which we vary parameters associated with the geometry of the tree (e.g., branch lengths, number of sister branches, number of branches distal to a given bifurcation), with the strength of each synaptic input, and with the relative time of firing of each input. A number of examples will be presented which will illustrate how the voltage transient at a given point depends on these parameters. These examples will be used to examine the concept of the dendritic tree as a computational device, and how the computation which occurs depends on spatial integration, temporal integration, synaptic efficacy, and dendritic geometry. I shall also indicate how this method can be generalized to take account of other parameters, especially the electrical cable parameters.

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49.1 REGULATION OF RECEPTOR POPULATIONS IN FEEDING. E. Omand and J. Zabara. Temple University Health Sciences Center, Depts. of Physiology and Biophysics, Philadelphia, PA 19140

In that the fly's sensorium consists of single chemoreceptors extending into projecting hairs, it is possible to measure individually the output of numbers of single receptors within an overall population. This can be done in succession, since, for instance, most labellar hairs can be sampled in a single preparation. This results in the response of a single type of distributed receptor, i.e., the sugar, salt or water receptor. We recorded the single unit potentials of the chemoreceptors of the fly (Phormia or Musca) to attempt to determine the response profile of the receptor population. Receptor response was measured by placing a glass micropipette containing the stimulus over a sensory hair on the mouth parts. A second, indifferent, glass capillary electrode containing (Bodenstein's) saline was inserted into the proboscidal sinus. The stimulus was chosen to be largely effective for the sugar, salt or water receptors. Feeding and photophase conditions were altered to determine possible changes in membrane receptor sensitivity. Our results indicate that all receptors of a single type do not respond identically. Rather there is a frequency range of response, including a null, which is characteristic of a critical condition of feeding, photophase or age. In the sugar receptor, the range of frequency response is least after eclosion and for several hours thereafter. The range increases with continued fasting to reach its largest value (100 Hz) at approximately 78 hours after eclosion. This is approximately the upper limit of recording during fasting before morbidity effects set in. Then, after feeding these animals, the range of response decreases to approximately half (50 Hz). In addition, the number of null responses is much greater following feeding. Thus, with fasting, there is not only a greater range of frequency responses, but also, a greater variation of frequency response between receptors. In all conditions, null responses were present, but these decreased greatly as fasting continued. During the course of fasting, the receptors increasingly tend toward higher frequencies. Also, the number of receptors with null responses progressively decreases during fasting. After feeding, there is a marked increase in the number of receptors demonstrating a null response. The increase or decrease in the frequency range and the number of null responses are in direct correspondence. A similar effect to fasting and feeding was observed with light and darkness. The similarities of effects do indicate a similar, or same, mechanism involved in both modalities, which possibly might explain their strong interactive effects. These results support the hypothesis of a regulatory factor which can progressively increase or decrease the sensitivity of the receptors.

49.2 MEMBRANE RECEPTOR POLARIZATION IN FEEDING. J. Zabara and E. Omand Temple University Health Sciences Center, Depts. of Physiology & Biophysics, Philadelphia, PA 19140.

The nature of the regulation of chemoreceptors in feeding is at present unknown. It would appear that irrespective of whether the efferent regulation of the receptor is humoral, ionic or neural, the net effect is to change its response characteristics. A possible experimental approach is based on the assumption that the change in the receptor is related to an alteration of the polarization of the receptor membrane. This polarization might be related directly to a change in the receptor potential. The receptor preparation of the fly offers the advantage here over the mammal in that the receptor potential and membrane impulse discharge can be correlated directly. Single unit and dc potentials were measured by placing a glass micropipette containing the stimulus over a sensory hair on the mouth parts (Musca). Another glass capillary containing (Bodenstein's) saline was inserted into the proboscidal sinus to serve as a second, indifferent electrode. With dc coupling of the amplification system, it is possible to observe a steady potential which represents either the actual receptor potential or the postulated generator current between the receptor and spike initiating sites. In these experiments, a combined dc recording at relatively low amplification to monitor this steady potential is compared with a simultaneous ac recording at relatively high amplification to monitor the spike discharge. The dc potential is initiated by contact of the capillary electrode with the sensillum, and the initial oscillating current (surge) usually observed in ac recordings is absent. The current surge often will block temporarily the preamplifier preventing initial observation of receptor response. This indicates that the current surge oscillation is based on a relatively short time constant of the recording system. The rise of the dc potential apparently represents partially a contact potential between electrode and sensillum. Our observation that the polarization change is of opposite polarity to the spike potential is consistent with a receptor process in this preparation based apparently on a separation between receptor and spike initiation sites. Further, after the rapid, initial rise in potential at electrode contact, a slow, steady increase in the level of polarization (dc recording) parallels a corresponding decrement of impulse frequency. A linear relationship between spike frequency and dc potential change would indicate a direct action of this potential on the spike generation site. This dc potential shift reflects a membrane change which might be the basis of observed receptor sensitivity control. These results are incorporated into a model for the regulation of receptor populations based on an apparent time constant effect in membrane receptor state.

49.3 AFFERENT AND EFFERENT PROJECTIONS OF THE NUCLEUS MEDIANUS: AN HRP STUDY. Victor M. Grazi*, Richard R. Miselis. Animal Biol. Sch. Vet. Med., Inst. Neurol. Sci., Univ. of Pennsylvania, Philadelphia, PA 19104.

The nucleus medianus (NM) of the medial preoptic area is a prominent structure of the anteroventral third ventricular (AV3V) area which is involved in the behavioral and physiological control of water balance. Little is known of its connectivity; however, recent anatomical studies have demonstrated projections from the subfornical organ (SFO) to the NM, and from the NM to the supraoptic nuclei (SON) (Miselis et al. Science, 205:1022, 1979). The connectivity of the NM was examined using horseradish peroxidase (HRP) histochemistry. 100 nl. of HRP (100 ug/ul; Sigma type VI, lot #20F-9600) were injected via a stereotaxically placed 33g cannula aimed for the anterior border of the NM. An anteriorly angled approach was used to avoid disruption of fibers directly above the NM. Ten SD male rats were included in this study and were sacrificed 48 hrs. post-injection. The brains were sectioned at 40 um and processed histochemically using the tetramethyl benzidine procedure. Three animals were injected with a cannula terminating just anterior to the NM. These injections primarily involved the ventral and middle portions of the NM and there was some spread of HRP into the medial septal nuclei (MS) and the nucleus of the vertical limb of the diagonal band (DBB). Both retrograde and anterograde labelling occurred. We observed retrogradely labelled HRP-positive neurons lying peripherally within the SFO, confirming the previously reported projections from the SFO to the NM. These labelled neurons were vertically oriented bipolar neurons. In addition, retrogradely labelled cells were observed in the horizontal DBB, and within the vicinity of the SON, initially lateral to the SON and then dorsolaterally and lastly medial to it at its caudal end. Efferent fibers were observed leaving the injection site and passed along the same trajectory adjacent to the SON where retrogradely labelled neurons were observed. Labelled fibers of passage orient from this area to the SON and terminate just short of it, but sparse terminals within the SON do occur. Terminals from these efferent projections of the NM also occur along this course. Differences and similarities of the projections from the NM from those of the MS and DBB will be discussed. This data further supports the involvement of the NM in the neuronal circuitry of thirst and the physiological control of body fluid balance and extends the description of this neural substrate.
(Supported by the Sloan Foundation, RR-07083, and Grass Foundation).

49.4 GLUCORECEPTORS FOR FEEDING AND HYPERGLYCEMIA: EVIDENCE AGAINST THEIR LOCATION IN THE FOREBRAIN. Peter G. Slusser*, Steven L. Stone* and Robert C. Ritter (SPON: R.W. Clark). Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, College of Veterinary Medicine, Washington State University 99164 and Department of Veterinary Science, University of Idaho, Moscow, ID 83843.

Intracerebroventricular (ICV) infusion of 2-deoxy-D-glucose (2DG) causes increased feeding and hyperglycemia in the rat. However, the anatomical loci of the receptors which mediate these responses are unknown. The traditional bias toward the hypothalamus as the receptor site is not supported by convincing evidence. In addition, the relatively high molar concentrations of ICV 2DG required to cause feeding and hyperglycemia limit the usefulness of this analogue in the search for the glucoreceptors. Recently, we have demonstrated that ICV infusion of 5-thioglucose (5TG) is 10 to 100 times more effective than 2DG for eliciting feeding and hyperglycemia. Therefore, we have been using 5TG in a series of experiments designed to localize the brain glucoreceptors which mediate feeding and hyperglycemia. We have compared the feeding and hyperglycemia produced by lateral ventricle infusion of 5TG, with that which occurs in response to fourth ventricular infusion. Furthermore, we have examined the effect of lateral ventricle infusions of 5TG prior to and subsequent to blockade of the cerebral aqueduct with silicone plugs. We have found that fourth ventricular infusions of 5TG (45, 90, or 180 µg/3 µl) cause greater elevations of blood glucose than do lateral ventricle infusions. Also, we have found that rats which feed in response to lateral ventricle infusion of 5TG prior to occlusion of the cerebral aqueduct fail to respond after aqueductal occlusion. Rats with aqueductal obstruction also fail to exhibit hyperglycemia in response to lateral ventricle infusion of 5TG. Aqueduct-obstructed rats do exhibit both feeding and hyperglycemia in response to subcutaneously injected 2DG. Our data suggest that the brain glucoreceptors which mediate glucoprivic feeding and hyperglycemia may not be located in the forebrain. Rather, these receptors may be located in the vicinity of the fourth ventricle. Experiments in progress are designed to test this hypothesis.

49.5 CONTRIBUTIONS OF PERIVENTRICULAR STRUCTURES OF THE ROSTRAL THIRD VENTRICLE TO THE MAINTENANCE OF DRINKING RESPONSES TO HUMORAL DIPSOGENS AND BODY FLUID HOMEOSTASIS. E.E. SHRAGER* & A.K. JOHNSON (SPON: L.D. MITCHELL). Dept. of Psychol., Univ. of Iowa, Iowa City, Iowa 52242.

Both the subfornical organ (SFO) and the periventricular tissues surrounding the anteroventral third ventricle (AV3V) have been implicated in the regulation of body fluid homeostasis and, in particular, the drinking responses to specific humoral stimuli, e.g., angiotensin(AII) and hypertonic saline (HTS). Moreover, neural and vascular connections between these regions have been reported. The purpose of the present analysis was to compare the effects of systematic ablation of subsections of tissue along the dorso-ventral extent of the rostral third ventricle on the drinking responses to AII and HTS. The acute effects of differential brain damage on ad lib water intake and fluid related body weight loss were also examined.

Rats were screened presurgery to 4 doses each of subcutaneously administered AII and HTS. Subjects were then divided into 4 lesion groups: 1) Dorsal placements in which electrodes were aimed at the SFO; 2) Midrostral placements aimed to include the median preoptic nucleus (MnP); 3) Ventral placements in which electrodes were directed at the organum vasculosum of the lamina terminalis (OVLT); and, 4) Sham lesions. Approximately 6 wk. after surgery all surviving subjects were again tested to the range of doses of AII and HTS.

Lesions of the ventral-rostral third ventricular region, but not the MnP or SFO regions, produced severe adipsia and associated weight loss. This was the only lesion placement which resulted in terminally adipsic animals. Dose-response analyses for drinking to AII and HTS indicated that damage limited to the ventral level produced no long term response deficits to these thirst challenges. Lesions at both the level of the MnP and of the SFO produced deficits to AII but not HTS.

These data support the independence of the drinking responses to AII and HTS and indicate, furthermore, that the acute period of adipsia associated with AV3V lesions is independent of chronic deficits to either humoral dipsogenic stimulus. The results suggest that initially, adipsia may reflect a primary disruption of thirst mechanisms but that extended adipsia may be largely related to the dehydration which results from the inability to conserve fluid. Finally this study found that tissue of both the SFO and the MnP are necessary, but neither alone is sufficient, for the maintenance of the drinking response to systemically administered AII. (USPHS NIH HLP-14388 & 1 RO1-HL24102; NIMH 1-KO2-MH00064).

49.6 TRANSIENT APHAGIA FOLLOWING LESIONS TO QUINTO-FRONTAL TRACT IN GROWING CHICKS. Wayne J. Kuenzel*(SPON: S. Brauth). Dept. of Poultry Science, University of Maryland, College Park, MD 20742.

The quinto-frontal tract (QFT) is reported to be a critical structure controlling feeding behavior in the pigeon. Lesions of quinto-frontal structures produce periods of aphagia ranging in duration from several days to several weeks (Zeigler, H.P. and H. J. Karten. J. Comp. Neurol., 152:59, 1973). The QFT and adjacent neural structures were ablated in growing chicks, Gallus domesticus, to evaluate their effects on food intake.

Three groups of chicks (2-3 weeks of age) were bilaterally lesioned using stainless steel insect pins (#1), anodal D.C., 1 mA for 15 or 30 sec. A fourth group served as sham-operated controls. An unpublished chick atlas was used to direct lesions to three areas of the brain: QFT near mid-hypothalamus, far-lateral hypothalamic area, and posterior hypothalamic-midbrain region. Group 1 was designated QFT chicks. Stereotaxic coordinates used were comparable to A 7.5, D 4.0, L 2.0 published for adult hens (vanTienhoven, A. and L. Juhasz. J. Comp. Neurol., 118:185, 1962). Group 2 included the far-lateral hypothalamic chicks. Stereotaxic coordinates used were comparable to A 7.5, D 3.5, L 2.5 (vanTienhoven and Juhasz, 1962). Group 3 was designated the posterior hypothalamic-midbrain chicks. Coordinates used were comparable to A 4.5-5.0, D 2.5, L 1.75 (vanTienhoven and Juhasz, 1962).

The QFT chicks (n = 6) were aphagic for an average of 3.7 days, lost 15.7% body weight, and took an average of 5 days to return to their pre-operational weight. Neural structures damaged included the QFT, ansa lenticularis (AL), n. intercalatus (IC), stilus corpus geniculatus (SG), and lateral hypothalamic n. (LH). The far-lateral hypothalamic chicks (n = 5) were aphagic for 1.2 days and lost only 6.8% body weight. Neural structures damaged included the ventrolateral geniculate body (GV), SG, IC, AL, n. entopeduncularis superior (ES) and the medial portion of the n. rotundus (ROT). The posterior hypothalamic-midbrain chicks (n = 6) were aphagic for 5 days, lost 24.0% body weight, and took an average of 7 days to return to their pre-operational weight. Neural structures damaged included the QFT, AL, and stratum cellulare externum (SE).

It is concluded that large bilateral lesions are necessary to render growing chicks aphagic. Although QFT lesions disrupt feeding behavior, more permanent aphagia occurs when other structures are destroyed concurrently such as the AL and SE.

49.7 ALTERED DIETARY PREFERENCE BUT NORMAL CALORIC REGULATION IN RATS WITH AREA POSTREMA LESIONS. Robert C. Ritter and Gaylen L. Edwards. Department of Veterinary Medicine, University of Idaho, Moscow, ID 83843 and Department of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

The area postrema (AP) is a circumventricular organ (CVO) of the caudal brain stem. We have previously shown that lesions of the AP cause rats to over-ingest a preferred diet during short (30 min) tests (Edwards, G. and Ritter, R., Neurosci. Abstr., Vol. 5, p. 215, 1979). In subsequent studies, we have found that AP-lesioned rats also ingest significantly more than intact rats when offered 23.7% glucose during 30 min ingestion tests. These results suggested an enhanced responsiveness to preferred tastes in AP-lesioned rats. The results presented below were obtained in order to determine whether exaggerated ingestion of a normally preferred diet by AP-lesioned rats had any consequences for their caloric regulation. Male AP-lesioned and intact rats ingested virtually identical amounts of lab chow and water ad lib or after 21 hr food deprivation. When given continuous ad lib access to lab chow and a preferred liquid food (milk-instant breakfast), AP-lesioned and intact rats displayed the same total caloric intake. However, intact rats consumed 40% of their calories as lab chow, whereas lesioned rats took at least 90% of their calories as instant breakfast and virtually ignored the lab chow. This difference between the two groups of rats was apparent immediately upon presentation of the preferred diet and persisted for the entire 14-day conduct of the experiment. When caloric concentration of the liquid diet was halved or doubled, both groups adjusted their intake and kept caloric consumption constant. However, under both calorically diluted or concentrated conditions, the lesioned rats ate more liquid diet than intact rats and consumed very little lab chow.

Our data demonstrate that over-ingestion of a preferred diet by AP-lesioned rats persists when that diet is continuously available. Our results also suggest that caloric control of intake is not disrupted in AP-lesioned rats. The findings reported here and those previously reported raise the possibility that the AP's role in the control of ingestive behavior may be to modulate responsiveness to taste cues rather than to adjust caloric intake.

49.8 FEEDING DEFICITS FOLLOWING LESIONS TO THE AREA POSTREMA AND ADJACENT PORTIONS OF THE NUCLEUS OF THE SOLITARY TRACT. Thomas M. Hyde* and Richard R. Miselis. Dept. of Anatomy, Sch. of Med. and Sch. of Vet. Med., University of Pennsylvania, Phila. PA 19104.

The area postrema (AP), a circumventricular organ, lies caudal to the 4th ventricle and dorsomedial to the nucleus of the solitary tract. The AP lacks a blood-brain and a total CSF-brain barrier making this structure a suitable receptor for substances in the blood or the CSF. There is a reciprocal connectivity between the AP and NTS. The subdiaphragmatic vagal afferents may project directly into the AP and to the NTS ventrolateral to the AP. These features suggest a role for the AP in ingestive behavior either as a chemoreceptor or as an integrator of peripheral visceral afferent input via the vagus. Aspiration lesions were made under direct visual guidance in male and female adult rats. All rats were maintained on an ad libitum diet of laboratory chow and water. Sham lesioned males returned to preoperative levels of food intake and body weight by the 3rd day after surgery. Lesioned rats remained hypophagic for at least 3 weeks postoperatively and were at 75% of prelesion levels on day 21. Body weights declined 25% below prelesion levels on day 12 and then progressively increased by 2 g/day to 78% of prelesion body weight on day 21. Lesioned rats displayed a mild hypodipsia for the first 7 days after surgery whereas sham lesioned rats return to baseline levels on day 2. Female rats displayed a similar pattern. All lesioned rats displayed no obvious postural or motor deficits. Lesioned rats were tested with 2-deoxyglucose (160 mg/kg of body weight, injected IV via a jugular catheter) within 10 days of surgery. Lesioned rats did not increase feeding from saline controls within the 1st hour after injection. When tested with insulin (4 U Iletin, injected SC), they responded normally except for a longer latency to feeding. In order to assess reactivity to aversive stimuli, lesioned and sham lesioned rats were given access to 0.01% Quinine-adulterated water for a 3 hr. period after 22 hrs. of water deprivation on day 6 postoperatively. Lesioned rats drank only 28% (in ml/g of body weight) of the sham totals. Under a similar paradigm, lesioned rats drank 58% of the sham totals when given access to plain water. In another group, prelesion experience with this test attenuated this difference. Histological examination of lesioned rats revealed a loss of the AP and varying degrees of damage to the NTS. Ingestive behavior is disturbed by loss of the AP and adjacent NTS. Since this area is a likely site for the termination of abdominal vagal afferents, this lesion may provide a means of studying the effect of the loss of abdominal afferents without major loss of vagal motor fibers.
(Supported by the Sloan Foundation, RR-07083 and ITG GM-07170).

49.9 ALTERATIONS IN FEEDING BEHAVIOR AND BODY WEIGHT FOLLOWING GLOBUS PALLIDUS LESIONS IN RATS: EFFECTS OF PREOPERATIVE DIETING. Carlos V. Grijalva. Dept. of Psychology, University of California, Los Angeles, CA 90024.

Lateral hypothalamic (LH) damage produces a well-defined syndrome characterized by initial periods of aphagia, anorexia, and adipsia, followed by recovery of ingestive behaviors although certain homeostatic deficits persist (Teitelbaum & Epstein, Psychol. Rev. 69: 74, 1962). Powley and Keesey (J. comp. physiol. Psychol. 70: 25, 1970) showed that rats preoperatively reduced in body weight by dieting recovered feeding behaviors sooner after LH lesions than rats at normal body weight prior to surgery. However, groups of rats either preoperatively dieted or at normal body weight maintained equivalent lower body weight levels after LH lesions relative to nonlesioned controls. Because lesions of the globus pallidus (GP) produce feeding deficits quite similar to those following LH lesions (e.g., Morgane, Am. J. Physiol. 201: 420, 1961; Neill & Linn, Physiol. Behav. 14: 617, 1975), the present study was conducted to examine the effects of preoperative dieting on feeding behavior and body weight maintenance after GP lesions.

Four groups of 8 male hooded rats were matched for initial body weight. Two groups were reduced to 80% initial weight by dieting (Grijalva et al. J. comp. physiol. Psychol. 90: 505, 1976) over a 14 day period. The remaining two groups were fed ad lib during the same calendar period to maintain normal weight. One dieted and one normal weight group received bilateral electrolytic GP lesions. The remaining dieted and normal weight group were given control operations.

The results showed that preoperative dieting significantly shortened the period of aphagia induced by GP lesions. The GP lesion-dieted group was aphagic for 2.4 days while the GP lesion-normal group was aphagic for 5 days. Both groups, however, were anorexic for about 2 to 3 days. Two months following surgery both the GP lesion-dieted group and the GP lesion-normal group were approximately 20% lower in body weight relative to both control-operated groups which were at similar body weight levels.

These findings indicated that dieting attenuated the period of aphagia but did not alter body weight maintenance which was chronically reduced following GP lesions. These findings support the view that certain disruptions in feeding behavior and body weight regulation after LH lesions may be due to interruption of fibers of passage originating in the globus pallidus or other structures of the corpus striatum.

49.10 FEEDING AND DRINKING FOLLOWING ELECTROLYTIC LESIONS OF THE MEDIAN RAPHE NUCLEUS, Karen E. Asin and David Wirtshafter. Dept. of Psychology, Univ. Illinois-Chicago, Chicago, IL. 60680

Since the median nucleus of the raphe (MR) appears to be profusely interconnected with forebrain structures, including the hypothalamus, this study sought to investigate the effects of electrolytic MR lesions on ingestive behaviors under various conditions.

Albino, male rats served as subjects. One group of rats had A.M. and P.M. food and water intakes and body weight recorded and, after a baseline period, the animals were either sham operated (C) or given a MR lesion (1ma/8sec). Intakes and body weight were followed for several weeks. Postoperatively, only a few rats showed a transient hyperdipsia. Lesioned subjects lost significantly more weight than C's for the first 4 days postop.,after-which body weight was maintained at a lower level but weight gain paralleled that of C's. Following a 4-day decrease in overall food intake, MR lesioned rats consumed as much food as C's during a 24 hour period but ate significantly less during the day. Later tests revealed no difference in food intake between groups during a 24 hour water deprivation period or 1,2 or 6 hours after an injection of insulin or 2-deoxy-D-glucose (2DG), although lesioned rats showed a greater suppression of food intake 24 hours after 2DG compared to controls. All MR lesioned rats were hyperactive.

Another group of rats had water intakes recorded before and after surgery; again, only an occasional subject was hyperdipsic, although all were hyperactive in the open field. During 24 hours of food deprivation, lesioned subjects sharply reduced their water intakes, often to less than 5mls. There were no differences in intakes following injections of hypertonic NaCl, isoproterenol, or polyethelene glycol when measured at 1 and 3 hours after treatment.

A third group of rats was allowed a 2 week postsurgical recovery period and was then placed on a 23 hour food deprivation schedule. Again, lesioned subjects drank significantly less in the absence of food and as the deprivation schedule continued, MR lesioned rats drank almost exclusively during the 1 hour feeding period in amounts greater than C's. Furthermore, during feeding MR lesioned rats took significantly more drinks and showed significantly more spillage than C's, although the amount eaten was the same.

In a final group, MR's and C's were maintained in a cage which monitored the amount and pattern of food and water intake.Lesioned rats showed marked prandial drinking, with small draughts occuring frequently within a meal, sometimes after each 45mg pellet. This behavior is reminiscent of that seen in an LH lesioned rat.

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50.1 ALTERATIONS OF SODIUM-POTASSIUM ATPase DURING CEREBRAL ISCHEMIA AND RECIRCULATION. R.B. Schwartz*, A. Spatacco*, and F.A. Welsh* (SPON: B. Uzzell). Division of Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104.

The purpose of this study was to determine whether cerebral ischemia alters the activity of Na^+,K^+-ATPase measured in vitro and, if so, whether that alteration persists in recirculated brain which has been irreversibly damaged. Incomplete ischemia, of sufficient severity to deplete tissue levels of ATP and phosphocreatine, was produced in cat brain by occlusion of the common carotid arteries plus rapid arterial hemorrhage to a mean arterial pressure of 30 torr. After 30 min of ischemia, the brain was recirculated for 2-4 hours prior to in situ freezing with liquid N_2. The frozen brain was sampled regionally for analysis of Na^+,K^+-ATPase activity and of ATP content.

Thirty min of ischemia caused Na^+,K^+-ATPase activity in cerebral cortex to increase from control values of 7.4 ± 0.7 to 11.5 ± 1.1 mmol/min per kg wet weight ($p < 0.01$). In recirculated brain, the activity of Na^+,K^+-ATPase returned to control (6.4 ± 0.4 mmol/min kg) in regions which resynthesized ATP to greater than 63% of preischemic levels. However, in brain regions with severe energy failure (ATP less than 40% of control), Na^+,K^+-ATPase activity remained markedly elevated: 12.1 ± 1.0 mmol/min kg ($p < 0.01$). The activity of Mg^{++}-activated (ouabain insensitive) ATPase did not change significantly during ischemia or recirculation.

These results demonstrate that ischemia causes a 55% increase in the in vitro activity of Na^+,K^+-ATPase and that this increase persists only in brain regions which have undergone irreversible energy failure. We speculate that the activation of Na^+,K^+-ATPase may result in excessive hydrolysis of ATP during the post-ischemic recivery period, thereby preventing restoration of tissue levels of ATP and, thus, contributing to the ultimate energy failure.

50.2 RELATION BETWEEN MEAN ARTERIAL BLOOD PRESSURE, LACTIC ACID ACCUMULATION, AND BRAIN INJURY FROM MARKED HYPOXIA IN RHESUS MONKEYS. K.R. Wagner, M.E. Brown*, S. Yamaguchi, R.E. Myers. Lab. of Perinatal Physiology., NIH, Bethesda, Md. 20205

Animals exposed to 25 minutes of marked hypoxia (PaO_2=15 mmHg) not accompanied by reductions in mean arterial blood pressure (MABP) below 65 mm Hg do not injure their brains (Myers, et al, J Neuropath Exp Neurol, 1980). Animals exposed to marked reductions in MABP and high serum glucose concentrations during hypoxia elevate lactic acid of cisternal CSF to values above the 16-18 mM threshold required for brain injury (deCourten, et al, J Neuropath Exp Neurol, 1980) as established previously (Myers, Adv Neurol 26:195, 1979). The present study exposes food-deprived rhesus monkeys to marked hypoxia (PaO_2=12-18 mm Hg) by respiring them with 3.5% oxygen for 2.5 to 25 minutes. Their brain metabolite concentrations were preserved by in situ freezing (Welsh & Rieder, J Neurochem 31:299, 1978) and tissue extracts assayed for glycogen, glucose, lactate, ATP and phosphocreatine using enzymatic-fluorometric methods. The animals exposed to 2.5 and 5 minutes of hypoxia elevated their cortical lactic acid (control=1.5-2.5 µmoles/g) to 7 and 9-10 µmoles/g, respectively. They then showed no further increase in lactic acid concentration for up to 25 minutes of hypoxia unless they experienced blood pressure troughs (MABP < 60-80 mm Hg) or a cardiovascular collapse. They decreased phosphocreatine at all times during hypoxia (3.5 µmoles/g; control=4.0 µmoles/g) and maintained glycogen, glucose and ATP concentrations unchanged from control values (5.0, 3.5, 2.1 µmoles/g, respectively) as long as they maintained blood pressure > 60-80 mm Hg. Animals that reduced MABP < 60 to 80 mm Hg at any time during hypoxia markedly elevated their cortical tissue lactic acid to concentrations >20-30 µmoles/g. They also significantly reduced their brain tissue glycogen, glucose, and high energy phosphates. These results, which strongly support our pathologic studies, indicate that animals exposed to hypoxia that maintain MABP > 60-80 mm Hg do not accumulate lactic acid to concentrations sufficient to injure their brains. Animals that experience marked reductions in MABP, and, therefore, in cerebral perfusion producing a near-anoxia of their brains, elevate lactic acid concentrations to values sufficiently high (>18-20 µmoles/g) as to induce brain injury.

50.3 COLUMNAR ALTERATIONS OF NADH FLUORESCENCE DURING HYPOXIA-ISCHEMIA IN NEWBORN RATS. A. Spatacco*, F.A. Welsh*, R.C. Vannucci*, and P. Campbell* (SPON: T.W. Langfitt). Division of Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104 and Department of Pediatrics, Hershey Medical Center, Hershey, PA 17033.

Exposure of 7-day postnatal rats with unilateral carotid artery ligation to 8% oxygen for 3 hours causes necrosis in the cerebral cortex, which frequently occurs in columns oriented at right angles to the pial surface (Rice et al., submitted to Ann. Neurol.). A similar columnar pattern of NADH fluorescence has been demonstrated during incomplete ischemia in cat brain (Welsh et al., Science 198, 951-953). In order to determine whether columnar alterations in metabolism occur in the above model of hypoxia-ischemia in newborn rats, we have measured NADH fluorescence and regional levels of ATP and phosphocreatine in brain frozen at various times during the insult.

At 60 min of hypoxia-ischemia, intensely fluorescent columns of NADH appeared in the cortex ipsilateral to the carotid occlusion. However, since tissue levels of ATP and phosphocreatine were depleted throughout the ipsilateral cortex, it was apparent that hypoxic alterations of high energy phosphate levels were not restricted to brain regions with increased NADH fluorescence. At 3 hours of hypoxia-ischemia, NADH fluorescence intensity had returned to normal levels, except in 2 of 5 animals in which faint columns of increased NADH persisted. At the same time, tissue levels of ATP and phosphocreatine remained lower than 0.1 mmol/kg.

Although the regional pattern of NADH fluorescence during hypoxia-ischemia corresponded well with the ultimate pattern of neuropathology, columnar necrosis cannot be explained simply by columnar depletion of high energy phosphates. Rather, the secondary decline of NADH fluorescence intensity between 1 and 3 hours may signify, in the presence of continued energy depletion, irreversible damage to the citric acid cycle-catalyzed generation of NADH.

50.4 NEUROCHEMISTRY OF IODOTHYRONINES. J. T. Gordon*, F. L. Crutchfield*, A. S. Jennings* and M. B. Dratman. V. A. Hospital, Philadelphia, Pa. 19104.

The work to be reported was aimed at clarification of the action of thyroid hormones in the autonomic and central nervous systems. It has been reported that accumulation of 3',3,5-triiodothyronine (T_3) derived from thyroxine (T_4) occurs more extensively in brain than in other tissues. Sucrose gradient fractionation of rat brain homogenate in this laboratory showed further enhancement of T_3 relative to T_4 in synaptosomes (Dratman, M. B. and Crutchfield, F. L., Am. J. Physiol. 4:E638, 1978). We now report the presence in rat brain of additional iodothyronines, 3',5',3-triiodothyronine (rT_3) and 3',3-diiodothyronine (3',3-T_2). In two separate experiments, rat pups reared perinatally on $^{125}I^-$ were sacrificed at intervals beginning at 5 days from birth up to 17 days. In each experiment brain, muscle, liver and serum were extracted and evaluated for ^{125}I-compounds. Extraction was by $CHCl_3$-CH_3OH (2:1, v/v) and was followed by solvent partitioning by addition of 0.05% aq. $CaCl_2$ and freeze-drying of the separated aq. CH_3OH layers. Samples were redissolved in CH_3OH for analysis by two-dimensional paper chromatography and autoradiography with subsequent excision of ^{125}I-regions for quantitative measurement of radioactivity. The chromatographic systems were 1-butanol, ethanol, 0.5 N NH_4OH (5:1:2, ascending) followed by t-amyl alcohol, hexane, 2 N NH_4OH (5:1:6, descending). Co-chromatography of authentic ^{125}I-rT_3 and ^{125}I-3',3-T_2 with duplicate extracts established the identity of the metabolites.

At 5 and 7 days rT_3 and 3'3-T_2 were present in brain and not detected in muscle, liver or serum. Thus it can be concluded that at those ages concentrations of rT_3 and 3',3-T_2 relative to T_4 are larger in brain than in the other tissues studied. In 13 to 17 day old pups rT_3 decreased to a trace in brain and appeared as a trace in the other tissues, while 3',3-T_2 continued to be detected only in whole brain, brain subcellular fractions and individual brain regions. That levels of 3', 3-T_2 relative to T_4 varied from 10 to 24 (average 16) % while rT_3 levels decreased from 21% to a trace is attributed to an increase in T_3 relative to T_4 after day 13. Ingbar and associates (J. Clin. Endocrinol. Metab. 46:916, 1978) have shown that T_4 deiodination in humans is a sequential process. Our findings suggest the nerve cell or nerve ending as a functional site for sequential deiodination of thyroid hormones. These observations may be relevant to understanding the potent analeptic and ß-adrenergic effects of thyroid hormones. Supported by funds from the NIMH Grant #MH29549, and Med. Res. Service, VA Hospital, Philadelphia, PA.

50.5 INTERACTIONS BETWEEN GLUCOSE AND KETONE BODY USE BY DEVELOPING BRAIN. A.L. Miller, C.A. Kiney,* D.H. Corddry* and D.M. Staton.* Mailman Research Center, McLean Hospital, Belmont, MA 02178.

Human and rat brain have considerable capacity to use blood acetoacetate and β-hydroxybutyrate (BHB) as fuels. Use of these ketone bodies by brain is most rapid in developing brain and, at all ages, varies directly with their blood levels. Some studies have found that loss of lactate from brain occurs when ketone body uptake increases. Whether brain glucose utilization is also affected has been unclear.

To examine the interactions between glucose and ketone body use by developing brain, we injected 20 day old rats with sodium DL-β-hydroxybutyrate (0.4 mmoles/rat, I.P.) and studied them under normocapnic and hypercapnic (20% CO_2, 21% O_2, 59% N_2) conditions. Brains were obtained with a freeze-blowing apparatus at 2, 6, 10 and 15 min after BHB administration. Brain glucose utilization was determined with [2-^{14}C]glucose and [^{3}H]deoxyglucose as tracers. Metabolites were assayed enzymatically.

In normocapnic rats, rates of glucose utilization by brain progressively decreased after BHB administration. These decreases were found with both radioisotopes, but were greater with [2-^{14}C]glucose as tracer. Brain concentrations of glucose 6-phosphate, pyruvate, citrate, α-ketoglutarate, malate and glutamate increased in the BHB-treated animals. During hypercapnia brain glucose utilization fell to a greater extent in BHB-treated rats than in saline-injected rats. Decreases in brain concentrations of pyruvate, lactate, citrate, α-ketoglutarate, malate and glutamate were not so rapid, however, in the BHB-treated hypercapnic rats as in the hypercapnic saline group.

We conclude that ketonemia produced by BHB injection into 20-day old rats results in decreased brain glucose utilization and increased loss of labeled lactate from brain. The decrease in glucose utilization is quantitatively more important. The increased size of the pools of Krebs cycle intermediates and of glutamate indicated an imbalance between the rate of entry of ketone bodies into the Krebs cycle and the rate of oxidative decarboxylation. The inhibition of brain glucose utilization that occurs during hypercapnia is not lessened by concurrent ketonemia but the rate of depletion of intermediary metabolites and glutamate is slowed. These observations confirm the ability of ketone bodies to partially replace glucose as an oxidative fuel for brain, but also show the limitations on short-term regulation of their use. Thus, in the normocapnic, ketonemic rats, the decrease in glucose carbon entry into the Krebs cycle did not balance the increase from ketone bodies and in hypercapnic, ketonemic rats entry of ketone bodies did not increase sufficiently to compensate for the inhibition of glucose utilization.

50.6 TEMPERATURE EFFECTS IN THE RESPIRATORY CHAIN OXIDATION OF REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE OF NEURONS. Carlos Rodríguez-Estrada. Cátedra de Fisiología. I.M.E. Fac. Medicina, U.C.V., Caracas, Venezuela.

Previous work (Brain Res. 6:217) has shown in soma neurons that excitation at 5°C produced a metabolic response characterized by a single change, oxidation, but re-warming at 25°C this metabolic response shows its normal metabolic change, that is oxidation, followed by reduction of nicotinamide adenine dinucleotide. This work studied the effect of temperature on the steady state level of reduced nicotinamide adenine dinucleotide (NADH). Fluorometric determinations of NADH were done on in vitro preparations of dorsal root ganglion neurons of frogs (Rana palmipes spix). Each preparation was placed in a moist chamber at a constant temperature of 25°C controled by a water flow under the floor of the chamber. This temperature was changed to 5°C and controled in the same way. A record of the temperature of the chamber was taken near the preparation with a thermistor. This chamber was provided with an outlet and inlets for gases. Recordings were done of the pO_2 at the outlet of the chamber. A sudden change of temperature from 25°C to 5°C in aerobic conditions produced a sudden increase of NADH level. This level did not stayed high and constant. It decreased (oxidation) slowly towards the level observed previously to temperature change. Returning the temperature to 25°C there was a sudden decrease of NADH level. This level did not stayed constant; it returned slowly towards the level observed previously to temperature change. A sudden change of temperature from 25°C to 5°C in anaerobic conditions produced a sudden increase of the NADH level. The new level stayed constant. Returning the temperature to 25°C there was a sudden change of NADH; the NADH level returned to a level previously recorded to temperature change. In anaerobic conditions at 5°C a change to aerobic conditions produced a sudden decrease (oxidation) of NADH level. The rate of change of NADH oxidation observed at 5°C is similar to that one observed at 25°C. Indicating that the rate of change of respiratory chain oxidation at 25°C as well as at 5°C is similar.

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50.7 CONSTANCY OF OXYGEN CONSUMPTION DURING TOTAL ISCHEMIA IN THE GERBIL CEREBRAL CORTEX. R. Martin and J.H. Halsey*. Dept. of Neurology, Univ. of Alabama in Birmingham, The Medical Center, Birmingham, Alabama 35294.

The constancy of oxygen consumption of the cerebral cortex during total ischemia was determined by analysis of the rate of falling pO_2 as measured by an oxygen microelectrode during transient bilateral carotid occlusion in barbiturate-anesthetized gerbils. Measurements were performed in animals under hyperbaria, heavily barbiturate-suppressed metabolism, or Metrazol seizure.

Occlusion produced an immediate (within .5 second) fall in pO_2. Under normoxia, oxygen was depleted usually within 5 seconds. However, as the pO_2 of occlusion was raised via hyperbaria, some measurements exhibited an initial linear fall in pO_2 followed by a "quasi-exponential" decay indicating slowing metabolism. In several instances this decay continued until the slope became zero leaving residual oxygen within the tissue. The EEG during this decay became increasingly inactive.

In the hyperbaric experiments, the rate of oxygen consumption was calculated for those measurements where the occlusion was performed at tissue pO_2 above 100 torr. This was necessary to avoid the slowed rate of oxygen disappearance found at normal tissue pO_2 due to the desaturation of capillary hemoglobin. In ten animals, the range of oxygen consumption for parietal cortex was found to range from 1.6-7.4 cc/100 cc tissue-min. with a mean of 4.4.

Both the barbiturate-suppressed group and Metrazol group had control and treatment phases. Of all control measurements, 47% were found to exhibit a period of decreasing slope of these, 45% changed slope within the first 2 seconds following ischemic onset and 55% changed in 2-5 seconds. In the barbiturate-suppressed phase, 37% had a change in slope with 86% changing within 2 seconds and 14% changing in 2-5 seconds. Of the measurements made during the Metrazol seizures, 47% had a change in slope and all of these changed within 2 seconds of ischemic onset. It may be that this slowing metabolic rate is a result of metabolite accumulation due to the ischemia. If true, the higher metabolic rate produced by Metrazol administration should generate more metabolite per unit time while the highly suppressed metabolism following high doses of barbiturate should require a longer period to produce this critical level. At low pO_2 measurements, the oxygen may be depleted before any change is evident, and as the pO_2 of measurement is raised, enough oxygen is available for this level to be reached. Supported in part by NINCDS Grants NS 07123 and NS 08802.

50.8 SEX DIFFERENCE IN BRAIN IRON. Joanna M. Hill* (SPON: P. D. MacLean). Laboratory of Brain Evolution and Behavior, National Institute of Mental Health, Bethesda, MD 20205

Iron, stored in the form of iron-ferritin, is unevenly distributed in the brain, the highest levels occurring in the globus pallidus (GP) and substantia nigra (SN). Rat brains were analyzed for iron content to determine if a sex difference, as has been reported in liver and serum iron, exists in brain iron levels. Ovarian hormones affect the synthesis of both ceruloplasmin, an iron-mobilizing factor, and ferritin, the iron storage protein.

Brain tissue was analyzed histochemically with Perl's method for ferric iron to obtain a qualitative measure of iron as well as to localize high iron areas. A more intense Perl's reaction was obtained with the female brain; however, in both sexes, iron was evident in the globus pallidus and substantia nigra.

Quantitative measures of iron levels were made by spectrophotometry. The tissues analyzed include the serum, liver, and both high iron (GP + SN) and low iron (cortex) areas of the brains of 10 female and 10 male 14 week old rats. The results of the spectrophotometric determination of iron appear below (all Student's t-tests df=18). Units are μg of Fe/g wet weight except serum which appears as μg of Fe/ml.

	Female	Male	
GP + SN	9.2 ± 2.81	5.7 ± 1.70	p<.01
Cortex	5.7 ± .50	5.3 ± .91	NS
Liver	139.7 ± 12.35	75.9 ± 8.31	p<.01
Serum	2.8 ± .45	1.8 ± .35	p<.01

These results demonstrate a sex difference in brain iron which parallels that in the serum and liver. Ovarian hormones, through their effects on ferritin and ceruloplasmin, are implicated in the establishment and maintenance of this sex difference. The availability of iron possibly has important consequences in those aspects of cerebral respiration and neurotransmitter metabolism in which iron plays a role.

A detailed analysis of changes in brain iron during the estrous cycle and pregnancy is in progress.

50.9 AMMONIA AND METHIONINE SULFOXIMINE INTOXICATION. W. Raabe and G. Onstad*. Dept. Neurology, VA Med. Center, Univ. of Minnesota, Minneapolis, MN 55417.

Methionine sulfoximine (MSO) has been reported to protect against the neuro-behavioral signs of ammonia intoxication (Hindtfeldt, B. and Plum, F., J. Pharm. Pharmacol., 27:456, 1975). This effect is not well understood because MSO itself increases cerebral ammonia concentrations by inhibiting glutamine synthethase, the enzyme promoting the removal of ammonia from the CNS via formation of glutamine.

The ability of ammonia to inactivate neuronal Cl^- extrusion and thus to abolish action potential suppression by postsynaptic inhibition (Raabe, W. and Gumnit, R., J. Neurophysiol., 38:347, 1975) was used to determine the neuronal tolerance to i.v. ammonia loads before and after treatment with MSO (35 mg/kg i.v.). In nembutal anesthetized cats recurrent postsynaptic inhibition of pyramidal tract cells was used to suppress extracellularly recorded antidromic action potentials of pyramidal tract cells. Ammonium acetate (NH_4-Ac) was given i.v. until inhibition no longer suppressed action potential generation, i.e., "disinhibition" occurred. At that time, the cerebral cortex was frozen in situ to determine ammonia and glutamine concentrations. In untreated cats disinhibition occurred after administration of 2.6 mM/kg of NH_4-Ac. With disinhibition cerebral ammonia increased from 0.20 ± 0.02 (S.E.) µM/g to 0.92 ± 0.10 µM/g, and cerebral glutamine increased from 3.55 ± 0.20 µM/g to 6.10 ± 0.64 µM/g. Thirty minutes after MSO treatment only 1.5 mM/kg of NH_4-Ac was needed to produce disinhibition; cerebral ammonia and glutamine concentrations were 0.73 ± 0.10 µM/g and 4.85 ± 0.38 µM/g respectively. One hundred and twenty minutes after MSO treatment 0.76 mM/kg of NH_4-Ac was sufficient to produce disinhibition; cerebral ammonia and glutamine concentrations were 0.89 ± 0.14 µM/g and 3.16 ± 0.07 µM/g respectively

MSO treatment significantly decreased the ammonia loads needed to produce disinhibition. The longer MSO affected glutamine synthesis, the less ammonia had to be infused to increase cerebral ammonia concentrations sufficiently to produce disinhibition. MSO treatment did not significantly alter the ammonia concentrations required to produce disinhibition. The results show that MSO does not change the ammonia concentrations neurons can tolerate. The protective effect of MSO on ammonia intoxication remains to be clarified.

50.10 STATUS OF SARCOPLASMIC RETICULUM CALCIUM-ACTIVATED ATPase IN HIBERNATION. R. Tamburini, Jr.*, S. S. Deshpande*, E. X. Albuquerque and F. C. Kauffman. (SPON: R. F. Mayer). Dept. of Pharmacol. & Exp. Therap., Univ. of MD School of Medicine, Baltimore, MD 21201.

Hind limb muscles from both hibernating and nonhibernating ground squirrels (Citellus tridecemlineatus) were removed under chloral hydrate anesthesia (400 mg/kg; intraperitoneally). Ca-stimulated ATPase activity was determined using a slightly modified method of Froelich and Taylor (J. Biol. Chem., 250:2013, 1975). The purpose of the present study was to examine possible relationships between thyroid status and Ca-activated ATPase in the sarcoplasmic reticulum of hibernating and non-hibernating animals. In deep hibernation the basal level of ATPase activity without the addition of Ca was significantly lower than the control value seen in the muscles of non-hibernating squirrels (301 + 49 vs 642 + 93 nmol/mg protein/min). Addition of Ca to the incubation medium stimulated the enzyme activity by 137% in the muscles of nonhibernating animals whereas it increased by only 43% of the basal activity in the muscles of hibernating squirrels. The level of Ca-stimulated ATPase activity was related to the level of hibernation of the animal. In experiments where the waking process was monitored from a deep hibernating state to the completely awake state, the activity of the enzyme was lowest at a body temperature of 7 °C, increasing to 1,300 at 21 °C and reaching a value of 1,445 at 35 °C, comparable to that seen in the muscles of nonhibernating squirrels.

Since administration of thyroxine to rats is known to increase Ca-activated myosin ATPase in cardiac muscle (Limas, C., Am. J. Physiol., 235: 745, 1978) and soleus muscle (Ianuzzo, D., et al., Nature, 270: 74, 1977) it was likely that decreased thyroid activity during hibernation could be responsible for the observed decrease in Ca-stimulated ATPase. Hind limb muscles of rats treated for 7 days with daily intramuscular injection of thyroxine (Na salt, 80 µg/100g) showed a 40% increase in enzyme activity as compared to nontreated controls. Similar injections of the hormone in nonhibernating squirrels had very little effect if any in increasing the activity of Ca-stimulated ATPase. These results implicate a mechanism other than that involving thyroid modulation in maintaining lower activity of the enzyme in hibernation. Perhaps other neural factors similar to those associated with maintaining fast-twitch and slow-twitch myosin as two distinct proteins in rats (Barany, M. and Close, R.I., J. Physiol., 213: 455, 1971) could play a role. (Supported in part by USPHS Grant NS-12063, NS-14728, funds from the Paralyzed Veterans of America and Conselho Nacional de Pesquisa of Brazil.)

50.11 CHANGES IN THE REDOX STATE OF CYTOCHROME a,a_3 IN RAT CEREBRAL CORTEX DURING HYPOGLYCEMIA. R.M. Bryan, R.B. Duckrow, J.C. LaManna, M. Rosenthal, and A.L. Sylvia, Dept. of Physiology, Duke Univ. Med. Center, Durham, NC 27710 and Dept. of Neurology, Univ. of Miami Sch. of Med., Miami, FL 33101.

The effects of decreased substrate availability to the respiratory chain were studied in rat cerebral cortex by monitoring the redox state of cytochrome a,a_3 before and after insulin induced hypoglycemia. Hypoglycemia decreases the amount of substrate available to the glycolytic chain. Rats were fasted from 12 to 16 hours, anesthetized with pentobarbital (50mg/kg), paralyzed with curare, and artificially ventilated. Arterial blood was sampled for the determination of blood gas tensions, pH, and glucose concentration. Changes in the redox state of cytochrome a,a_3 and cortical blood volume were monitored from the surface of the cerebral cortex using dual-wavelength reflection spectrophotometry. The steady state redox level of cytochrome a,a_3 was determined by comparing the degree of oxidation induced by 95% O_2/5% CO_2 respiration to the total labile cytochrome a,a_3 signal, defined as the signal change from maximum oxidation to the level of reduction achieved after 30 sec. of respiration with 100% N_2. Hypoglycemia resulted in a decrease in the fraction of reduced/oxidized cytochrome a,a_3 in the steady state. An active metabolic state was induced by direct cortical stimulation and was characterized by a transient negative shift of the steady cortical potential, a transient oxidation of cytochrome a,a_3, and a transient increase in cortical blood volume. Hypoglycemia decreased the amplitude of the transient responses in both cytochrome a,a_3 and blood volume. The cytochrome a,a_3 amplitude was more severely attenuated than the blood volume amplitude. Both the "steady state" and "active" redox response of cytochrome a,a_3 to hypoglycemia could be reversed by an intravenous administration of glucose unless hypoglycemia had progressed to the point of cortical isoelectricity. Although cytochrome a,a_3 is the terminal member of the respiratory chain and responds primarily to changes in oxygen availability, its redox state is sensitive to a decrease in glycolytic flux in both the steady state and during increased metabolic activity. (Supported in part by PHS grants NS 06157, NS 14319, NS 14325, and a grant from the AHA of Greater Miami).

50.12 ETHANOL INDUCED CHANGES IN PROTEIN SYNTHESIS OF COX GLIOMA CELLS E. W. Fleming*, M. E. Woodson* and S. Tewari* (SPON: V. G. Carson) Dept. of Psychiatry and Human Behavior, Univ. of Calif., Irvine, CA 92717.

Inhibition in steps of brain protein and RNA synthesis in vivo and in vitro have been reported following chronic ethanol ingestion by rodents. To elucidate the effects of ethanol on a specific cell population derived from human brain, cultures of Cox glioma cells were grown for 10 days in the presence or absence of 100 mM ethanol. Both groups of cells showed rapid growth and mitosis. Following harvesting cell homogenates were fractionated and polyribosomes purified by magnesium precipitation and ultracentrifugation. While cell densities remained unchanged, polyribosomes had reduced RNA and protein contents following the ethanol treatment. The states of cellular synthesis of RNA and protein were analyzed by growing cells in the presence of (5-^{3}H) orotic acid and (^{14}C) leucine for 24 hours prior to harvesting. Ethanol treated cells showed reduced incorporation of the precursors into RNA and protein. The activity of the polysomes for the in vitro incorporation of (^{14}C) leucine into protein was also reduced following the ethanol treatment. Furthermore, ethanol treatment resulted in dissaggregation of polysomal chains analyzed on sucrose density gradients. In addition to ethanol, cells were grown in the presence of 0.017µgm/ml of cycloheximide (Cy) added 1 or 24 hours prior to harvesting. After 1 hour of Cy treatment of control cells, the cell densities were unchanged, polysomal protein and RNA yields decreased and in vitro protein synthetic activity increased, but all measures showed decreases after 24 hours of Cy. Cells grown in the presence of ethanol however, showed decreased polysomal protein contents after 24 hours, but increased cell densities and in vitro protein synthetic activities after 1 and 24 hours of Cy treatment. The incorporation of the ^{3}H and ^{14}C precursors into monomer and polysomal units were determined by analyzing post-mitochondrial supernatants on sucrose gradients. Cy treatment at one hour caused an increase in the incorporation into RNA and protein in the polysomal region of both groups. The results suggest that a general property of ethanol treatment is the inhibition of protein synthesis, both for brain and CNS cells in tissue culture. While cycloheximide appeared to inhibit the termination step in protein synthesis, the ethanol treatment lessened the cycloheximide effects. The significance of these results will be discussed. This work was supported in part by NIAAA Grant No.'s AA 00252, AA70899 and AA03506-03A1.

50.13 MITOCHONDRIAL ISOLATION METHODS: APPLICATION TO SPINAL CORD.
N.R. Clendenon, S.T. Palayoor*, W.A. Gordon*, M.E. Nesham* and N. Allen. Neurochem. Lab, Division of Neurology, Ohio State University, Columbus, Ohio 43210.

The overall objective was directed toward finding a suitable method for isolation of mitochondria from samples of traumatized spinal cord for oxidative phosphorylation studies. A critical assessment of 3 methods was made using rat spinal cord and the data compared with results obtained for rat brain. The best procedure was applied to dog cord.

Spinal cords (7-8) were removed from Ketamine anesthetized rats by the rapid method of DeSousa and Horrocks (Dev. Neurosci. 2:115, 1979). Respiratory function was assessed with a Clarke electrode on a Gilson oxygraph at 25°C using a 1.5 ml water jacketed reaction chamber. Respiratory control rates (RCR) and P/O ratios were determined as described by Chance and Williams (J.Biol.Chem. 217: 383, 1955). Protein and cytochrome oxidase (CO) were measured for all fractions. The lysosomal enzymes, acid phosphatase (AcP) and β-glucuronidase (β-G) were assayed in fractions from at least one preparation for each isolation procedure.

In general, rat spinal cord mitochondria isolated by the Bernard(salt-wash)method (Biochim.Biophys.ACTA 548:173,1979) gave the best results in terms of RCR,P/O and respiration rates, while the Holtzman method (J.Neurochem. 30:1409,1978) using Nagarse was the least desirable. The Clark-Nicklas procedure (J.Biol.Chem. 245: 4724,1970) using a density gradient prepared with Ficoll required a longer preparation time. RCR values were approximately 50% lower in the presence of NAD-linked substrates (glutamate-malate or pyruvate-malate) when compared with the Bernard method. RCR values and State 3 respiration were generally lower than brain. Mitochondrial protein content and yield were strikingly low for all procedures. Spinal cord mitochondrial RSA values for CO were *essentially equivalent to brain preparations*. Lysosomal contamination was also similar (β-G and AcP RSA between 2 and 3).

Lumbar spinal cord (7-9cm) from Suritol anesthetized dogs maintained on N_2O:O_2(4:1) during laminectomy, was used for mitochondrial isolation by the Bernard method. Excess blood, dura and nerve roots were removed in ice cold isolation medium. Pooled data for 3 experiments gave a mitochondrial yield of 10% with a RSA for CO of 3.33. Because the amount of mitochondrial protein was very low (0.49 mg/cm cord), oxidative phosphorylation values varied greatly with State 4 respiration dropping to near zero. The Clark-Nicklas method was even less satisfactory.

Mitochondrial isolation methods when applied to dog spinal cord have proved disappointing. The limiting factor in a study of respiratory function appears to be the very large amount of material needed to isolate sufficient numbers of mitochondria from either normal or injured dog spinal cord. (Supported by NINCDS #NS-10165).

50.14 A CRITICAL ASSESSMENT OF MITOCHONDRIAL ISOLATION TECHNIQUES.
S.T. Palayoor*, N.R. Clendenon, W.A. Gordon*, M.E. Nesham* and N. Allen (SPON: J.H. Hofteig), Neurochem. Lab, Division of Neurology, Ohio State University, Columbus, Ohio 43210.

An extensive study was carried out on 4 existing methods designed to isolate rat brain mitochondria. Comparisons were made in terms of percent yields and purity in addition to good coupling and oxidation rates. For the Ozawa method (J.Biochem.59:501,1966), mitochondrial isolation is based upon differential centrifugation in isotonic mannitol with repeated rinsing of the crude mitochondrial pellet to remove the light fluffy layer. The Clark-Nicklas method (J.Biol.Chem. 245:4724,1970) utilizes a density gradient separation procedure with Ficoll to remove synaptosomes and other nonmitochondrial material plus a gentle swirling of the purified pellet with small volumes of medium lacking BSA to remove the fluffy layer. The Bernard method (Biochim.Biophys.ACTA 548: 173,1979) employs a salt wash medium to purify the crude mitochondrial pellet. Holtzman's method (J.Neurochem. 30:1409,1978) adds an equal volume of isolation medium containing Bacillus proteinase (Nagarse) to the partially disrupted tissue prior to completing the homogenization procedure. In this case, the brown pellet remains firmly attached to the bottom of the centrifuge tube during the rinsing steps.

Rats were lightly anesthetized with ether before decapitation with utilization of 2 brains per separation experiment. A minimum of 3 experiments were run for each isolation procedure. Mitochondrial respiration function was assessed with a Clarke electrode on a Gilson oxygraph at 25°C using a 1.5 ml water jacketed reaction chamber. Protein and cytochrome oxidase (CO) were measured for all fractions. The lysosomal enzymes, acid phosphatase (AcP) and β-glucuronidase (β-G) were assayed in fractions from at least one preparation for each isolation procedure.

The rate of respiration was lowest in rat brain mitochondria isolated according to the Ozawa procedure. Addition of BSA to the incubation medium significantly improved all respiratory parameters using the Clark-Nicklas method. Mitochondria prepared by the Bernard procedure showed very high respiratory control rates (RCR) with succinate, but failed to respire in the presence of NAD-linked substrates, glutamate-malate or pyruvate malate. High RCR values and respiratory rates were found with all substrates using the simpler and quicker Holtzman method. Mitochondrial recoveries based on CO as a marker were highest using the Clarke-Nicklas procedure (30%) and lowest with the Holtzman (11.8%), while the Bernard method showed the highest relative specific activity (RSA=4.69) but intermediate yield (13.8%). All preparations were somewhat contaminated with lysosomes with RSA values of 2-3 for β-G and AcP. (Supported by NINCDS #NS-10165).

51.1 MYELIN BASIC PROTEIN IMMUNOCYTOCHEMISTRY IN CHEMICALLY INDUCED DEMYELINATING LESIONS. Lidia M.N. Macedo*, Constancia del Cerro*, Robert M. Herndon. Center for Brain Research, University of Rochester, Rochester, NY 14642.

Immunocytochemical study of myelin basic protein (MBP) was performed in rat spinal cords injected previously with 2 µl of a 1% solution of lysolecithin in saline.

The animals were sacrificed by perfusion at 6,12, and 24 hours and 3,5,8,12, and 28 days. The spinal cord tissue was either cut 20 to 25 µm thick in a vibratome or embedded in epon 812 and cut 1 to 2 µm thick.

The vibratome and plastic sections were stained immunocytochemically with rabbit anti-MBP and peroxidase anti-peroxidase complex (PAP) and developed with diaminobenzidine.

After immunocytochemistry with anti-MBP, the lysolecithin lesions were easily identified by the diminished concentration of brown precipitate in the white matter. This effect was observed to start at 6 hours and to continue through the evolution of the lesion. The pale appearance of the lesion at 6 hours was due largely to edema since most of the myelin sheaths stained normally. A few of the smaller sheaths appeared to have lost staining. At 12 hours the myelin breakdown was obvious and most of the sheaths were fragmented and stained poorly. By 24 hours the myelin sheaths in the central portion of the lesion had lost most of their staining properties. In the vibratome sections, a very dark brown precipitate at the border of the lesion was observed. This was interpreted as myelin debris with a large number of antigenic sites exposed to the anti-MBP. It also suggested that the myelin breakdown was occurring more slowly at the edges of the lesion.

The phagocytized myelin debris in macrophages reacted very intensely with the antibody. In the vibratome sections this reaction was so intense that it was difficult to recognize the cell type under the brown precipitate. In plastic sections (1µm), cells with faintly stained cytoplasm appeared in the lesion at 8 and 12 days. They had the size and shape of oligodendrocytes, but EM remains necessary for definitive identification. Remyelination was apparent between 8 and 12 days after the lesion and its progression could be followed by increasing stain around axons. At 28 days the lesions remained paler than the surrounding white matter, but every axon had a thin ring of new myelin around it.

51.2 EXPRESSION OF MOSAICISM IN FEMALES HETEROZYGOUS FOR JIMPY. R. P. Skoff* and I. Nowicki Montgomery* (SPON: J. Rafols). Depts. of Anatomy and Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI 48201.

The Jimpy mouse is a sex-linked, recessive, juvenile lethal mutant that is characterized by a paucity of myelin throughout the central nervous system (CNS). The cause of the myelin deficit has not been determined although there have been numerous biochemical and morphological studies. Previous studies have been limited to affected males and there have been no investigations of female carriers of the Jimpy mutant gene. We describe here for the first time the pattern of myelination of females heterozygous for Jimpy. Myelin in the optic nerve shows a mosaic distribution which appears to be due, in part, to the effect of x-chromosome inactivation (XCI).

Mice ranging from 12 to 24 days postnatal were used in this study. More than 25 females heterozygous for Jimpy were compared to a collection of over 200 normal and 100 Jimpy littermates. Optic nerves and spinal cords, embedded in Araldite plastic, were analyzed using light and electron microscopy. Expression of the Jimpy gene in the optic nerves of heterozygous females varies considerably from animal to animal. They have a mosaic appearance where patches of tissue are identical in appearance to the hemizygote male while the remainder appears almost normal. Patches of unmyelinated fibers are always found around the periphery of the nerve but may extend into the central core. The unmyelinated areas may be all around the periphery, at opposite sides of the nerve, on one-side only or as a small isolated patch. Electron microscopic observations of the affected areas are identical in appearance to the Jimpy males. The vast majority of the axons are unmyelinated. The fibers are usually surrounded by astrocytic processes or sometimes by an incomplete wrapping of an oligodendroglial process. The number of oligodendroglial cells is reduced and they appear less differentiated than their counterparts in myelinated areas. Lipid laden microglia-like cells are present but they do not appear to be derived from oligodendrocytes. Myelinated areas of the optic nerves in the mosaics do not appear completely normal since the sheaths are often abnormally thick or thin when compared to the diameters of their axons. The intensity of the mosaicism ranges from a severe form identical in appearance to the hemizygote male to an almost normal condition. The demonstration of mosaicism in the female heterozygotes provides a unique system to study the interactions between a normal population of cells and those affected by the mutation. Supported by a Basil O'Connor grant from the March of Dimes and by U.S.P.H.S. grant NS 15338.

51.3 MYELIN SYNTHESIS FOLLOWING CHRONIC POSTNATAL METHYLZANTHINE ADMINISTRATION. G.N. Fuller and R.C. Wiggins., Dept. of Neurobiol. & Anat., Univ. of Texas Med. School, Houston, Texas 77025.

The litters of 6 pregnant Long Evans rats were adjusted to 10 pups each at birth. Five pups from each litter were given an oral dose of 1 methylzanthine(aminophylline, theophylline, or caffeine; 40 or 80 mg/Kg) daily from postnatal day 2 through weaning (20 days). The remaining 5 pups served as littermate controls. Chronic drug treatment produced no deficit in the gross body weight of experimental pups, and brain weights on the day of sacrifice were also normal. At ages 21-26 days randomly selected experimental pups were injected intraperitoneally with ^{3}H-leucine as a precursor of protein synthesis and controls received ^{14}C-leucine. Following 3 hrs incubation, the animals were killed, test and control brains were paired, and subcellular fractions prepared. For each subfraction, a $^{3}H/^{14}C$ (test/control) ratio was determined and normalized to the corresponding microsomal value for that double brain homogenate. The resultant percentage values give a measure of the relative synthesis of each drug-treated subcellular fraction.

Results for individual test/control brain pairs are shown:

Dose (mg/Kg)	40			80		
Drug	A*	T	C	A	T	C
Myelin	103	92	87	89	96	81
	102	102	89	93	93	98
				96	100	
Nuclear	100	87	96	94	92	88
	106	95	87	84	88	90
				95	95	
Synaptosomal	97	91	98	101	101	101
	97	95	100	93	98	93
				100	100	
Mitochondrial	103	99	93	99	96	99
	101	96	100	93	102	93
				96	95	
Microsomal	100	100	100	100	100	100

A*=aminophylline, T=theophylline, C=caffeine

The results show low myelin ratios for caffeine at 40 mg/Kg and for aminophylline and caffeine at 80 mg/Kg. Low ratios are also noted for the nuclear fraction. No indication of depressed synthesis is seen in the synaptosomal or mitochondrial fractions. (Supported by USPHS NS-14355 and NS-13799 to RCW).

51.4 TRYPTIC PEPTIDE MAPS OF MAJOR STRUCTURAL PROTEINS FROM MYELIN OF MICE. Marvin W. Luttges and Douglas E. Groswald*. Dept. of Aerospace Engineering Sciences, Univ. of Colorado, Boulder, CO 80309.

It is now generally recognized that there exist differing populations of myelin in the central nervous system (CNS) and peripheral nervous system (PNS) as characterized by buoyancy on sucrose gradients. Less certain, however, are the differences between the apparent microheterogeneity of the myelin proteins from these different buoyant fractions. Also in many species differing numbers of basic proteins have been reported for both CNS and PNS myelin. The major glycoprotein in PNS-myelin has been linked to two other closely electrophoretically migrating proteins as a consequence of changes in their relative concentrations during degeneration and their periodic acid schiff (PAS) sensitivity. The present study examines the relative concentration of the major structural proteins from PNS myelins which are recovered from continuous sucrose gradients, their tryptic peptide maps and the peptide maps between the basic proteins from CNS and PNS myelin from mice. PNS myelin was found to be normally distributed on continuous density gradients. The major glycoprotein accounted for approximately 40% of the recoverable protein, 15% for the basic proteins, and approximately 20% for the two major PAS-sensitive proteins. Comparisons between the mole concentrations for the different protein-species to the mole concentration of the major glycoprotein, revealed that a basic complement of protein is apparent in PNS myelins of differing buoyant densities. Tryptic peptide maps between the major glycoprotein and two PAS-sensitive proteins exhibited significant overlap. Also tryptic maps between the three major basic proteins (two slow migrating and the faster migrating one) show major similarities between themselves within their respective myelins and between the two different myelins. These studies suggest that in the PNS, protein contributions are not primarily responsible for differing myelin buoyancies, and that a similar gene in the myelin producing cells of the CNS and PNS is active in mice.

51.5 EFFECTS OF CELL DENSITY ON LIPIDS OF HUMAN GLIOMA AND FOETAL GLIA-LIKE CELLS. V.A. Liepkalns*, A.J. Yates, C. Icard*, D.K. Thompson*, and R.W. Hart*. Depts. of Pathology and Radiology, Ohio State Univ. Sch. of Med., Columbus, OH 43210.

A human glioblastoma multiforme (12-18) and human foetal glia-like cells (CH) were grown in vitro to sparse density, when few cells are in contact, and confluent density when all cells are in contact with adjacent cells. Ganglioside sialic acid, phospholipid phosphorus, and cholesterol levels were determined on both glioma and foetal cell lines at both cell densities. Distributions of sialic acid among ganglioside species and phosphorus in the major phospholipid classes were also determined. Total ganglioside sialic acid, phospholipid phosphorus and cholesterol increased in the glioma cell on a per cell number, mg protein and mg total lipid extract basis by 2 to 3-fold as cell density increased 25-fold. Ganglioside sialic acid, phospholipid phosphorus and cholesterol levels increased with cell density on a per cell and mg protein basis in the foetal cell but not on a total lipid extract basis. In both cell lines ethanolamine plasmalogen comprised a slightly greater proportion of phospholipids at all confluent densities. The proportion of sphingomyelin was higher in the glioma (9.4 $\pm$ 0.5%) than in CH (6.0 $\pm$ 0.6%) at all cell densities. Distributions of ganglioside sialic acid indicated a consistent trend towards an increased proportion of complex gangliosides in the glioma cell as density increased. The set of simpler gangliosides (GM_4 + GM_3 + GM_2) decreased with cell density from 50% of total ganglioside sialic acid to 36% during log phase to 30% at confluency. The proportions of both (GM_1 + GD_{1a}) which are in one biosynthetic pathway and the set (GD_3 + GD_2 + GD_{1b}) which are synthesized along another pathway were commensurately higher in confluent glioma cells. In the foetal glia-like cells the set of simpler gangliosides (GM_4 + GM_3 + GM_2) was about 48% of total ganglioside in both sparse and confluent preparations. The foetal cell had a 2-fold higher proportion of GM_3 (32.4 $\pm$ 2.1%) than the glioma cell (16.8 $\pm$ 1.6%) but a lower proportion of GM_1 (9.1 $\pm$ .7 vs. 18.2 $\pm$ 1.8%) at all cell densities considered. We conclude that between these two cells of glial origin, the glioma (12-18) has a greater response to increasing cell density in terms of the synthesis of more complex membrane lipid constituents.

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51.6 APPLICATION OF SENSITIVE GAS CHROMATOGRAPHY WITH NITROGEN PHOSPHOROUS DETECTOR FOR THE ESTIMATION OF SPHINGOLIPIDS. Basalingappa L. Hungund* (SPON: Dale Deutsch). Long Island Res Inst, SUNY, Stony Brook, N.Y. 11794.

The sphingolipids comprise an important lipid class which are believed to be localized predominantly in the surface membranes. Quantitative determination of these lipids is an important prerequisite for many biochemical studies. Most methods described in the literature for quantitation rely on the colorimetric and fluorimetric measurements of the long chain bases (LCB) released after hydrolysis. These techniques are sensitive but they are nonspecific and unreliable.

A simple and very sensitive gas chromatographic (gc) method is presented which utilizes the sensitivity and selectivity of recently introduced nitrogen-phosphorous (NP) detector. The sphingoid base is released from individual sphingolipid by acid hydrolysis. The free base thus released is derivatized with trifluoroacetic anhydride which is then injected onto gc column for quantitation. The method is highly sensitive (5-6 pmol) per injection, specific and can provide information on chain length and degree of unsaturation of sphingoid bases derived from individual sphingolipid.

52.1 NUCLEIC ACID SEQUENCE OF A PREDOMINANT mRNA FROM CHICK EMBRYO BRAIN. R.J. Milner*, J.G. Sutcliffe*†, T. Shinnick*†, J. Ray *†, R.A. Lerner *† and F.E. Bloom. A.V. Davis Ctr., The Salk Inst. and Scripps Clinic and Research Foundation†, San Diego, CA 92037.

The embryonic chicken brain contains several predominant mRNA species. These include mRNAs coding for tubulin and actin as shown by protein synthesis in vitro. A third predominant mRNA species codes for a protein with a molecular weight of 33,000 daltons which we have called p33. The identity of this protein is not known. Recombinant DNA techniques now provide the means to analyze eukaryotic genetic systems. Using these methods in combination with DNA sequencing techniques, it is possible, in many cases, to obtain primary structural information for a given protein more rapidly than by conventional protein sequencing. We are using this approach to characterize p33 and other gene products expressed in the nervous system.

Double stranded DNA produced from p33 mRNA by reverse transcriptase and DNA polymerase was inserted into the Hind III site of the plasmid pBR322 and cloned in E.coli. Digestion of the cloned plasmid with restriction enzymes showed that the insert contained sites for the enzymes Hpa II, Alu I, Hinf I, Mbo I and Hae III. Fragments of the insert were prepared with these enzymes, end labelled with 32p using polynucleotide kinase and sequenced by the partial chemical degradation method. Sequences of overlapping fragments were aligned to generate the complete DNA sequence of the insert. The sense strand of the insert, identified by a polyadenine sequence at its 3' terminal, corresponds to 550 bases of the p33 mRNA and includes the 3' non-coding region and approximately half of the coding region of the mRNA. The amino acid sequence of the carboxy terminal half of the p33 protein was obtained by translation of an open reading frame in the coding region. Comparison of the sequence with known amino acid sequences has not revealed any identity or significant homology. Experiments are in progress to determine the identity, function and location of this protein.

We thank D.W. Cleveland (Dept. of Biochem. UCSF) for clones used in these studies. These experiments were conducted according to NIH Recombinant DNA Research Guidelines and supported by grants from McNeil Laboratories and the Sun Oil Company.

52.2 MESSENGER RNA POPULATIONS IN VARIOUS REGIONS OF THE RAT BRAIN: PARTIAL PURIFICATION OF A DIENCEPHALON-SPECIFIC mRNA. Robert Bruce Moffett* (SPON: C. Bauer-Moffett). Dept. Molecular Biology, Roswell Park Memorial Inst., Buffalo, N. Y. 14223.

In order to delineate the genetic activity of different CNS cell types, the protein and mRNA populations of different brain regions were examined. The mRNA population of the rat cerebellum, cereberum, diencephalon, and brain stem was compared by two dimensional electrophoretic separation of their cell-free translation products. Fluorographs indicated that only a few significant differences existed among the species of abundant mRNA present in these various regions. Species of mRNA which were enriched in a particular brain region were denoted as "cell specific" products. One such mRNA was predominantly confined to the diencephalon. This mRNA was among the more abundant mRNAs present in the diencephalon and coded for a 30,000 Dalton peptide. This diencephalon "specific" mRNA was partially purified by oligo(dT)-cellulose chromatography and urea-sucrose gradient selection. These studies are preliminary to the production of cDNA and recombinant DNA probes for investigating CNS genetic activity. (NIH grant GM-07093).

52.3 NEUROCARCINOGENESIS OF ETHYLNITROSOUREA. M.J.W. Chang, A. Koestner and R.W. Hart*, Department of Veterinary Pathobiology and Department of Radiology, The Ohio State University, Columbus, Ohio 43210.

Ethylnitrosourea (ENU) is a potent single-dose perinatal neurocarcinogen in rats. The susceptibility for induction of neurogenic tumors in fetuses increases with advancing pregnancy and culminates toward the termination of the gestation period approaching a 100% tumor incidence. While newborn rats are equally susceptible, this susceptibility declines with advancing age and single-dose induction of neurogenic tumors becomes almost impossible beyond the age of 30 days. In the present study DNA was isolated from the brains of rats following i.v. exposure to radiolabeled ENU (45-180 mg/kg b. wt.) and analyzed by dilute acid hydrolysis and Sephadex G-10 column chromatography. N^7-ethylguanine, N^3-ethyladenine and O^6-ethylguanine were identified. Of these ethylated bases, O^6-ethylguanine is particularly interesting since it represents a promutagenic lesion which, if not repaired before DNA replication may result in a point mutation. Two time points were, therefore, studied: one hour and 7 days following ENU exposure to determine initial damage and repair. Three age groups of rats were compared: a) fetuses or neonates, b) 30 day old rats and c) adult rats. The persistence of brain DNA O^6-ethylguanine was similar in all three age groups (60-85% of initial O^6-EtGua/Gua). Within the 7 day period, however, the brain DNA content in neonates increased 300%, in 30 day old rats 10% and less than 1% in adults. Considering the great difference in brain tumor incidence in rats exposed to a single dose of ENU at different ages (100% in neonates and 0% in adults) and in the light of equal retention of ethylated O^6-guanine the following hypothetical conclusion has been reached: carcinogenesis by ENU and perhaps other alkylating agents is a function of both persistence of DNA adducts and rate of DNA replication in target cells. (Supported by USEPA Grant No. R-805337-02).

52.4 INTERACTION OF DELTA-9-TETRAHYDROCANNABINOL WITH BRAIN RIBOSOMAL RNA. M. K. Poddar (SPON: D. A. Urquhart). Dept. of Physiol. Biophys., College of Med. & Dent. of New Jersey, Rutgers Med. Sch. NJ 08854, USA.

Delta-9-tetrahydrocannabinol (THC), the major active component of cannabis, is lypophilic in nature. To understand the molecular mechanism of action of delta-9-THC in brain, in terms of its structure function relationship, an in vitro interaction study between the delta-9-THC molecule and the brain ribosomal RNA (r-RNA) has been made. In the present investigation the interaction between delta-9-THC and r-RNA (isolated from rat cerebral cortex and by hypothalamus) was studied based on the principle that thermal dissociation of dyes or drugs from the mixture of nucleic acid and drug depends on the nature of their interaction and also on the melting temperature (tm) of nucleic acid. The in vitro interaction of delta-9-THC at two different concentrations (9.3 and 93.0 μM) with r-RNA isolated from rat brain cortex and hypothalamus suggests that delta-9-THC binds with r-RNA (from both brain cortex and hypothalamus) maximally at low concentration (9.3 μM) whereas at higher concentration (93.0 μM) binding is comparatively less. The measurement of thermal absorbances of mixture of delta-9-THC and r-RNA indicate that the binding of delta-9-THC, at very low concentration (9.3 μM), to r-RNA may occur through intercalation between the bases of r-RNA of localized helical region; at high concentration, on the other hand, delta-9-THC may interact with r-RNA through other type of binding, instead of intercalation. These concentration dependent interaction of delta-9-THC with brain r-RNA may be explained by the fact that at lower concentration, delta-9-THC molecules may remain in the single monomeric form and because of this planar structure of the benzene ring prevails; as a result, delta-9-THC may intercalate between the bases of r-RNA segment having localized helical region. At higher concentration the delta-9-THC molecule, instead of remaining in monomeric forms, tends to exist in micelle form (Garrett and Hunt. J. Pharm. Sci. 63, 1056, 1974 and Poddar et al. Toxicol. Appld. Pharmacol. 46, 737, 1978) which may alter the configuration of the molecule and hence the interaction of delta-9-THC with r-RNA through intercalation is not possible.

52.5 ANTI-ENCEPHALITOGENIC RAT SPINAL CORD PROTEIN (RSCP): IMMUNOCHEMICAL PROPERTIES. C.F.C. MacPherson and S. Yu* Dept. of Psychiatry, Univ. of Western Ontario, London Ontario, Canada

Immunoreactive rat spinal cord proteins occur in axons of brain (RB-SCP), spinal cord (RSCP) and peripheral nerve (RSCP-PN). The importance of RB-SCP and RSCP is due to the observation that if Lewis rats are pretreated with RB-SCP or RSCP, they do not develop experimental allergic encephalitis when they are challenged with the rat myelin basic protein (MacPherson & Armstrong, Nature, 266, 459, 1977). Originally, RSCP was isolated from 0.05 M ammonium acetate extracts of rat spinal cords that had been frozen for over a year (MacPherson & Armstrong, J. Immunol. 116, 398, 1976). Subsequently, it was found that only a fraction of the total RSCP present in fresh or recently frozen rat nervous tissues could be extracted with 0.05 ammonium acetate. This report will describe the immunochemical properties of RB-SCP, RSCP and RSCP-PN obtained from acetone defatted tissues by extraction with 0.05 M sodium acetate, pH 5.0. Contaminants were removed by absorption on CM-52 cellulose at pH 5.0 and by gel-filtration on Sephadex G-50. At this stage RSCP-PN formed one band after SDS-polyacrylamide electrophoresis but RB-SCP and RSCP required a final purification step consisting of passage through a column of cyanogen bromide activated Sepharose-4B coupled to anti-RSCP IgG. RSCP's have a molecular weight of 11,000 + 1,000 daltons and an isoelectric point of about pH 4.5. The amino acid compositions of the RSCP's are similar but not identical. They contain from 32-24% of acidic amino acids, 19-13% of basic amino acids and less than 0.5% of ½ cystine.

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52.6 SEROTONIN METABOLISM: CHARACTERIZATION OF SEROTONIN BINDING PROTEINS PREPARED FROM BRAIN AND PINEAL ORGAN. Yoshisuke Ozaki and Richard J. Wurtman. Lab. of Neuroendocrine Regulation, Dept. of Nutrition and Food Science, M.I.T., Cambridge, MA 02139.

It has previously been shown that mammalian brain contains cytoplasmic proteins which can bind serotonin (5HT). We are purifying such proteins from brain and pineal organ using Sephadex G25 filtration, affinity chromatography, ion exchange chromatogrpahy, polyacrylamide gel molecular sieving, and isoelectric focusing. We find that brain contains at least three distinct macromolecules that bind 5-HT, with apparent equilibrium dissociation constants (Kd) of approximately 10^{-7}, 10^{-8}, and 10^{-9} M, and apparent molecular weights of 11,000, 30,000, and 150,000 daltons. We are currently investigating the functions of these proteins (e.g., 5HT receptors; carrier molecules mediating axoplasmic flow or synaptic uptake; storage sites; enzymes). Pineal organs also contain proteins which bind 5HT with apparent affinities of 10^{-7}, 10^{-8}, and 10^{-9} M. The 5HT binding of the highest affinity pineal macromolecule (10^{-9}M) is decreased if rats are pretreated with norepinephrine (2.5 mg/Kg, SC). This could increase the amount of stored or bound 5HT available for metabolism by melatonin-forming enzymes (serotonin-N-acetyltransferase; hydroxyindole-O-methyltransferase) or monoamine oxidase.

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52.7 THE Ca^{2+} IONOPHORE, A23187, INHIBITS PROTEIN SYNTHESIS IN GUINEA PIG HIPPOCAMPAL SLICES. Barbara Koons* and Peter Lipton. Dept. of Physiology, Sch. Med., Univ. of Wisconsin, Madison, WI 53706.

We are attempting to study the dependence of brain protein synthesis on cytosolic Ca^{2+} using the Ca^{2+} ionophore A23187. We are using the isolated transverse slice of the guinea pig hippocampus. Slices are incubated in a modified 95% O_2/5% CO_2 Krebs buffer at 37°C and total protein synthesis is measured as the rate of incorporation of ^{14}C-lysine into PCA-insoluble material. Statistical comparison of incorporation rates between ionophore treated and untreated slices is facilitated by a "double-label" protocol in which each slice effectively serves as its own control: slices are exposed to ^{3}H-lysine in a control period prior to exposure to the ionophore. $^{14}C/^{3}H$ in PCA precipitable material is then compared for treated and untreated tissue. Treated tissues are exposed to A23187 dissolved in DMSO; untreated tissues are exposed to DMSO.

A23187 added to the bathing medium 30 minutes prior to adding ^{14}C-lysine, produces a dose dependent inhibition of ^{14}C incorporation into proteins. Maximal inhibition occurs at 20μM ionophore. 10μM A23187 inhibits synthesis by about 25% and does not affect the uptake of ^{14}C-lysine into the PCA-soluble fraction of the cell.

Incubating slices in a Ca^{2+}-free buffer elevated basal levels of protein synthesis slightly but did not alter the % inhibition caused by A23187. Thus, an A23187-mediated influx of extracellular Ca^{2+} is probably not the basis for the inhibition of synthesis. Incubating slices in a Mg^{2+}-free buffer depressed basal levels of protein synthesis (~20%) and enhanced the inhibition of synthesis caused by A23187. This indicates that the inhibition does not result from an ionophore-mediated Mg^{2+} influx into the cell. The increased inhibition in 0 Mg^{2+} is consistent with the ability of Mg^{2+} to activate protein synthesis and to compete with Ca^{2+} for binding to the ionophore.

Incubating slices in 2.5mM EGTA for 2 hours prior to adding the ionophore reduced basal levels of protein synthesis by about 60%; however, the percent inhibition of synthesis by A23187 was the same as in slices which were not exposed to EGTA. These data suggest either i) that the ionophore-mediated inhibition of protein synthesis is independent of a rise in cytosolic Ca^{2+} or ii) that the ionophore is causing release of Ca^{2+} from intracellular stores which are unaffected by the EGTA treatment (e.g., mitochondria) and that the increased cytosolic Ca^{2+} then inhibits tissue protein synthesis. Experiments are being carried out to resolve these two possibilities.

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52.8 ISOLATION OF NEUROFILAMENT PROTEINS FROM BRAIN BUFFER EXTRACTS BY IMMUNOAFFINITY CHROMATOGRAPHY. DEMONSTRATION OF IMMUNOLOGICALLY ACTIVE DEGRADATION PRODUCTS IN THE 50K RANGE. D. Dahl. Spinal Cord Injury Research Laboratory, Harvard Medical School and West Roxbury VA Medical Center, Boston, MA. 02132.

Neurofilament proteins were extracted at low ionic strength (1 mM sodium phosphate buffer) from brain and spinal cord of 4 mammalian (human, bovine, rabbit and rat) and 4 submammalian species (chicken, turtle, frog and shark). The extracts were applied to Sepharose 4B coupled to neurofilament antisera raised to degraded chicken neurofilament proteins (Dahl, D. and Bignami, A., J. Comp. Neurol., 176:645, 1977; Dahl, D, BBA, 622:9, 1980). Neurofilament polypeptides at approximately 150K and 70K were tightly attached to the column and eluted at pH 2.5. In rat and rabbit the 150K dalton protein was smaller compared to human and bovine as indicated by co-migration experiments on SDS polyacrylamide gel electrophoresis. Omission of EDTA and EGTA in the homogenization buffer resulted in the degradation of the neurofilament proteins into multiple components in the 50K range. The degraded proteins maintained their immunological activity and tightly attached to the anti-neurofilament column. Additional evidence as to the limited proteolysis of neurofilament proteins into immunologically active components was obtained in rat optic nerves undergoing Wallerian degeneration. Following enucleation of the eyes neurofilament proteins at 70K and 150K were no longer identifiable in SDS extracts of the degenerated optic nerves. However, axonal debris was still stained by immunofluorescence with neurofilament antisera in cryostat sections of the degenerated nerves. Supported by a grant from the National Science Foundation (BNS-7912962) and by the Veterans Administration.

53.1 MASS SPECTROMETRIC CHARACTERIZATION OF DERIVATIZED BIOGENIC AMINES PREPARED FOR QUANTITATIVE GAS CHROMATOGRAPHIC ANALYSIS. R. T. Coutts*, G. B. Baker and S.-F. Liu* (SPON: W. F. Dryden). Neurochem. Res. Unit, Fac. of Pharmacy and Pharm. Sci. and Dept. of Psychiatry, Univ. of Alberta, Edmonton T6G 2N8, Canada.

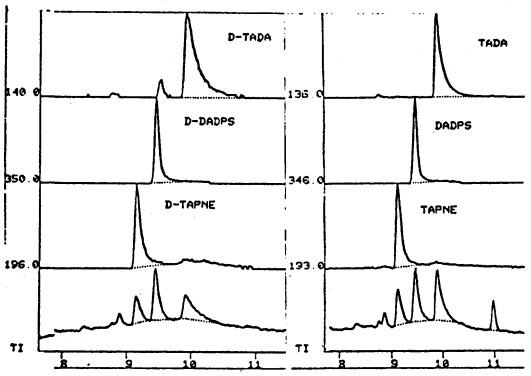
Various classes of biogenic amines have been detected in trace amounts in biological samples. Some can be considered to be simple derivatives of 2-phenylethylamine or analogous compounds, e.g. tryptamine, histamine; alcoholamines and phenolic amines are also encountered. The quantitative analysis of mixtures of biogenic amines presents some difficulties. They must be extracted efficiently and derivatized with a reagent which improves the sensitivity and specificity of their detection and quantitation. We have developed an analytical procedure which permits the quantitative analysis of mixtures of biogenic amines in which the amines are acetylated in aqueous solution then perfluoroacylated to give products which are readily analyzed using gas chromatography with electron-capture detection (EC-GC). An essential feature of the analytical procedure is the unequivocal identification of the derivatized products. This was accomplished mass spectrometrically.

Evidence is presented that 2-phenylethylamines are converted to N-acetyl-N-perfluoroacyl derivatives during the analytical procedure. Phenolic amines initially form N, O-diacetyl products which are, in most cases, subsequently converted to N, O-diacetyl-N-perfluoroacylated compounds. With 5-hydroxytryptamine, however, the final product is an O-acetyl-N-perfluoroacylated beta-carboline derivative (Baker, G.B. et al., J. Pharmacol. Meth., 3:173, 1980). Derivatization of histamine produces a structurally related cyclized product. Acetylation of alcoholamines results only in N-acetylation; the alcohol group remains underivatized. Subsequent perfluoroacylation of the acetylated intermediate yields an N-acetyl-N, O-di(perfluoroacyl) derivative. The product obtained from phenylethanolamine, for example, was deduced by mass spectrometry to be $PhCH(OCOC_nF_{2n+1})$-$CH_2N(COCH_3)COC_nF_{2n+1}$. This compound is not stable. On standing in solution, it decomposes to a compound which is insensitive to detection by EC-GC. Interpretation of the mass spectrum of the decomposition product revealed that it was an oxazoline derivative. A mechanism which explains its formation is suggested. Fragmentation pathways of various examples of derivatized biogenic amines, which are compatible with the recorded mass spectra, are also suggested. Many of the diagnostic ions retain the perfluoroacyl group, although a consistent fragment in most of the spectra is the result of the expulsion of a molecule of structure $CH_3CONHCOC_nF_{2n+1}$ from the molecular ion.

(Supported by Medical Research Council of Canada)

53.2 GC/MS METHOD FOR SIMULTANEOUS DETERMINATION OF PICOGRAM LEVELS OF NOREPINEPHRINE, DOPAMINE AND SEROTONIN IN BIOLOGICAL SAMPLES. Lelland C. Tolbert and George B. Brown, Neurosciences Program, University of Alabama in Birmingham, Birmingham, Alabama 35294.

A method has been developed for the simultaneous determination of norepinephrine, dopamine and serotonin in biological samples. The method involves the derivatization of the amines by acetylation with acetic anhydride followed by acylation with pentafluoroproprionic (PFP) anhydride. Dopamine is measured as the triacetyl derivative (TADA) (3-O, 4-O, N) norepinephrine as the triacetyl-mono PFP derivative (TAPNE) (as with dopamine plus the PFP addition to the β-hydroxyl position), and serotonin as the diacetyl-di-PFP derivative (DADPS). This method has the advantage of stability of the derivatives when compared to other GC/MS methods. Quantitation is achieved in the selected ion monitoring mode with reference to internal deuterated standards. Diagnostic fragments chosen for this analysis are at m/e 136 and 140 for proteo and deutero dopamine, m/e 193 and 196 for proteo and deutero norepinephrine, and m/e 346 and 350 for proteo and deutero serotonin. A representative chromatogram is given below showing the separation of the three derivatives and the specificity of the selected ions. This method also has the advantage of not requiring an extensive column purification procedure before derivatization. Because the initial reaction can occur in aqueous solutions it is also possible to directly derivatize tissue perfusates. Applications will be discussed.



53.3 PRESENCE OF CONJUGATED CATECHOLAMINES (CA) IN RAT BRAIN: A NEW METHOD OF ANALYSIS OF CA SULFATE. N.T. Buu*, L. Truong* and O. Kuchel*. (SPON: A. Barbeau). Clinical Research Institute, Montreal, Quebec H2W 1R7.

Sulfation of CA has been shown to occur readily in mammals (Rutledge & Hoehn, Nature, 244:447, 1973), but the physiological meaning of CA conjugation remains unknown. Phenolsulphotransferase (PST), the enzyme responsible for the sulfation of phenols and CA was found in rat brain (Meek & Neff, J. Neurochem., 21:1, 1973) and most recently in human brain (Renskers et al., J. Neurochem., in press, 1980). Yet despite the large concentrations of free CA in various regions of the brain no CA sulfate in brain has been reported, in contrast to the large quantities of conjugated CA found in mammalian plasma, urine and peripheral tissues, including the vesicular fraction of dog's adrenal medulla (Unger et al., Can. J. Physiol. Pharmacol., 58:22, 1980). This may be due to the lack of a sensitive method of measurement of conjugated CA in samples containing large quantities of free CA. The present paper reports an efficient method of isolating and measuring CA sulfate and the finding of CA sulfates in three different regions of rat brain.

Homogenate of brain was subjected to alumina adsorption at pH 8.6. The supernatant was acidified and transfered to Dowex 50X-8 (H^+ form) column. Eluate from Dowex column was analyzed for CA sulfate by a radioenzymatic technique in the presence and absence of a sulfatase. Values of CA sulfate from seven rat brains, expressed as means ± SEM (ng/g tissue) are as follows:

Areas	DA sulfate	NE sulfate	E sulfate
hypothalamus	77.6 ± 25.0	15.9 ± 2.7	10.2 ± 6.6
striatum	50.2 ± 20.7	4.4 ± 2.8	7.9 ± 4.1
hippocampus	25.4 ± 7.0	ND	ND

DA:dopamine, NE:norepinephrine, E:epinephrine; ND:not detectable

Concentrations of free CA are comparable to those reported in the literature. Sulfate esters of DA, NE and E account for only about 5-10 percent of the total CA in the brain. The small degree of conjugation of brain CA stands in contrast with the much higher percentage of conjugation found in the periphery (e.g. 99 percent for plasma DA). This difference in the degree of conjugation cannot be explained by the difference in PST activity alone or by a faster turnover of CA sulfates since they are not readily metabolized either by COMT or MAO. It may, however, suggest that CA sulfates are quickly evacuated from the brain. Sulfation seems to represent therefore a rapid method of removing excess of free CA from the brain to the periphery.

In summary this paper: 1) describes a rapid and sensitive new method of extracting and measuring CA sulfate; 2) reports finding of CA sulfates in three specific areas of rat brain.

53.4 AVAILABILITY OF TYROSINE AND PHENYLALANINE FOR BRAIN NOREPINEPHRINE SYNTHESIS IN PROTEIN MALNOURISHED RATS. Maravene Miller, Rachelle Hasson* and Oscar Resnick*. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The availability of tyrosine (Tyr) and/or phenylalanine (Phe) for norepinephrine (NE) synthesis from birth to age 30 days was examined in regional brain areas of rats fed a normal (25% casein) or a low protein (8% casein) diet. The malnourished group, whose dams received the low protein diet 5 weeks prior to conception, showed significantly elevated NE levels at birth (Table below) and at older ages. Although the amount of brain Tyr was markedly lower at birth and at some other ages during development in the malnourished rats, these animals consistently showed marked increases in their brain Phe levels at all ages examined compared to the normal rats. Also, the plasma Phe levels of the malnourished rats were significantly higher than the normal animals from birth to 30 days, whereas their plasma Tyr levels only showed significant decreases at some ages compared to the controls. These data seem to indicate that: (1) more Tyr has been withdrawn from peripheral circulation in the deprived rats in an attempt to maintain their brain Tyr levels at near normal values, and/or (2) the increased availability of plasma Phe represents a decrease in its conversion to Tyr in the liver in order to provide enough of this essential amino acid for metabolic purposes including brain NE formation.

Day of Birth (Mean ± S.E.)

Diets:	8%	25%
(n)	(8)	(8)
	NE ng/g	
Telencephalon	265 ± 10[a]	149 ± 15
Brainstem	778 ± 24[a]	193 ± 18
	Tyr ng/g	
Telencephalon	27284 ± 1523[a]	38899 ± 2035
Brainstem	30338 ± 941[a]	41894 ± 954
	Phe ng/g	
Telencephalon	41408 ± 1405[a]	25627 ± 1713
Brainstem	52199 ± 1482[a]	34365 ± 721
	Plasma Precursors ng/ml	
Tyrosine	22798 ± 1046[b]	24182 ± 951
Phenylalanine	15301 ± 1101[b]	12011 ± 272

[a] $p < 0.001$, [b] $p < 0.01$

Supported by grant HD 06364.

53.5 MANGANESE-INDUCED CHANGES IN BRAIN AMINES. G. Gianutsos, M. T. Murray* and M. D. Poulin* Section of Pharmacology & Toxicology, University of Connecticut, Storrs, CT 06268.

Manganese has been reported to alter brain amines, especially dopamine, when administered orally in an animal's drinking water (Bonilla et al., J. Neurochem. 22:297, 1974). In the present study, the effect of manganese on the concentrations of dopamine (DA) and GABA and on the activity of choline acetylase, a marker for cholinergic neurons, was measured. Manganese was administered in 3 different ways: I. by incorporation of 4% manganous chloride into the animal's food for up to 6 months; II. by direct unilateral intrastriatal injection of 10-20 ug of manganous chloride; and III. by subcutaneous injection of the gasoline additive methylcyclopentadienyl Mn tricarbonyl (MMT). In Exp. III, MMT was diluted in propylene glycol and injected on alternate days for 3 weeks.

Regardless of the route of exposure, manganese produced a small (15-25%) but significant decrease in striatal DA content. Decreases in DA were also noted in the olfactory tubercle and hypothalamus in Exp. I and III. Hypothalamic norepinephrine remained unchanged. On the other hand, striatal GABA concentrations were elevated in the manganese-treated mice, while GABA in the cerebellum remained unchanged. Choline acetylase also showed transient changes, tending to increase in the striata of manganese-exposed mice while remaining essentially unchanged in the hippocampus.

It is concluded that exposure to high concentrations of manganese may induce a change in DA neurons which may secondarily affect striatal GABAergic and cholinergic neurons. It is suggested that these changes may be related to the extrapyramidal symptoms associated with manganese toxicity.

53.6 EFFECTS OF SODIUM DEPLETION ON BRAIN ISO-RENIN AND CATECHOLAMINES. K.B. Brosnihan, C.M. Ferrario, R.R. Smeby,* & J.M. Saavedra. Res. Div., Cleveland Clinic, Cleveland, OH & NIMH, Bethesda, MD.

Reduction of sodium intake may alter the point of equilibrium between the renin-angiotensin and sympathetic nervous systems in the control of cardiovascular function. Recent studies from our laboratory have given support to the idea of depressed sympathetic nervous system function following severe dietary sodium depletion that caused a 15-fold increase in plasma renin activity (PRA) and was associated with elevated cerebrospinal fluid (CSF) norepinephrine (Brosnihan et al., The Physiologist 21: 13, 1978). The latter findings suggest an alteration in central noradrenergic activity; however, information regarding the concomitant change in brain renin-angiotensin is altogether lacking. Therefore, we determined the influence of sodium depletion on brain iso-renin of different brain regions and on catecholaminergic activity of the area postrema, a circumventricular area of the 4th ventricle known to be sensitive to angiotensin II.

Mongrel dogs were placed either on a normal (65 mEq Na+/day) or low (4 mEq Na+/day, supplemented with furosemide) sodium diet. Samples for PRA were taken in the conscious animal. The animals were anesthetized with sodium pentobarbital, and samples of CSF were taken from the cisternum magnum for renin determinations. Brains of animals were then perfused with cold isotonic saline. The area postrema, pyramidal tract and choroid plexus were assayed for catecholamines, PNMT, and serotonin. The brainstem, cortex, cerebellum, and spinal cord were assayed from brain iso-renin by the method of Smeby (unpublished) and for brain cathepsin D.

After sodium depletion norepinephrine concentration of the area postrema decreased from 9.17 $\pm$ 0.96 ng/mg protein to 5.23 $\pm$ 0.80 ng/mg protein ($p < 0.01$); whereas the concentration of epinephrine, dopamine, serotonin, and PNMT did not change. No alteration in catecholamines was found in the pyramidal tract during sodium depletion. As expected PRA rose from 1.3 $\pm$ 0.5 to 17.9 $\pm$ 4 ng/ml ($p < 0.01$) during sodium depletion. The concentration of brain iso-renin was significantly lower in the upper and lower brainstem of sodium depleted dogs; whereas cathepsin D concentration of all brain areas studied was unaltered by sodium depletion.

Our findings of a reduction in brain iso-renin concentration in the presence of marked elevation of PRA support the hypothesis of a negative feedback relationship between the kidney and the brain. Additionally, this study reveals for the first time an alteration in the brainstem of both iso-renin and noradrenergic activity during sodium depletion.

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53.7 COMPARISON OF THE EFFECTS OF TRANYLCYPROMINE AND PHENELZINE ON SEVERAL AMINES IN RAT BRAIN. G. B. Baker, J. M. Baker*, D. F. LeGatt* and R. T. Coutts*. Neurochemical Res. Unit, Dept. of Psychiatry and Fac. of Pharmacy and Pharmaceutical Sciences, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2G3.

The amine hypotheses of depression propose that depression is due to a decreased functional availability of one or more amines at central synapses. Amines which have been proposed as being important in this regard include the catecholamines (primarily norepinephrine), 5-hydroxytryptamine (5-HT) and the "trace" amines 2-phenylethylamine (PEA) and tryptamine (T). Studies are presently being conducted in our laboratories in which all these amines are being assayed simultaneously in rat brain tissue. We describe in this report a study of the effects of chronic administration of the monoamine oxidase inhibitors tranylcypromine (TCP) and phenelzine (PLZ) on the brain concentrations of dopamine (DA), norepinephrine (NE), 5-HT, PEA and T. Drugs were administered ip once daily (10 mg/kg for TCP and 15 mg/kg for phenelzine) for 7 days, and rats were killed 6 h after the last dose. Brains were homogenized in ice-cold 0.1 N perchloric acid containing 10 mg % EDTA, and after centrifugation a small portion was taken for analysis of catecholamines using a radioenzymatic procedure (Martin, I. L. et al., Biochem. Pharmac., 27: 1519, 1978). The remainder of the sample was prepared for simultaneous analysis of PEA, T and 5-HT using electron-capture gas chromatography with a glass capillary column (Calverley, D. G. et al., Can. J. Neurol. Sci., in press). Briefly, this consists of isolation of the amines using di-(2-ethylhexyl) phosphoric acid, elution into HCl, acetylation in the aqueous phase with acetic anhydride and extraction into ethyl acetate. The organic phase is dried under nitrogen and the residue is reacted with pentafluoropropionic anhydride. The resultant derivatives are separated on a 10 m WCOT glass capillary column, OV-101.

Results showed that with this dosage schedule concentrations of all amines had risen significantly above controls, but there were a number of interesting differences between the effects of the two drugs. Brain concentrations of 5-HT and DA were not significantly different between the two drug-treated groups, but NE concentrations were significantly higher in the brains from TCP-treated rats ($p<0.02$). The most noticeable differences between the two drugs were their effects on the levels of PEA and T. Increases in concentrations of both amines were several times greater with TCP than with PLZ.

(The support of the Medical Research Council of Canada is gratefully acknowledged).

53.8 PHENCYCLIDINE EFFECTS ON THE SYNTHESIS/RELEASE OF SYNAPTOSOMAL DOPAMINE CONTINUOUSLY APPEARING FROM PHENYLALANINE: SENSITIVITY TO RESERPINE. S. P. Bagchi. Rockland Research Institute, Orangeburg, NY 10962.

Phencyclidine (PCP) is an abused drug and it may reactivate schizophrenic psychosis and produce treatment resistant schizophrenia like behavior. In view of the evidence implicating dopamine (DA) in schizophrenia, PCP effects on the synthesis/release of synaptosomal DA continuously appearing from ^{14}C-phenylalanine (Phe*) were examined. Influence of reserpine (Res) on the PCP actions was also tested since Res may modify the action of amfonelic acid, a nonamphetamine stimulant like PCP, on synaptosomal DA. The published methods (Brain Res. 187, 403 (1980))consisted of the incubation (10 min) of synaptosomal (P2) preparation from rat caudate nuclei in tris-salt buffer (pH 7.4) containing pargyline, sucrose, glucose and Phe* and filtration of the mixture on Millipore filter postincubation to separate synaptosomes from the medium. Separated fractions were analyzed for labelled DA and the particulate level of Phe*. Results show that PCP may stimulate the total (particulates plus medium) ^{14}C-DA synthesis at 36.4 (166% of control) and 9.1 (154%) but not at 0.91 (95%) micromolar concentration. PCP at each of these concentrations significantly increased the release (medium/total x 100) of synaptosomally formed ^{14}C-DA beyond that occurring spontaneously. A PCP congener, Ketamine, at 36.4 and 9.1 micromolar did not stimulate the synthesis and only slightly increased the release. Res (5.5 to 90 nanomolar) had an inhibitory effect on the total DA formation and a stimulatory one on the release. The PCP stimulation of DA synthesis was not only blocked by a coaddition of Res but an inhibition beyond that due to Res alone was also observed. The release index in the presence of Res plus PCP was higher than that with Res alone. After pretreating the rats with Res, PCP also showed an inhibitory effect on the total DA synthesis and stimulated the release. None of the drugs affected particulate Phe*. In summary, the results show that a) PCP may stimulate the synthesis of synaptosomal DA and its release and b) the synthesis stimulation is Res sensitive, suggesting a catecholamine storage vesicle participation in PCP action. A comparison of tetrabenazine, another amine depletor, and Res actions further suggest that the Res sensitivity may be related to, not amine depletion, but possibly an interaction of PCP with what has been termed Res receptor. (Kindly supported by the Office of Mental Health, State of New York).

53.9 CHRONIC PHENYLETHYLAMINE TREATMENT AND BRAIN CATECHOLAMINES: A POSSIBLE BIOCHEMICAL MODEL OF SCHIZOPHRENIA. F. Karoum, S. G. Speciale, Jr., L-W. Chuang,* and R. J. Wyatt. Lab. of Clinical Psychopharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032; Dept. of Psychiatry, Univ. of Texas Health Center, Dallas, Texas.

Recent reports of increased urine excretion of phenylethylamine (PEA) and of increased norepinephrine (NE) concentration and metabolism in the hypothalamus and the nucleus accumbens in the post-mortem brains of paranoid chronic schizophrenics, have prompted us to evaluate the effects of chronic PEA treatment on brain catecholamines and metabolites. PEA (100 mg/kg) was administered intragastrically twice daily to rats for 10 days. The rats were killed 2 hours after the last dose, long after any stereotypy had subsided. The brain areas analyzed were: hypothalamus, nucleus accumbens, olfactory tubercle, caudate, and frontal cortex.

Chronic PEA significantly increased NE concentration in the hypothalamus (35%) and the nucleus accumbens (95%) but not in any of the other brain areas analyzed. In the hypothalamus, 3-methoxy-4-hydroxyphenylglycol (MHPG) was also increased (44%).

Chronic phenylethylamine had no effect on dopamine (DA) concentration in any of the brain areas analyzed except in the caudate where a slight but significant increase was observed (10%). The concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) was increased in the hypothalamus, nucleus accumbens, and caudate, thus suggesting increased DA turnover in these areas. DOPAC remained unchanged in the olfactory tubercle.

The changes in brain NE observed after chronic PEA treatment resemble remarkedly the post-mortem results reported first by Farley et al. (Science, 200:456, 1978) and confirmed by us. We therefore suggest that the increased NE concentration found in the hypothalamus and nucleus accumbens of paranoid chronic schizophrenics may be attributed to sustained increased brain PEA secondary to both central and peripheral causes. Increased stimulation of DA neurons in the hypothalamus, nucleus accumbens, and caudate by PEA in schizophrenia may also play a role in the etiology of the disease.

53.10 PERIPHERAL CORRELATES OF CENTRAL NORADRENERGIC ACTIVITY. J. D. Elsworth*, R. H. Roth, J. M. Stogin*, D. J. Leahy*, M. R. Moore*, D. E. Redmond, Jr. Departments of Pharmacology and Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06510

One approach to the study of human brain transmitter turnover is the measurement of transmitter metabolite concentration in CSF, plasma or urine. Previous studies in rodents and primates have suggested a significant correlation among the concentrations of the norepinephrine (NE) metabolite 3-methoxy-4-hydroxyphenethylene glycol (MHPG) in plasma, CSF and two NE rich brain regions[1,2]. In addition Maas et al.[3,4] have found arterio-venous differences in MHPG content from cerebral circulation in non-human primates and man, indicating that venous plasma MHPG might reflect brain NE turnover. However, since a limited fraction of plasma MHPG is derived from brain such measurements might reflect uncontrolled peripheral sympathetic sources instead. Direct measurements in brain regions and peripheral pools under a variety of conditions might determine whether CSF and plasma MHPG content are reliable indices of central NE activity. Toward this end we have measured and correlated the quantity of MHPG in several brain areas, CSF and plasma in monkeys (Cercopithecus aethiops) before and after pharmacological manipulation of central noradrenergic systems.

Twenty-nine monkeys received one of the following treatments: saline, the α_2 agonist clonidine, the α_2 antagonist piperoxane, morphine, naloxone and/or withdrawal from morphine addiction. MHPG concentration was determined by gas chromatography-mass spectrometry using a single ion monitoring technique[3].

A significant correlation was found between plasma and CSF MHPG ($r=0.66$, $p < .001$) and between the MHPG content of hypothalamus and plasma ($r=0.55$, $p < .005$) and hypothalamus and CSF ($r= 0.63$, $p < .001$). Hippocampal MHPG also correlated with the concentration in plasma ($r=0.74$, $p < .001$) and CSF ($r=0.83$, $p < .001$).

These striking relationships further validate the technique of monitoring plasma and CSF MHPG in order to assess central NE metabolism. Similar data with other brain regions and on dopaminergic systems will also be presented. (Supported by DA 02321, MH-14092 and a grant from the Harry Frank Guggenheim Foundation)
(1) Redmond et al. (1979) Neursci. Abst. 5, 348; (2) Crawley et al. (1978) Brain Res. 141, 380; (3) Maas et al. (1976) Brain Res. 118, 167; (4) Maas et al. (1979) Science 205, 1025.

53.11 THE EFFECT OF CYCLOHEXIMIDE AND ANISOMYCIN ON MONOAMINE SYNTHESIS IN MOUSE BRAIN SYNAPTOSOMES. M. M. Schweri* and L. A. Carr. Dept. Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY 40292.

Many protein synthesis inhibitors have been shown to inhibit catecholamine (CA) and 5-hydroxytryptamine (5-HT) synthesis in the brain when administered *in vivo*. In an effort to elucidate the mechanism of action of these drugs, the effect of cycloheximide (CXM) and anisomycin (ANISO) on the synthesis of dopamine (DA), norepinephrine (NE), and 5-HT in synaptosomes was measured following both *in vivo* and *in vitro* administration of these drugs.

In order to study the effects of *in vivo* administration of these drugs on CA formation, CXM (100 mg/kg) and ANISO (50 mg/kg) were administered s.c. to mice 1 hr. prior to sacrifice. Crude synaptosomal pellets (P_2) were prepared from the brains and the synaptosomes were incubated for 20 min. with ^{3}H-tyrosine (Tyr) at pH 7.4. Synaptosomal content of ^{3}H-Tyr and endogenous Tyr, and ^{3}H-DA and ^{3}H-NE in synaptosomes and medium were determined. In the *in vitro* study, CXM and ANISO were added to synaptosomes prepared from untreated mice in a concentration of 10^{-3}M. In parallel experiments, ^{3}H-tryptophan (Trp) was added as a substrate. Synaptosomal content of ^{3}H-5HT and ^{3}H-Trp was measured.

In vivo and *in vitro* administration of both CXM and ANISO caused an inhibition of ^{3}H-NE synthesis. ANISO also suppressed ^{3}H-DA synthesis under both conditions. CXM, but not ANISO, caused an elevation of synaptosomal ^{3}H-Tyr content when added *in vitro*. No effect on ^{3}H-Tyr or endogenous Tyr content was observed after *in vivo* administration of the drugs. Neither of the protein synthesis inhibitors affected endogenous Tyr following *in vitro* addition. When given *in vivo*, both protein synthesis inhibitors decreased synaptosomal ^{3}H-Trp and ^{3}H-5HT content. ANISO, but not CXM, caused similar effects when added *in vitro*.

The results indicate that these protein synthesis inhibitors do not require prior conversion to their metabolites in order to inhibit CA synthesis, as has been suggested, but may act directly on the biosynthetic enzymes. The data also suggest that CXM and ANISO may inhibit 5-HT synthesis by interfering with Trp accumulation in the nerve terminal.

53.12 ESTIMATION OF SYNTHESIS RATE AND TURNOVER OF ENDOGENOUSLY LABELED CATECHOLAMINES USING LIQUID CHROMATOGRAPHY ELECTROCHEMICAL DETECTION. Barbara A. Bennett* and David K. Sundberg* (SPON: Melvin Levitt). Dept. of Physiol. Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

Catecholamine turnover has been measured using steady-state methods by: (a) following the decay of tissue levels of injected labeled amines; (b) following the rate of formation of labeled amine after injection of labeled precursor; or (c) using nonsteady-state systems by following decline or accumulation of amine levels after inhibiting synthesis or degradation, respectively.

We have developed a technique for quantitating the turnover of catecholamines in various rat brain regions *in vivo*. This procedure involves the administration of 5 μCi of ^{3}H-tyrosine directly into the lateral cerebral ventricle in chronically cannulated rats. The tissues were weighed and extracted for catecholamines. Separation was by high performance reverse phase liquid chromotography and content analyzed with electrochemical detection. Eluant samples were collected for tritium monitoring.

Areas examined were the anterior hypothalamus (AH), medial basal hypothalamus (MBH), striatum, and brain stem. Both dopamine (DA) and norepinephrine (NE) were synthesized as indicated by co-migration of tritiated label with DA and NE retention times. Thirty minutes after administration of ^{3}H-tyrosine, DA synthesis was greater than NE in the MBH (1,916 fg vs 828 fg), AH (1,030 fg vs 560 fg), and striatum (7,420 fg, NE not measureable). The brain stem, on the other hand, showed greater NE (532 fg) synthesis than DA (110 fg). These levels are a conservative index of biogenic amine synthesis since specific activity of total tissue tyrosine could not be established at this time. However, they are a reliable index of amine turnover.

This method of analysis allows for pharmacological studies of synthesis inhibition and consequent changes in catecholamine turnover.

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53.13 HYPOTHALAMIC CATECHOLAMINE TURNOVER IN VITRO MEASURED BY ELECTROCHEMICAL DETECTION. David K. Sundberg*, Laurie S. Pittman* and Barbara A. Bennett* (SPON: P. Blaise Smith). Dept. Physiol. Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

Catecholaminergic innervation to the hypothalamus has been implicated in the regulation of many neuroendocrine parameters. Many investigators have studied neuropeptide secretion by radioimmunoassay from isolated hypothalami in vitro and have administered exogenous catecholamines to determine their effects on release.

In the following communication we have developed a system to quantitate changes in content and the biosynthetic rate of norepinephrine (NE), L-DOPA and dopamine (DA) in vitro. Medial basal hypothalami (MBH) were incubated in Earls balance salts solution (pH 7.2, 95% O_2 5% CO_2) with 5 µCi of ^{3}H-tyrosine for different lengths of time and in the presence of various enzyme inhibitors involved in catecholamine metabolism (alpha methyl para-tyrosine, 10^{-3}M, αmpt) or catabolism (iproniazid, 10^{-4}M). Prior to, or after incubation the MBH was weighed and extracted for catecholamines using a modification of the alumina adsorption technique. The purified catecholamines were separated by high performance liquid chromatography, quantitated by electrochemical detection, and the eluant was collected for tritium monitoring. During incubation amine content fell during the first hour of incubation and approached a state of equilibrium at 2.5 hrs. NE and epinephrine content decreased by 50% when compared to non-incubated controls (3.26 ± 0.27 to 1.73 ± 0.18 ng/mg and 101 ± 12 to 46 ± 13 pg/mg, respectively). DA content, however, was reduced by 68% during incubation (1.03 ± 0.04 to 0.380 ± .05 ng/mg). Appreciable amounts of tritiated label were found to co-migrate with NE, L-DOPA and DA. During the first thirty minutes the majority of label appeared with L-DOPA (.39 ± 64 x 10^3 dpms) and particularly DA (6.45 ± 1.7 x 10^3 dpms). The newly labeled DA pool increased during incubation and plateaued at 2.5 hrs (10.7 ± .29 x 10^3 dpms), while the labeled L-DOPA pool remained essentially unchanged. The label that co-migrated with NE continuously increased (0.92 ± .10 x 10^3 dpms) but never approached that level found in the DA pool. Iproniazid reduced incorporation of ^{3}H-tyrosine into DA (61%), probably via feedback inhibition of tyrosine hydroxylase consequent to increasing amounts of the cytoplasmic amine. Direct inhibition of tyrosine hydroxylase by αmpt virtually abolished (93%) DA biosynthesis.

These studies indicate that elevated hypothalamic dopamine turnover continues in vitro using a steady-state index, while norepinephrine turnover (quantitatively the major amine) is lower. This rate of amine turnover undoubtedly affects neuroendocrine parameters as measured in vitro. (Supported by NIH grant HD-10900).

53.14 ACETAMINOPHEN AS AN INTERNAL STANDARD FOR CALIBRATING IN VIVO ELECTROCHEMICAL ELECTRODES IN RAT CAUDATE. M.E. Morgan* and C.R. Freed* (SPON: D.G. Whitlock). Depts Medicine and Pharmacology, Div. Clinical Pharmacology, U. Colorado Health Sciences Center, Denver, CO 80262.

In vivo electrochemistry has been shown to be useful for measuring relative changes in extracellular dopamine concentration in the caudate. Electrodes have been calibrated to dopamine concentrations in the beaker, but electrode responsiveness in tissue has been difficult to assess. Because acetaminophen is electrochemically active and does not interact with catecholamine neurons, we felt it would be a good compound for in vivo electrode calibration. Male Sprague Dawley rats 200 to 300 gm were anesthetized with urethane 1.0-1.5 gm/kg. A 200 micron carbon paste electrode was inserted stereotaxically in the left caudate. The electrochemical response was generated and measured with a DCV-4 cyclic voltammetry amplifier modified to provide nanoamp/volt sensitivity (Bioanalytical Systems). The amplifier output was processed by a semi-differentiator constructed by Bioanalytical Systems. Semi-differentiation has been shown to produce a more easily interpretable voltammetric readout (Lane et al. J. Electroanal.Chem. 95:117, 1979). The dopamine peak appeared at 0.42 volts. Acetaminophen electrochemical oxidation occurred at 0.55 volts. Acetaminophen was injected i.p. in doses of 25, 50, 75 and 100 mg/kg. Voltammetric scans were done every 10 minutes and maximum voltammetric response was seen approximately 0.5 hour after drug injection. Incremental doses were given at 2 hour intervals. Because the drug half-life is 1.5 hour, incremental peak heights were estimated after subtracting the extrapolated signal from the preceding dose. Results show a linear dose response relationship to acetaminophen administration. Actual electrode sensitivity was measured by killing the animals and assaying caudate acetaminophen concentrations. The ratio of the acetaminophen peak height just before sacrifice to the acetaminophen concentration assayed in the caudate is an absolute measure of electrode sensitivity. This value correlated well with the maximal current response produced by a single dose of 100 mg/kg acetaminophen (coefficient of variation 15%). Preliminary experiments with bilateral electrochemical detectors in rat caudate indicate that the acetaminophen response predicts the magnitude of the amphetamine response on each side. These data show that intraperitoneal acetaminophen can generate an electrochemical response that occurs at a potential distinct from dopamine and that is useful for predicting in vivo electrode sensitivity.

53.15 IN VIVO VOLTAMMETRY: STUDIES OF DOPAMINE RELEASE IN THE RAT STRIATUM AFTER ADMINISTRATION OF L-DOPA. F. Hefti, E. Melamed, T. Maher, and R.J. Wurtman. Lab. Neuroend. Reg., MIT, 56-245,Cambridge, MA.

We studied the time-course of dopamine (DA) release after systemic administration of L-DOPA in the rat striatum. Recordings were obtained from anesthetized animals with carbon paste microelectrodes (diameter (100 µm). Working and reference electrodes were prepared as described by Conti et al. (Life Sci. 23, 2705, 1978). Chronoamperometric measurements were made by applying a potential of 0.8V for 1 sec, with an interval of 5 min between measurements. In later experiments, the potential was scanned every 5 min from 0 to 1.0V (scan rate: 50 mV/sec); this procedure allowed us to measure DA plus DOPAC without interference from methylated catecholamine metabolites. Administration of L-DOPA (50 mg/kg) after pretreatment with carbidopa (an inhibitor of peripheral DOPA decarboxylase, DDC) produced a large rise in chronoamperometric currents, lasting for approximately 6 hours. We used different approaches to determine whether DOPA, DA, or DA metabolites (which are all oxidized at the potential chosen) were responsible for these increases. 1) Rats were killed at different times after L-DOPA administration, and their striata were analyzed biochemically for DOPA, DA, and DA metabolites. Comparison of time-courses revealed that the rise in chronoamperometric responses correlated with elevations in striatal DOPAC and HVA levels rather than with those of DOPA or DA. L-DOPA administration also raised chronoamperometric responses in the cerebellum, where only very small increases in DA levels but large elevations in DOPAC and HVA concentrations were measured biochemically. These findings suggest that DA metabolites contribute significantly to the increases in chronoamperometric responses observed after administration of L-DOPA. 2)L-DOPA given after inhibition of central DDC, a treatment that results in large increases in striatal DOPA levels (measured biochemically)without formation of DA and DA metabolites, failed to increase chronoamperometric responses, indicating that exogenous L-DOPA entering the striatum is not detected with this technique. 3) In animals pretreated with an inhibitor of monoamine oxidase, L-DOPA administration produced only a small and short-lasting increase in chronoamperometric responses, despite sustained and large increases in striatal DA concentrations. (Using the potential-scanning technique, it could be shown that the increases in electrochemical responses occur at a potential at which DA and DOPAC but not methylated catecholamine metabolites are oxidized.) Consequently, DA appears to contribute only a small fraction limited to the initial rise of the large increases in chronoamperometric responses observed after administration of L-DOPA. Our results suggest that administration of L-DOPA (after pretreatment with carbidopa) produces only a short-lasting increase in striatal DA release, despite a prolonged rise in striatal DA concentration.

53.16 IN VIVO MONITORING OF DOPAMINE (DA) RELEASE AFTER NIGRAL STIMULATION OR AMPHETAMINE USING CYCLIC VOLTAMMETRY. S.H. Butcher, C.D. Hull, R.F. Lane. Mental Retardation Research Center, School of Medicine, UCLA, Los Angeles, CA 90024 and Department of Chemistry, University of Oregon, Eugene, OR 97403.

Interest in voltammetric techniques for the in vivo, continuous measurement of endogenously released catecholamines has gained widespread interest over the last few years. Lane et al. (J. Electroanal. Chem. 95: 117, 1979) have demonstrated in the rat that d-amphetamine produces an increase in release of DA in the caudate nucleus, while producing no change in the release of serotonin.

In these experiments the effects of d-amphetamine or stimulation of the substantia nigra were examined using voltammetric techniques in the cat. Adult cats were anesthetized with sodium methohexital, a short-acting barbiturate. A four-pronged stimulating comb was stereotaxically introduced into the right substantia nigra, and a carbon paste electrode was introduced into the right and left caudate nucleus (tip diam. 100µm). Potentials are measured with respect to a reference-auxiliary electrode combination which is placed on the surface of the posterior cortex. Semiderivative voltammograms were then recorded every ten minutes until a stable baseline was reached.

After a stable baseline was achieved the substantia nigra was stimulated unilaterally for 5 min (10pps, 0.5ms duration, 10 V). The release of DA, both ipsilateral and contralateral to the stimulation was monitored immediately after stimulation, and at 10 min intervals thereafter. DA release after nigral stimulation increased 128-133% when compared with control release. However, 10 min after nigral stimulation DA release reached maximum levels, which ranged from 180-225% of control.

The increase in released DA ipsilateral to the nigral stimulation did not return to control release levels for approximately 20-30 min.

The effect of nigral stimulation on the contralateral caudate nucleus was equivocal. These effects ranged from an increased DA release (101-105% compared to control) to a decrease in DA release (80-94% of control).

Amphetamine (0.2mg i.v.) also produced an increase in release of DA (144-151%). This effect lasted for approximately 15 min. (Supp. by USPHS HD05958, NS5316).

53.17 THE DYNAMICS OF ENDOGENOUS DOPAMINE (DA) RELEASE FROM THE CAT CAUDATE NUCLEUS IN VIVO. K. G. Lloyd, B. Le Roux* and G. Bartholini*, Research Dept., Synthelabo-LERS, Paris, France.

The dynamics of the striatal DAergic synapse has been previously studied by indirect biochemical methods. The present study examines directly the "in vivo" release of endogenous DA by means of a push-pull cannula placed in the caudate nucleus through which an artificial CSF is perfused, as previously described (Experientia 31, 560, 1975). In the present study, adult cats of either sex were anesthetized, placed in a stereotaxic apparatus and the push-pull cannulae implanted (A: 15.5 ; D: + 5 ; L: + 4.5). Samples were collected over 20 min periods (200 µl/min) and the DA estimated by radioenzyme assay. All injections were by the i.v. route and 3-5 cats were used per dose per drug. The DA content of the striatal perfusate varied considerably from cat to cat, but for a given animal the release was stable for a period of at least 5 hours. Saline injection did not alter DA release. Several neuroleptics (haloperidol, pimozide, chlorpromazine) produced a large, immediate, increase in DA release which lasted for at least 3 h post-injection. Administration of pimozide (ip) daily for 10 days before the experiment greatly reduced ($p < 0.02$) the striatal release of iv pimozide injection. Amphetamine (0.5-10 mg/kg) resulted in an immediate, dose-dependent increase (to > 3000 percent of control at 10 mg/kg) in DA release. Nomifensine, (5-10 mg/kg) which inhibits DA uptake, also increased DA release, but had a slower onset of action and was less potent than amphetamine. Reserpine (2 mg/kg) produced an initial release of DA (to 300 percent of control) which was of a short duration. Gamma-hydroxybutyrate (G-OH) produced an initial small decrease in striatal DA release which was followed later (2.5 h) by a significant increase. G-OH retarded but only slightly reduced the effect of amphetamine (2.0 mg/kg) on DA release. GABA agonists (SL 76 002 or muscimol) produced a decrease in striatal DA release.SL 76 002 completely blocked the DA release induced by chlorpromazine and partially blocked that due to amphetamine. These results provide direct evidence for : (1) the enhanced striatal DA release after DA receptor blockade ; (2) the inhibition of ongoing DA neuron activity and the blockade of the neuroleptic-induced feedback activation by GABA agonists ; (3) the tolerance upon repeated neuroleptic administration to the feedback-induced release of striatal DA ; (4) the immediate effect of amphetamine on endogenous DA release "in vivo" ; (5) that nomifensine enhances the synaptic concentrations of DA : (6) that reserpine can produce an initial pulse of DA release ; and (7) G-OH initially decreases DA release, followed by a rebound augmentation. Thus, this method provides a direct "in vivo" analysis of striatal DA synaptic dynamics and can be used to define the mechanism of action of psychotropic drugs.

53.18 ELEVATION OF NIGRAL GABA CONCENTRATION BY GABACULINE: EFFECT ON STRIATAL DOPAMINE RELEASE. B.S. Glaeser, W.R. Millington, and R.J. Wurtman. Laboratory of Neuroendocrine Regulation, Dept. of Nutrition and Food Science, M.I.T., Cambridge, MA 02139.

Sprague-Dawley rats received IP injections of water or Gabaculine (100 mg/kg), an irreversable inhibitor of GABA-transaminase. Four hours later rats were sacrificed by decapitation; striatia and substantia nigrae were dissected and removed within 3.0 min. GABA was analyzed by a modified amino acid analysis method utilizing one buffer (pH = 4.53, 0.6 N Li+) with cation exchange chromatography and ninhydrin detection. Dopamine, DOPAC, and HVA were analyzed by HPLC/electrochemical techniques. Four hours after Gabaculine administration, nigral GABA levels were markedly elevated ($p \leq 0.05$) when compared with those in control animals (see table). Striatal dopamine concentrations were not altered by Gabaculine treatment; however, striatal DOPAC and HVA levels were significantly elevated ($p \leq 0.05$). In a second experiment, Gabaculine-induced elevations of GABA were not associated with significant changes in striatal acetylcholine concentrations after four hours. These data suggest that Gabaculine induced elevations of GABA levels are not associated with direct inhibition of nigrostriatal dopamine neurons. The GABA may inhibit another inhibitory neuron in the substantia nigra or striatum.

	Nigral GABA Concentrations	Striatal concentrations ng/mg tissue (X + SD) OF		
Group	nmol/mg tissue (X+SD)	DA	DOPAC	HVA
H_2O	4.87 ± 1.81 (n=8)	7.5 ± .84 (n=8)	1.32 ± .29 (n=8)	.80 ± .14 (n=8)
Gabaculine (100 mg/kg)	15.30 ± 3.42* (n=9)	7.8 ± .75 (n=10)	1.92 ± .6* (n=10)	1.19 ±.41* (n=10)

*$p \leq 0.05$ different from controls

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53.19 HIGH-AFFINITY GLUTAMATE TRANSPORT IN THE STRIATUM: POSSIBLE DOPAMINERGIC REGULATION. L.C. Murrin and V.J. Robertson*, Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68105.

A high-affinity glutamate transport system (K_T=10 µM) has been demonstrated in synaptosomal preparations from rat brain. In striatum there is evidence that this system is associated, at least in part, with the cortico-striatal glutamatergic pathway. High-affinity glutamate transport can be activated in synaptosomal preparations by depolarization (Mol. Pharmacol. 12:1082, 1976; Life Sci. 22:2009, 1978). The ability to alter rate of glutamate transport in response to depolarization is analogous to what has been found for high-affinity choline transport in cholinergic neurons and for tyrosine hydroxylase activity in noradrenergic and dopaminergic neurons. The cortico-striatal pathway has been shown to have dopaminergic receptors on its terminals (Nature 271:766, 1978; Eur. J. Pharm. 60:229, 1979). Thus it seems possible that rate of glutamate transport may reflect the state of activity in glutamatergic neurons and the activity of these neurons in the striatum may be modified by a dopaminergic input. We sought to examine this possibility. Rats were injected i.p. with the dopaminergic agonist, apomorphine, or the antagonist, haloperidol, and were decapitated at various times following the injection. A synaptosomal preparation (P_2) was prepared from brain regions by standard techniques. High affinity glutamate transport was measured as previously described (Mol. Pharmacol. 12:1082, 1976; Life Sci. 22:2009, 1978). Zero-sodium blanks were used throughout and were found to be essentially the same as 0°C blanks. A relatively low dose of apomorphine produced a significant increase ($p<.02$, Student's two tailed t test) in glutamate transport in the striatum. Ten and 30 minutes after 0.1 mg/kg of apomorphine, glutamate transport was 12.8 ± 1.6 pmol/2 min-mg prot (n=3) and 10.2 ± 1.0 pmol/2 min-mg prot (5) while control levels were 7.3 ± 0.5 pmol/2 min-mg prot (21). This effect was apparently dose-dependent since the alteration in glutamate transport was greatly diminished at 0.5 mg/kg and no significant alteration was seen at 1 and 10 mg/kg (10, 30 and 60 min). Haloperidol at a relatively low dose (0.1 mg/kg for 30 min) also produced a significant increase in glutamate transport while less of an effect was seen with 0.25 mg/kg for 30 min and no effect was seen with 1 and 10 mg/kg (10, 30 and 60 min). Neither drug at any of the doses or times examined produced significant alterations in glutamate transport in cortex or hippocampus. This data suggests that the dopaminergic input to the striatum may affect the high-affinity glutamate transport in that region and so may in some way modulate the glutamatergic neurons in striatum. The specificity of this effect for dopaminergic receptors and whether this is a direct or indirect effect of these drugs remains to be examined. Supported by BNS-7921105, MH-33390 and March of Dimes Foundation.

53.20 DIAZEPAM INHIBITS THE LIGHT-INDUCED INCREASE IN RETINAL DOPAMINE TURNOVER. C. W. Kamp and W. W. Morgan. Dept. Anat. Univ. Texas Health Sci. Ctr., San Antonio, TX 78284.

Light stimulates dopamine (DA) turnover in dark-adapted rat retinas, and this effect is blocked by the GABA agonist, muscimol (MU), in a dose-dependent fashion (Morgan and Kamp, 1980). Pentobarbital and phenobarbital potentiate this action of MU while picrotoxinin, a GABA antagonist, reverses the combined effect of MU and pentobarbital (Kamp and Morgan, 1980). These data suggest that light-activated DA turnover in the rat retina may offer an ideal in vivo model for testing drugs which purportedly interact with the GABA system. Here we present the effects of diazepam (DZ) on the light-induced increase in retinal DA turnover. Rats were dark adapted for 15 hr and divided into 4 groups. The first group was injected with DZ (22 mg/kg, i.p.) 90 min before sacrifice; the second received 3 equal injections of MU beginning 75 min prior to sacrifice (6.6 µmole/kg, i.v. cumulative); the third was treated with both DZ and MU, and the last group received saline or vehicle injections at all appropriate time points. All rats were exposed to light for 1 hr, and half the rats in each group were treated with α-methylparatyrosine (αMPT, 250/kg, i.p.) just before light exposure. Following sacrifice, the retinas were quickly removed and frozen. Later DA contents were assayed radioenzymatically. The decline in DA after αMPT treatment served as an estimate of DA turnover. MU alone produced no significant change in the light-mediated decline of DA. This dose of MU was also ineffective in our earlier work, but in combination with barbiturates, which were also ineffective alone, it produced a dramatic decline in DA turnover. In contrast to the barbiturates, DZ significantly reduced DA turnover in the light ($p<.05$), but this effect was not enhanced in the presence of MU. We have recently found that intraocular picrotoxinin or bicuculline methiodide enhance the light-induced increase in DA turnover (unpublished). These results indicate that there is a tonic GABAergic input to the retinal DA neurons. DZ may be potentiating the action of this endogenous GABAergic input. We are currently conducting experiments which should answer this question. Supported by DA 00755, NS 14855 and RSDA MH 00028 to WWM.

53.21 EFFECTS OF RESERPINE ON TURNOVER OF MONOAMINE OXIDASE IN MOUSE BRAIN AND LIVER. T.R. Hall*, P.B. Yurgens*, D.K. Newton*, J. Korducki* and H.R. Figueroa*. (SPON: E. Stein). Dept. of Biology, Marquette University, Milwaukee, WI 53233.

Disturbances in activity of monoamine oxidase (MAO), an enzyme important in the regulation of levels of monoamines in the central nervous system and circulation, have been implicated in a variety of affective disorders. It is important, therefore, to understand the regulation of production of this enzyme. Adult mice were injected once with the irreversible MAO inhibitor pargyline at a dose of 30 mg/kg ip., which was shown to be the minimum dose to produce maximum inhibition of MAO activity; or every 3 days with the monoamine depletor reserpine at a dose of 1 mg/kg ip., which was shown to produce greater than 75% depletion of 5-hydroxytryptamine (5HT) in the brain; or with both treatments. MAO was determined fluorometrically in hypothalamus, cerebellum, brainstem, rest of brain and liver. 5HT was determined fluorometrically in brainstem and rest of brain. In all tissues MAO activity increased in a time-dependent fashion after pargyline inhibition. The calculated half-life of the enzyme, assuming first order rate kinetics, was tissue dependent, with liver < hypothalamus < cerebellum < rest of brain < brainstem. 5HT content was elevated after pargyline, low after reserpine and showed intermediate values after both drugs for at least 2 weeks. However, 5HT levels after pargyline + reserpine were significantly depressed, compared to pargyline treatment only, in both brainstem and rest of brain. Following reserpine, turnover of MAO, as determined by the slope of the recovery curve following pargyline administration, was significantly slower in hypothalamus and cerebellum, showing a 2-3 fold increase in the time for 50% recovery of steady state activity. Reserpine alone had no significant affects on MAO activity in any tissue studied, nor did it affect the rate of recovery of MAO following pargyline in the liver. We conclude that (a) turnover of MAO varies between different brain regions and liver, (b) the content of monoamines may regulate synthesis of MAO in hypothalamus and cerebellum. Synthesis of MAO in other regions may be independent of monoamine control.

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53.22 CHARGE ISOZYMES OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE: MECHANISM OF ACTIVATION BY PHOSPHATE. D. Park, T. Joh, E. Baetge, K. Chiang* and D. Reis. Laboratory of Neurobiology, Dept. of Neurology, Cornell Univ. Med. College, New York, NY.

It is known that adrenal phenylethanolamine N-methyltransferase (PNMT) from different species has different biochemical characteristics, e.g., electrophoretic mobility, stability, etc. Even within the same species, several charge isozymes exist. The mechanisms governing the different biochemical characteristics of the enzyme are still unknown. In order to further elucidate the molecular mechanisms accounting for these differences, we characterized purified PNMT from both rat and bovine adrenal glands. (1) The subunit molecular weight, by SDS-PAGE, was 31,000 daltons for both forms. (2) Antibody to the rat form strongly cross-reacted to rat PNMT but poorly to the bovine form. Nevertheless, antibody to the bovine form crossreacted strongly to both forms. (3) When PNMT was synthesized in vitro by the translation of mRNA purified from bovine adrenal medulla, antibodies to either form precipitated PNMT, thereby suggesting that the basic protein structure of PNMT of both species is similar. (4) The electrophoretic mobility of the enzyme at pH 8.2 in Tris-glycine buffer in a 7.5% polyacrylamide gel was Rm = 6.3 toward the anode for the rat and 4.5 for the bovine enzyme, indicating that the rat form is more negatively charged. Since the molecular weight of both forms is similar, they are charge isozymes. (5) When phosphate buffer (0.1 M) was used for the activity assay at pH 8.6, the specific activity of pure PNMT from both species was similar (2.0 umol/mg/h for bovine and 2.1 for rat form). However, when Tris-Cl buffer was used the specific activity of the bovine form was only one-third that of the rat form (0.7 umol/mg/h for bovine). Addition of phosphate to Tris-Cl buffer in the assay mixture increased PNMT activity of only bovine origin and the increase was concentration dependent. At 100 mM phosphate, the activity increased three-fold. Since the bovine form is less negatively charged, phosphate ion, which is a polyanion, may influence the total charge of the bovine form to become more negatively charged. It is probable that the more negatively charged form has higher specific activity.

The data indicate that: (1) PNMT in bovine and rat adrenal medulla is a charge isozyme, and the rat form is more negatively charged; (2) phosphate, a polyanion, increases the specific activity of the bovine form of PNMT, probably by increasing the negative charge on the enzyme; and (3) the differences in the biochemical characteristics of PNMT isozymes are caused by post translational modification of the enzyme, probably due to carbohydrate content which changes the charge of the enzyme.

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53.23 REGULATION OF TYROSINE HYDROXYLASE ACTIVITY IN WHOLE RETINA, RETINAL CELL SUSPENSIONS, AND RETINAL HOMOGENATES. P.M. Iuvone* and P.B. Marshburn* and N.H. Neff. Dept. of Pharmacology and Ophthalmology, Emory Univ. Sch. of Med., Atlanta, GA 30322; Preclinical Pharmacology Lab, NIMH, St. Elizabeths Hosp., Washington, D.C. 20032 (SPON: J. Sutin).

Dopamine (DA) is localized in a subpopulation of amacrine neurons in the retinas of most mammalian species. Photic stimulation increased the release and biosynthesis of retinal DA in vivo (Kramer, Invest. Ophthalmol. 10: 438, 1971; Iuvone et al., 1978), suggesting that these neurons are activated as a consequence of light exposure. Associated with the light-evoked stimulation of DA biosynthesis was an activation of tyrosine hydroxylase (TH). To determine if TH could be activated by depolarization of the DA-containing neurons, retinas were dissociated into cell suspensions for in vitro studies. Dissociation of the retinas disrupted most synaptic connections, and greater than 90% of the cells in suspension were present as single cells. In these suspensions, high concentrations of K^+ resulted in an activation of TH.

GABA and muscimol, injected intravitreally, inhibited the light-evoked activation of TH. Intravitreal injections of bicuculline or picrotoxin activated TH in the dark, indicating that GABA plays a role in the inhibition of the DA-neurons in darkness. Incubations of retinal cell suspensions with muscimol decreased tyrosine hydroxylase activity in a picrotoxin-sensitive manner, suggesting that the GABA receptors responsible for inhibiting the DA neurons in darkness are located on the DA neurons, rather than on other neurons that interact with the DA cells.

Incubation of retinal homogenates with cAMP, ATP and Mg^{+2} increased TH activity. Incubation of the retinal cell suspensions with 8-Br-cAMP also increased TH activity, indicating that the DA neurons contained sufficient cAMP-dependent protein kinase in the proper subcellular location to activate TH in situ.

These studies illustrate the usefulness of retinal cell suspensions in pharmacological and biochemical studies of the regulation of synaptic processes.

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53.24 ACUTE AND CHRONIC EFFECTS OF β-PHENYLETHYLAMINE (PEA) ON RAT STRIATAL TYROSINE HYDROXYLASE ACTIVITY. David M. Stoff and Karen Gale. Lab of Clinical Psychopharm., St. Elizabeths Hosp., and Dept. of Pharmacol., Georgetown Univ. Schools of Medicine & Dentistry, Washington, DC 20007.

β-phenylethylamine (PEA), a naturally occurring non-catechol sympathomimetic amine, has structural and pharmacological features in common with amphetamine. In order to further characterize the actions of PEA on the nigrostriatal dopaminergic system, we investigated 1) the ability of acutely administered PEA to reverse both the catalepsy and the activation of tyrosine-hydroxylase (TH) induced by chlorpromazine, and 2) the effects of chronically administered PEA on striatal TH activity.

In the acute study, rats were pretreated with a dose of chlorpromazine (20mg/kg i.p.) which was maximally effective in causing catalepsy and activation of striatal TH, when measured between one and two hrs after injection. At 20-45 min after injection of chloropromazine, the animals were treated with various doses of either PEA, apomorphine, d-amphetamine or saline (controls). Rats were decapitated 1-1.5 hr after chlorpromazine and striatal TH was assayed (within 24 hr after killing) in the presence of a subsaturating concentration of $DMPH_4$ cofactor (0.2mM) and a saturating concentration of tyrosine. We obtained a dose-dependent reversal of both catalepsy and TH activation with all 3 drugs tested. PEA, in a dose of 150mg/kg i.p., caused approximately a 50% reduction in both the duration of catalepsy and degree of TH stimulation produced by chlorpromazine. PEA was 40 times less potent than amphetamine and 80 times less potent than apomorphine.

In the chronic study, large doses of PEA (600 mg/kg) were applied intragastrically twice daily for 14 consecutive days. This dosage regimen produced behavioral stimulation lasting for 5-7hrs per day. Rats receiving this treatment were found to have a 20% increase in V_{max} of striatal TH when compared with controls which had received daily intragastric injections of saline. The increased TH activity could be measured for up to 10 days after cessation of chronic PEA treatment. Concurrent chronic administration of haloperidol (1.0mg/kg i.p.) did not influence the stimulation of TH by chronic PEA, suggesting that the effect is not dependent upon dopamine-receptor stimulation.

Thus, we have obtained neurochemical evidence which indicates that PEA is capable of augmenting nigrostriatal dopaminergic transmission acutely: the ability of PEA to reverse the effects of dopamine receptor blockade is similar to that of known dopamine agonists. In addition, our chronic studies suggest that PEA may be able to augment DA transmission in a long-term fashion by a mechanism independent of dopamine-receptor stimulation.

53.25 STRIATAL SYNAPTOSOMAL TYROSINE HYDROXYLASE ACTIVITY: A MODEL FOR STUDY OF PRESYNAPTIC DOPAMINE RECEPTORS. Brian A. McMillen, Dept. Pharmacol., U. Texas Health Sci. Center, Dallas, Tx 75235.

The concept of an inhibitory presynaptic dopamine (DA) receptor on DA nerve endings is now well accepted. Stimulation of this receptor by DA or DA agonists causes (in vivo) decreased tyrosine hydroxylase activity (Tyr-OH) which in turn will cause decreased DA release. This autoreceptor is presumed to be on the outside of the nerve ending membrane, but evidence for this supposition is scant. If the autoreceptor is an external membrane receptor, DA re-uptake inhibitors should inhibit Tyr-OH activation due to small doses of neuroleptics by potentiating the amounts of DA released into the synapse. Furthermore, a non-diffusable DA agonist should inhibit synaptosomal Tyr-OH, in vitro. The activation of Tyr-OH by haloperidol (Halo, 0.025 mg/kg) in vivo (determined by 30 min L-DOPA accumulation after decarboxylase inhibition with NSD-1015) is inhibited by DA re-uptake blockers (amfonelic acid, nomifensine, cocaine, benztropine, etc.). Either no change or an enhancement of L-DOPA accumulation occurs when these drugs are combined with 1.0 mg/kg Halo. Thus, potentiation of DA in the synaptic cleft, by inhibiting DA re-uptake, prevents Tyr-OH enhancement by small doses of Halo. After the larger dose of Halo, DA cannot overcome the presynaptic receptor blockade and Tyr-OH is increased because of presynaptic receptor blockade and increased impulse flow. Tyr-OH, determined in synaptosomal preparations, is inhibited by apomorphine (Apo) or DA with IC_{50} values of 4.5×10^{-7}M and 2.5×10^{-6}M, respectively; and the inhibition is reversed by Halo. The potent DA uptake inhibitor, amfonelic acid, at 3×10^{-6}M did not alter the IC_{50} of DA for inhibition of synaptosomal Tyr-OH. Thus, the data indicate that the DA presynaptic receptor is indeed an external membrane receptor. Synaptosomal Tyr-OH may provide a system for study of neuroleptic affinity for presynaptic receptors using a pharmacological response (reversal of Apo inhibition of Tyr-OH) instead of ligand displacement. Comparison of neuroleptic drug interaction in this system with radio-ligand binding studies may prove useful for determining whether apparent specific binding may be at presynaptic DA receptors. Experiments on the ability of neuroleptic drugs to reverse Apo inhibition of synaptosomal Tyr-OH will be presented and the usefulness of this presynaptic DA receptor system discussed.

53.26 DIFFERENTIAL EFFECTS OF GABA-ELEVATING AGENTS ON THE NEUROLEPTIC-INDUCED ACTIVATION OF STRIATAL TYROSINE-HYDROXYLASE: EVIDENCE THAT Di-N-PROPYLACETATE AUGMENTS GABAERGIC NEUROTRANSMISSION. Mariano Casu* and Karen Gale (Spons: R.A. Gillis). Dept. of Pharmacology, Georgetown Univ. Schools of Med. & Dent., Washington, D.C. 20007.

Di-n-propylacetate (sodium valproate, DPA), in contrast to many other agents which elevate brain GABA content, appears to increase GABA selectively in a compartment that is associated with nerve-terminals (Iadarola and Gale, Eur. J. Pharmacol. 59: 125, 1979). In order to determine whether the DPA-induced increase in nerve-terminal GABA in substantia nigra (SN) could augment GABAergic transmission in SN, we examined the ability of DPA to influence nigrostriatal dopamine function.

Neuroleptic drugs, such as haloperidol, cause an allosteric activation of striatal tyrosine hydroxylase (TH) which can be measured in vitro as a decrease in the K_m of TH for cofactor ($DMPH_4$). This effect can be reversed by treatment with GABA-receptor agonists (Gale et al., JPET 206: 29, 1978). We therefore examined the ability of DPA to reverse the activation of striatal TH induced by haloperidol (0.5mg/kg i.p., 40min prior to decapitation). DPA was administered i.p., 10min after haloperidol. The data obtained (see Table below) demonstrate the ability of DPA to reverse the haloperidol-induced activation of striatal TH in doses which caused a 30-50% increase in GABA in SN. The action of DPA was completely antagonized by treatment with bicuculline (BIC) (3.5 mg/kg s.c., 5min after DPA), a GABA receptor antagonist, indicating that the effect of DPA is mediated via GABA receptors.

The effect of amino-oxyacetic acid (AOAA, injected i.p. 80min. before haloperidol) was also examined, since our previous evidence suggested that the GABA increase after this agent was largely associated with compartments of GABA other than nerve terminals. As shown below, AOAA was less effective than DPA in preventing the haloperidol-induced activation of TH, despite the fact that nigral GABA was increased by 30-100%.

K_m of Striatal TH for $DMPH_4$ (mM):

Treatments	SAL	DPA 200	DPA 300	DPA 400	DPA 400+ BIC 3.5	AOAA 20	AOAA 40	AOAA 60(mg/kg)
control	.75	.76	.75	.78	.73	.75	.70	.73
haloperidol	.30**	.29**	.43*	.76	.31**	.28**	.34**	.41*

**significantly different from control
*significantly different from control and from haloperidol + saline (SAL) $p < .05$

(Supported by USPHS grants DA 02206 and MH 32359)

53.27 GLUCOCORTICOID EFFECTS ON BRAIN TYROSINE HYDROXYLASE. K. A. Markey*, A. C. Towle* and P. Y. Sze. Dept. of Biobehavioral Sci., Univ. of Connecticut, Storrs, CT 06268.

An "inductive" effect of glucocorticoids on brain tyrosine hydroxylase (TH) was demonstrated in the pontine region of mouse brain during early postnatal development. Administration of corticosterone (20 mg/kg, i.p., twice daily) to 7-day-old mice for 3 days led to an increase of TH activity (by 50%) in the pons but not in the substantia nigra. Similar inductive effects on pontine TH could be produced by other glucocorticoids, including hydrocortisone, cortisone, dexamethasone and triamcinolone. Steroids such as estradiol, testosterone, 11-deoxycortisol and 21-deoxycortisol were all ineffective. Developmentally, the responsivity of pontine TH to corticosterone was limited to a brief postnatal period (approximately days 7-12). Hormone administration during postnatal days 2-5 or after day 12 failed to produce an increase of enzyme activity. Interestingly, midbrain tryptophan hydroxylase responds to exogenously administered glucocorticoids also only during the second postnatal week, as previously reported.

To examine the possible involvement of a cytoplasmic steroid receptor, the binding of ^{3}H-glucocorticoids to the receptor was determined in vitro. A 100,000 g cytosol preparation from dissected locus coeruleus region was incubated in the presence of 2×10^{-7} M ^{3}H-steroid at 0°C for 3 hrs. Corticosterone, hydrocortisone, dexamethasone and triamcinolone were all found to bind to a protein binding site with a specific binding capacity of 160-190 fmoles/mg cytosol protein (from day 10 animals). The binding of these glucocorticoids was mutually competitive, indicating binding to the same site. Cortexolone and progesterone (two steroids with "anti-glucocorticoid" actions) were found to block ^{3}H-corticosterone binding. When animals were pretreated with either cortexolone or progesterone (20 mg/kg, i.p., twice daily), the effect of corticosterone to produce the increase of TH activity was totally prevented. These data support the contention that the steroid effect on TH may be mediated by an intracellular glucocorticoid receptor.

In addition to TH, several enzymes such as liver arginase are known that respond most effectively to exogenously administered glucocorticoids only during the developmental period preceeding adrenocortical maturation. It is possible that after steroidogenesis, some enzymes have already maximally responded to endogenously available steroids. The response of TH to glucocorticoids as demonstrated in the immature animals could signify a hormone component in the regulation of the enzyme.

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53.28 DIFFERENT TRENDS IN TYROSINE HYDROXYLASE ACTIVITY IN CELL BODIES AND TARGET SITES DURING DEVELOPMENT AND AGING. L. Yurkewicz, J.M. Lauder, E. Giacobini and M. Marchi*. (SPON: M. Wilson). Lab. of Neuropsychopharmacology, Dept. of Biobehavioral Sci., Univ. of CT, Storrs, CT, 06268.

Tyrosine hydroxylase (TH) activity was measured in the locus coeruleus (LC), cerebellum, cervical spinal cord, lumbar sympathetic ganglia and iris throughout most of the lifespan of the chick (8 days of incubation to 5 years after hatching) in order to compare the trends in TH activity in noradrenergic cell bodies and terminals in both the CNS and PNS. Fluorescent histochemistry and horseradish peroxidase tracing were used to characterize the LC-cerebellar projections. TH activity was detected in the cerebellum as early as 8 days of incubation, which is the earliest stage so far reported. The greatest increase in total TH activity in the LC (21-fold) and cerebellum (31-fold) occurred during the embryonic period with a greater increase in the target organ, whereas the increase in total TH activity in the cervical spinal cord was approximately the same in the embryonic (4-fold) and post-hatch (5-fold) periods. The cerebellum and cervical spinal cord displayed different trends in TH activity throughout development and aging which suggests the possibility that enzyme transport from the LC may depend upon specific characteristics of the target site during maturation. In both structures examined in the PNS, the greatest increase in total TH activity occurred during the posthatching period (1 day post-hatch to 5 yrs.) with a greater rise in the cell bodies of the lumbar sympathetic ganglia (16-fold) than in the noradrenergic terminals of the iris (13-fold). In the 3 noradrenergic target sites examined, total TH activity continued to increase long after hatching, reaching the highest levels at 7 mo. when the chicken is considered fully mature. This rise in enzyme activity may be due to continual growth of the innervating fibers up to 7 mo. as opposed to synthesis and accumulation of TH in pre-existing terminals. During adulthood and aging, cell bodies and noradrenergic target sites showed different patterns in enzyme activity, in that total TH activity declined in the LC and cerebellum after 16 mo., declined earlier in the iris after 7 mo. and continued to rise in the ganglia up to 5 yrs. During aging (16 mo. to 5 yrs.), the terminals of noradrenergic neurons seem to be more affected than the cell bodies in both the CNS and PNS, which is consistent with age-related changes in the cholinergic system (Marchi, M., Hoffman, D.W. and Giacobini, E., Dev. Neurosci., 1980. 3 in press). This phenomenon is more marked in the PNS than in the brain. Supported by the Univ. of CT Research Foundation and NIH grant, NS-13481 to J.M. Lauder.

53.29 TYROSINE HYDROXYLASE INACTIVATION FOLLOWING cAMP-MEDIATED PHOSPHORYLATION ACTIVATION. Kent Vrana* and Robert Roskoski, Jr. Dept. Biochem., LSU Medical Center, New Orleans, Louisiana 70112.

The rate of catecholamine biosynthesis is limited by the activity of tyrosine hydroxylase (TH). We have studied the regulation of this enzyme by cAMP-dependent protein kinase in rat corpus striatum homogenates. Treatment of these extracts with cAMP and Mg·ATP produces a two to ten-fold increase in enzyme activity as determined in a subsequent TH assay. After maximal activation (3 min), continued treatment under phosphorylation conditions is associated with a loss of enzyme activity. This loss is complete following 30 minutes and the inactivated TH exhibits activity which is less than or equal to the activity of homogenates incubated in the absence of cAMP and Mg·ATP. In characterizing this phenomenon, we find that after activation and inactivation of TH with a 30 minute pretreatment with cAMP and Mg·ATP, addition of cAMP alone or purified protein kinase catalytic subunit alone will reactivate and inactivate the enzyme. One interpretation of this is that inactivation of activated TH is due to the loss of protein kinase activity following hydrolysis of cAMP by phosphodiesterase. However, when cAMP levels are maintained during the 30 minute preincubation by repeated addition of cAMP or by including a phosphodiesterase inhibitor (1 mM papaverine), the enzyme is activated and inactivated, but can not be reactivated by additional cAMP or the addition of purified catalytic subunit. Inactivation following activation, then, is mediated by two mechanisms the relationship of which is presently unknown. First, the inactivation which parallels cAMP degradation and loss of protein kinase activity may be related to dephosphorylation, and is reversed by cAMP or exogenous protein kinase. Second, inactivation under conditions of phosphodiesterase inhibition, continued addition of cAMP or addition of protein kinase catalytic subunit is associated with continuous protein kinase activity. Under these conditions, however, inactivated TH can not be reactivated.

Following filtration of a striatal homogenate on a Sephadex G-100 column, the TH is activated by protein phosphorylation conditions but is not subsequently inactivated. The activated TH is therefore inactivated in the striatal homogenate by some factor(s) present in the homogenate which may be resolved by gel filtration. The regulation of tyrosine hydroxylase activity is complex and involves more than the enzyme per se and cAMP-dependent protein kinase. Additional experimentation will be required to ascertain the exact regulatory mechanisms.

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53.30 REGULATION OF DOPAMINE β-HYDROXYLASE DEGRADATION BY ASCORBIC ACID. Dona L. Wong*, Steven J. Masover*, and Roland D. Ciaranello. Dept. Psych., Stanford Med. Ctr., Stanford, CA 94305.

Rat adrenal medullary dopamine β-hydroxylase (DBH) is subject to both hormonal and neuronal controls. Glucocorticoids regulate enzyme steady state levels by inhibiting the rate of protein degradation. Neuronal stimuli via the splanchnic nerve induces de novo enzyme synthesis. Both processes require an intact cholinergic receptor.

Further investigation of the hormonal control of DBH degradation indicates that glucocorticoids exert their effect via the oxidation-reduction cofactor, ascorbic acid, utilized by the enzyme in the production of norepinephrine. Tryptic proteolysis of DBH yields a biphasic degradation curve, with a 3 min half-life for fast phase degradation and a 15 min half-life for slow phase degradation. Ascorbate stabilizes the enzyme against trypsinization. At high concentrations of cofactor, slow and fast phase proteolysis merge yielding a monophasic degradation curve. Phenylethylamine, a substrate of DBH, confers minimum protection to the enzyme against proteolysis. However, when used simultaneously with ascorbic acid, this compound acts synergistically with ascorbate in preventing DBH proteolysis. Immunotitration studies suggest that native protein and protein from a 3 min tryptic digest represent a similar molecular species distinct from protein from a 15 min tryptic digest. Immunotitration of native protein and native protein, 3 min and 15 min digests executed in the presence of ascorbate indicate that ascorbate may prevent the conversion of native enzyme to a form which is less active catalytically but which still retains antigenecity.

The above data suggests that both DBH and phenylethanolamine N-methyltransferase (PNMT) are subject to similar hormonal controls. Adrenal medullary glucocorticoids regulate the steady state levels of these biogenic amine enzymes by controlling their respective cofactors. The cofactors, in turn, prevent the degradation of DBH and PNMT by binding to the enzymes and inhibiting proteolysis.

53.31 RESERPINE EFFECT ON THE AXONAL TRANSPORT OF DOPAMINE-B-HYDROXYLASE AND TYROSINE HYDROXYLASE. B. E. Levin. Dept. of Neurology & Neurosciences, VA Med. Ctr. & NJ Med. Sch., Newark, NJ 07019.

Reserpine induces the synthesis of dopamine-B-hydroxylase (DBH) and tyrosine hydroxylase (TH) enzyme protein in the locus coeruleus (LC). This is followed, after an initial delay, by increased enzyme activity in brain areas supplied by LC axons. To study the role of axonal transport in these changes, 6-hydroxydopamine (4 μg/μl in 2 μl) was injected into the decussation of LC axonal fibers in the far anterior rat hypothalamus on the left side. Axonal transport was measured by the differences in TH and DBH activities between the left hypothalamic sections, proximal to the injection site, and the comparable right, uninjected, hypothalamic sections. Both enzymes accumulated linearly, for up to 4 d proximal to the injection site. Rats were therefore injected in the left hypothalamus with 6-hydroxydopamine 24 h prior to death, preceded by reserpine (5 mg/kg, IP) from 1-22 d prior to death. Following reserpine injections, right hypothalamic (uninjected side) DBH levels were unchanged for 4 d, fell by 57% at 5 d, rose to 159% of control at 6 d and returned to control levels by 15 d post-reserpine injection. Axonal transport of DBH was uneffected by reserpine until the sixth post-injection day when it increased by 477%, with a peak increase of 593% at 7 d, and a gradual return to control levels by 22 d. Right hypothalamic TH levels began to increase on the sixth post-reserpine day, peaked at 269% of control at 8 d and returned to baseline by 15 d. Axonal transport of TH increased transiently by 108% at 2 d, was completely blocked at 5 d, increased to 389% and 493% of control at 6 d and 8 d respectively, and returned to baseline by 15 d after reserpine injections.

Therefore, the delayed increases of TH and DBH activities seen in the hypothalamus following reserpine treatment appear to be due to a delayed onset of the axonal transport of these newly synthesized enzymes from the LC.

53.32 METHAMPHETAMINE-INDUCED DEPRESSION OF BRAIN TRYPTOPHAN HYDROXYLASE: REGIONAL RECOVERY FOLLOWING ACUTE TREATMENT. C. Bakhit* and J.W. Gibb, Dept. Biochem. Pharm. & Toxicol., University of Utah, Salt Lake City, Utah 84112.

We have recently reported that acute administration of methamphetamine to rats produces large decreases in the activity of tryptophan hydroxylase (TPH) in regions that have serotonergic nerve terminals (Bakhit et al., Fed. Proc. 39:268, 1980; Hotchkiss and Gibb, In Press). In this study we report the recovery of TPH activity following acute methamphetamine treatment. Rats were administered a single injection of methamphetamine (10 mg/kg, s.c.) and sacrificed at the following time points after drug treatment: 3, 12, 36, 96 hrs and 2 weeks. To minimize the diurnal variation in enzyme activity, the experiments were designed so that groups of rats from the different time points were sacrificed on the same day between 10 a.m. and 2 p.m. Rats were sacrificed by decapitation and the following brain regions dissected: nucleus accumbens (NA), olfactory tubercle (OT), neostriatum (NS), hypothalamus (Hy), hippocampus (H), cerebral cortex (CC) and spinal cord (SC). TPH activity was measured by a modified $^{14}CO_2$ trapping method (Hotchkiss et al., Life Sci. 25:1373, 1979). Three hrs after the administration of methamphetamine, TPH activity was markedly depressed (expressed as % of control values): NA 36%, OT 50%, NS 47%, Hy 67%, H 44%, CC 42%, SC 74%. At 12 hrs enzyme activity recovered as follows: NA 61%, OT 100%, NS 74%, Hy 93%, H 62%, CC 69%, SC 85%. At 36 and 96 hrs following drug treatment, TPH activity was back to control values in all regions except the nucleus accumbens and hippocampus. When measured at 2 weeks, TPH activity had returned to control values in all regions studied. These data suggest that there is a time-dependent and differential recovery of enzyme activity in various serotonergic nerve terminal regions examined. (Supported by USPHS grants GM 07579 and DA 00869.)

53.33 SIMILAR POTENCY RATIOS OF AMPHETAMINE ISOMERS ON INHIBITION OF ^{3}H-DOPAMINE UPTAKE BY STRIATAL, OLFACTORY TUBERCLE AND FRONTAL CORTEX DOPAMINERGIC NERVE TERMINALS. R.T. Matthews and P.A. Shore. Department of Pharmacology, University of Texas Health Science Center, Dallas, Tx 75235.

Recent electrophysiological data suggest important differences between nigral (A-9) and ventral tegmental (A-10) dopamine (DA) neurons in their responses to drugs in vivo. Specifically, D-amphetamine (D-AMPH) is 5 to 6 fold more potent than L-AMPH in inhibiting A-9 impulse flow while A-10 impulse flow is inhibited equally by the AMPH isomers. It was therefore of interest to study the relative potencies of AMPH isomers on ^{3}H-DA uptake in nerve terminals of A-9 and A-10 DA neurons in the rat. A method for selective measurement of ^{3}H-DA uptake by DA nerve terminals was developed and the effects of AMPH isomers on DA uptake in striatal synaptosomes (A-9 terminals) was compared to uptake in olfactory tubercle and frontal cortex synaptosmes (A-10 terminals). Desipramine (DMI) had a biphasic dose-response effect on ^{3}H-DA (10^{-7}M) uptake in frontal cortex but only a monophasic effect at high concentrations in the other areas, suggesting substantial ^{3}H-DA uptake by norepinephrine (NE) neurons only in the frontal cortex. At a concentration of DMI chosen for maximal inhibition of NE uptake but minimal effects on DA uptake, fluoxitine (10^{-6}M), a potent serotonin reuptake inhibitor had no additional effect on DA uptake in any terminal area. In contrast, amfonelic acid, a potent DA uptake inhibitor, was additive with DMI in all areas (IC_{50} - $1\text{-}3x10^{-8}$M). In the presence of DMI and fluxotine, D-AMPH was 4 to 6 fold more potent than L-AMPH as a DA uptake inhibitor in all terminal areas. This suggests that A-9 and A-10 DA terminals are pharmacologically similar and that recently reported electrophysiological differences in AMPH isomer potencies on A-9 and A-10 neurons may be due to a NE-DA neuronal interaction.

53.34 PARANEURONS OF THE THYROID GLAND: A MODEL FOR STUDIES OF SEROTONIN STORAGE AND RELEASE. P. Bernd*, T. Tagliente*, E.A. Nunez*, M.D. Gershon and H. Tamir (SPON: E.B. Masurovsky). Department of Anatomy, Columbia University, New York, N.Y., 10032 and New York State Psychiatric Institute, Division of Neuroscience, New York, N.Y. 10032.

Parafollicular cells of the sheep thyroid gland are neural crest derivatives that have been referred to as paraneurons and contain the biogenic amine serotonin (5-HT) as well as the hormone calcitonin. These cells also contain a highly specific serotonin binding protein. The parafollicular cell, therefore, could serve as a model for studying 5-HT storage and secretion. We have devised a method to obtain an enriched and purified population of thyroid parafollicular cells. Thyroid glands of sheep were dissociated with trypsin (0.25%) and the cells were then incubated with thyroid stimulating hormone (TSH, 5 mU/ml). This hormone caused the follicular cells to extend pseudopods and become highly phagocytic. These TSH-stimulated cells were then passed through a column of Sepharose 6MB to which thyroglobulin had been coupled and fractions were collected (2 ml; 1 ml/min). The follicular cells presumably adhered to the beads and the cells that passed through the column were primarily red blood cells and parafollicular cells. The greatest number of cells was found in fraction 4, while the 5-HT concentration was highest in fraction 5 (30 fg/cell; a 10 fold enrichment over the dissociated cells). Quantitative electron microscopic evaluation of fraction 5 revealed that the ratio of parafollicular cells to follicular cells was five fold higher than the same ratio determined for the originally dissociated cells. The other fractions obtained from the column were enriched with parafollicular cells approximately two fold. Control experiments that did not include an incubation with TSH did not show an enrichment or separation of parafollicular cells from follicular cells in any of the fractions. In conclusion, cell chromatography has yielded a fraction that is greatly enriched with viable parafollicular cells. These 5-HT-rich paraneurons can now be used to study the mechanisms of 5-HT storage and release.

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53.35 THE UPTAKE AND FATE OF THE RADIOLABELED 5-HYDROXYTRYPTAMINE IN ISOLATED CEREBRAL MICROVESSELS. T. Abe*, W. D. Rausch*, N. Merkel*, K. Abe* and M. Spatz. Lab. of Neuropathology and Neuroanatomical Sciences, National Institutes of Health, Bethesda, MD 20205.

Only a slight amount of circulating 5-hydroxytryptamine (5-HT) was found to pass the blood-brain barrier (Axelrod and Inscoe, J. Pharmacol. Exp. Therap. 141, 161, 1963). To elucidate the mechanism responsible for these reported observations the uptake and metabolism of radiolabeled 5-HT were investigated in isolated cerebral microvessels, which were previously proven to be metabolically active and suitable for such studies (Mrsulja, Mrsulja, Fujimoto, Klatzo and Spatz, Brain Res. 111, 361, 1976).

The capillary uptake of ^{3}H 5-HT was found to be saturable since it could be inhibited by increasing the concentration of unlabeled (cold) 5-HT in the incubating medium containing the labeled substrate (estimated Km 2.3 µm). The addition of K^+ increased while the reduction of Na^+ or addition of ouabain decreased the ^{3}H 5-HT uptake in the isolated microvessels. Metabolic inhibitors (Na azide, NaFl, DNP, KCN) had little effect on either the uptake or metabolism of labeled 5-HT in the microvessels. However, hypothermia, phentolamine (1 mm), dichloroisoproterenol (1 mm) and imipramine (0.3 mm) reduced the capillary 5-HT uptake in 90, 86, 83 and 57 percent, respectively. Moreover, a cross inhibition of this uptake was seen by L-norepinephrine, DL metaraminol, L-epinephrine, L-dopa, dopamine and tryptamine but not by the amino acids of the L and A transport system. Bivalent ions (Co^{++} Mn^{++}) reduced both the uptake and metabolism of ^{14}C 5-HT. 5-HIAA was found to be the main metabolite extractable from the microvessels and from the incubating medium. The turnover rate of ^{14}C 5-HT was 1.89 nmoles/mg P/30 minutes. Anoxia markedly reduced the metabolic rate of the labeled 5-HT.

These results indicate that the capillary uptake of 5-HT takes place by a specific Na^+ and K^+ dependent carrier-mediated process (which may be shared by other amines). These features and the sensitivity of the 5-HT uptake to hypothermia and to metaraminol cross inhibition resemble the reported characteristic properties of the neuronal amines' uptake. However, the cerebral microvessels also showed the main characteristics of extraneuronal uptake, namely, the capability of metabolizing the 5-HT and releasing the deaminated metabolite. Thus, the cerebral microvessels have the capacity for 5-HT uptake and metabolism rather than for uptake and storage. Therefore, they are most likely responsible for regulating the inflow and outflow of 5-HT by inactivating the amine, which can be altered under pathological conditions.

53.36 UPTAKE OF 5-HYDROXYTRYPTAMINE AND NORADRENALINE BY SPINAL CORD SLICES IS REDUCED BUT NOT ABOLISHED BY CHRONIC SPINAL TRANSECTION. D.M. Wright* and R.S.G. Jones* (SPON: R.B. Malmo). Dept. of Anaesthesia Res., McGill Univ., Montreal, PQ. H3G 1Y6 and the Psychiatric Div., Univ. Hospital, Saskatoon, SK, S7N OW8 Canada.

It has been reported that 5-hydroxytryptamine (5-HT), but not noradrenaline (NA), evokes a significantly greater increase in the amplitude of the motoneurone field potential after chronic section of dorsal roots (Roberts & Wright, J. Physiol. (Lond.) 285 (1978) 21P). One possible explanation, the development of a motoneuronal supersensitivity to 5-HT, implicates 5-HT as a transmitter of primary afferent neurones.

To test this postulate further, monoamine uptake was measured in 200 µM slices of rat lumbar spinal cord 10 days after a 2 mm section of thorasic cord had been removed to cause the degeneration of descending monoamine pathways (*cf.* Haggendal & Dahlström, Neuropharmacology, 12 (1973) 349). Slices were placed in rapid transfer tubes for incubation in buffer (Krebs-Henseleit + 10^{-6}M Clorgyline) containing 10^{-7}M of either ^{14}C-5-HT or ^{14}C-NA. Radioactivity was determined on a Packard Tri-carb model 3320.

		5-HT UPTAKE$^+$	NA UPTAKE$^+$
CONTROL	37	628.0±91.9 (16)	870.6±146.7 (7)
	0	138.4±13.0 (24)	495.3± 42.6 (7)
	sig.	p < 0.001	p < 0.025
TRANSECTED	37	199.6±19.4 (28)	521.9±47.0 (13)
	0	125.0±11.0 (27)	351.6±34.8 (10)
	sig.	p < 0.001	p < 0.01

$^+$ Uptake expressed as (fmole.mg^{-1}.15min^{-1}; (n))

These results demonstrate the existence of active uptake systems for 5-HT and NA in the spinal cord following degeneration of descending pathways. This supports previous suggestions (Roberts & Wright, 1978,Lackovic Int.Cong.Pharm. Abs.2310; Nozaki et al., Psychopharmacology 67 (1980)25) that there are serotonergic and noradrenergic mechanisms within the spinal cord that are not supraspinal in origin.

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53.37 (-)METHADONE ANTAGONISM OF BRAIN SEROTONIN DEPLETION BY p-CHLOROAMPHETAMINE IN RATS. Kenneth W. Perry*, Ray W. Fuller and Martin D. Hynes. The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

Methadone is a potent inhibitor of the uptake of serotonin by brain synaptosomes in vitro (Ciofalo, J. Pharmacol. Exp. Ther. 189, 83, 1974), but attempts to demonstrate inhibition of serotonin uptake in vivo by methadone have generally failed (Fuller and Perry, Biochem. Pharmacol. 25, 360, 1976; Donzanti and Warwick, Eur. J. Pharmacol. 59, 107, 1979; Miranda et al., Pharmacol. Res. Commun. 11, 455, 1979). Using antagonism of p-chloroamphetamine-induced depletion of brain serotonin in rats as an index of inhibition of uptake into serotonin neurons in vivo, we have found that (-)methadone at high doses can inhibit uptake into serotonin neurons but the duration of action is short. (-)Methadone HCl at doses of 5 mg/kg i.p. given 10 min before and again 1.5 hrs after p-chloroamphetamine HCl (5 mg/kg i.p.) antagonized depletion of serotonin measured 3 hrs after p-chloroamphetamine. Serotonin concentration was 0.45 + .02 µg/g in the brains of control and methadone-treated rats. p-Chloroamphetamine lowered brain serotonin concentration to 0.17 + .01 µg/g in controls but only to 0.36 + .04 µg/g in (-)methadone-treated rats. A single dose of (-)methadone HCl given simultaneously with p-chloroamphetamine HCl (both at 5 mg/kg i.p.) protected against serotonin depletion at 1 hr and at 2 hrs, but by 4 hrs serotonin was depleted to the same extent with or without methadone co-treatment. Comparing p-chloroamphetamine antagonism and analgesic effects in a rat tail jerk assay at various s.c. doses of (-)methadone HCl indicated that inhibition of serotonin uptake by (-)methadone does not occur until doses higher than those needed for maximum analgesia are reached. A dose of 1 mg/kg (-)methadone HCl significantly prolonged rat tail jerk latency, and a dose of 2 mg/kg produced maximum effect. In contrast, no antagonism of serotonin depletion by p-chloroamphetamine HCl (5 mg/kg i.p.) occurred until a 4 mg/kg dose of (-)methadone HCl was reached. Thus inhibition of serotonin uptake probably has no role in methadone analgesia.

53.38 EFFECTS OF DRUGS ON 5-HYDROXYTRYPTAMINE, DOPAMINE AND THEIR METABOLITES IN CEREBROVENTRICULAR PERFUSATES OF UNANESTHETIZED, FREELY-MOVING RATS. J.A. Nielsen* and K.E. Moore. Dept. of Pharmacology & Toxicology, Michigan State Univ., E.Lansing, Michigan 48824.

The activity of aminergic neurons have been estimated from measurements of neurotransmitter metabolites in cerebrospinal fluid (CSF) or perfusates of the cerebroventricular system. These studies generally have been performed in anesthetized animals or have used radiolabelled precursors. To reduce problems inherent in these methods we have undertaken to characterize a procedure which would allow the measurement of endogenous biogenic amines and their metabolites in cerebroventricular perfusate of unanesthetized, freely-moving rats.

Male rats were implanted with permanent push-pull cannulas such that the tips were in the right lateral ventricles (Tilson and Sparber, J. Pharmacol. Exp. Ther. 181:387, 1972). After recovery from surgery the rats were perfused with artificial CSF at a rate of about 15 µl/minute. Perfusate samples were collected every 15 min in 0.1 M citrate-phosphate buffer (pH 3.5) and analyzed by high performance liquid chromatography utilizing a C-18 reverse phase column coupled with an electrochemical detector (Hingtgen et al., Soc. Neurosci. Abstracts 5: 559, 1979). The perfusates contain endogenous dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). The amounts of these compounds in perfusate are different from rat to rat but consistent for each rat over several weeks. Probenecid (250 mg/kg, ip) increased perfusate concentrations of all the acid metabolites without affecting DA or 5-HT. Tryptophan (100 mg/kg, ip) increased 5-HT and 5-HIAA but had no effect on DA or its metabolites. Pargyline (75 mg/kg, ip) decreased 5-HIAA, DOPAC and HVA while not altering 5-HT or DA. These results suggest that concentrations of endogenous amines and their metabolites in perfusates from the lateral ventricles may be used to estimate the effects of drugs on biogenic amine neuronal systems in brains of unanesthetized, freely-moving rats. (Supported by USPHS Grant NS15911.)

53.39 THE SELECTIVE RELEASE OF TRITIATED meta-TYRAMINE (m-TA) and para-TYRAMINE (p-TA) FROM RAT STRIATAL SLICES BY SOME AMINOTETRALINS (AT'S). L.E. Dyck* (Sponsor: A.A. Boulton), Psychiatric Research Division, University Hospital, Saskatoon, Saskatchewan, S7N OXO.

Cerebral dopamine (DA) systems are believed to play an important role in the mediation of drug-induced hyperactivity and stereotypy. To characterize the stereochemically effective configuration of DA at the receptor(s) responsible for eliciting such behaviors, rigid analogs of DA, such as the AT's, have been employed. For example, ADTN, 6,7-dihydroxy(diOH)AT, appears to be a highly specific and potent DA agonist. However, several other AT's, which have only one, or no, aromatic hydroxyl group, but which have a dipropyl (diPr) substituted amino group, are active as well. It is not yet clear whether the activity of these AT's is direct or indirect; therefore, several of these AT's were tested to see if they could release DA, or m- and p-TA.

The simultaneous release of m-TA-^{3}H and DA-^{14}C, or p-TA-^{3}H and DA-^{14}C, from 0.2mm slices of the anterior portion of rat striatum (10-15 mg wet wt.) was studied. The slices were preloaded by incubation for 15 min in oxygenated Krebs-Henseleit medium containing 100nM of each labelled amine at 37°C. The slices, then, were moved in succession through ten tubes containing the standard medium ± 10µM AT. Pargyline (10µM) was always present, and the AT's were in tubes 6 through 10. The percentage increases (mean ± S.E.M.) in tube 6, above the spontaneous release in tube 5, caused by the various AT's were:

	% Increase in the Release of			
Drug	p-TA-^{3}H	DA-^{14}C	m-TA-^{3}H	DA-^{14}C
ADTN	229±61	317±75	147±11	500±50
M8	281±34	377±19	164±18	327±36
5,6-diPr	90± 6	6± 9	65±14	17± 4
5OH	129±21	4± 4	124±27	42±23
6OH	104±24	35±12	103±39	40±24
7OH	181±31	20±12	62±14	43±14

ADTN and M8 released DA, as well as m- and p-TA. In contrast, the other four AT's, all of which are diPr substituted, released m- and p-TA, but did not release DA to a significant extent. diPr substituents are reported to enhance activity at post-synaptic central DA receptors and enhance the stereotypic potency of an AT; whereas they attenuate the hyperactive responses to the AT. It may be concluded from this study, that the hyperactivity induced by an AT involves DA primarily, since the diPr derivatives were not able to release DA. Stereotypy, however, may involve m- or p-TA, primarily, since the diPr derivatives released them, but did not release DA.

Supported by Saskatchewan Health.

54.1 DIVALENT IONS AND AMINO ACID RESPONSES IN FROG SPINAL CORD. A.L. Padjen and P.A. Smith. Department of Pharmacology, McGill University, Montreal, Quebec H3G 1Y6.

In an attempt to examine the possible role of divalent ions in amino acid responses, experiments were performed on the isolated hemisected superfused spinal cord of the frog (R.pipiens) at 15°C. The DC potentials of dorsal (DR) and ventral roots (VR) were recorded using the sucrose gap technique. A series of responses to glutamate (GLU), GABA (both 1 mM), D,L-homocysteate (DLH, 50 μM) kainate (KA, 50 μM), N-methyl D,L-aspartate (NMDLA, 0.1 mM) and K^+ (8 mM) were examined in the presence of 0.1-1 μM TTX to block indirect responses. Lowering of Ca^{++} to 1 μM or less (using an EGTA-Ca buffer) caused a time dependent change in the membrane potential (2-3 mV hyperpolarization followed by a depolarization of VR and particularly DR). Initially there was a general depression of the depolarizing responses of both roots to all acidic amino acids at a time when the response to $8K^+$ was little changed or even increased. The glutamate after hyperpolarization (GLU-AHP) was more than doubled and occasionally the usually monophasic response to DLH exhibited an AHP. The GABA depolarization of DR was depressed to 50% of control while the hyperpolarization of the VR increased by 20%. Prolonged washing (>3 h) with low Ca^{++} Ringer caused a nonspecific depression of all responses. These changes were completely reversed 1 h after return to normal Ca^{++} (2 mM) Ringer. Unlike Ca^{++} removal, Co^{++} (2-5 mM) caused an immediate and lasting depolarization of both roots. (DR 2-5 mV; VR 1-3 mV). Furthermore, the responses of both roots to DLH or NMDLA were selectively antagonized (50-100%) at the time when GLU and KA responses of VR were enhanced by 10-25%. Although the response to $8K^+$ was within 10% of control on VR, but reduced on DR, the GABA responses underwent a dramatic change, a 200% increase on DR and a reversal into a depolarization on VR. Cd^{++} (50 to 200 μM) enhanced GABA responses on both roots yet nonselectively depressed all responses to acidic amino acids. D600 was only effective at doses exceeding 0.1 mM and depressed all responses except the hyperpolarizing response of VR to GABA which was enhanced by up to 400%. Qualitatively similar results were sometimes obtained with La^{+++} (0.5-1 mM). These results raise the possibility that a Ca^{++} conductance may be involved in acidic amino acid responses particularly those to DLH and NMDLA on VR and GABA on DR. Perhaps Co^{++} can substitute for Ca^{++} in these events. The possible involvement of some other mechanism such as receptor modification, modulation of agonist induced conductance change, change of membrane resistance or potential, effect on pump mechanisms, or chelating effects cannot yet be excluded.

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54.2 KOJIC AMINE: A GABA AGONIST WITH DUAL ACTION. G.W. Bourne and A.L. Padjen. Dept. of Pharmacology, McGill Univ., Montreal, Quebec H3G 1Y6.

The GABA analog kojic amine (KJA) has been shown to mimic GABA in receptor binding assay and in bicuculline sensitive inhibition of cerebellar Purkinje cells and is more potent than GABA in reducing synaptic potentials in the amphibian spinal cord (Yarbrough et al.,Arch.Int.Pharm.Ther. 241:266,1979). The action of KJA was studied on the isolated hemisected superfused frog spinal cord. Synaptic, spontaneous and drug evoked responses were recorded on the ventral (VR) and dorsal roots (DR) by means of the sucrose gap technique. KJA (50-500 μM) produced a dose dependent depression of all synaptic potentials recorded (DR potentials evoked by DR or VR stimulation, and VR potentials evoked by DR stimulation). This action was completely reversible by 30 to 60 min washing. A 3 min application of 0.5 mM KJA completely abolished spontaneous activity recorded on both roots for 30 to 45 min, following an initial hyperpolarization of VR (1.5 mV) and a depolarization of DR (1.5 mV). This contrasts with the depression of spontaneous activity by GABA which does not extend beyond the time of application. Examination of direct responses to KJA (10 μM-0.5 mM; in the presence of 2 mM Mn^{2+}, 0.2 mM Ca^{2+} Ringer) revealed a dose dependent hyperpolarization of VR (2 mV with 0.5 mM). At concentrations above 0.25 mM KJA depolarized DR (2 mV with 0.5 mM), however, lower concentrations caused a 1 mV hyperpolarization of DR. Multiphasic responses were observed at intermediate concentrations (50 μM). All direct responses to KJA were characteristically slow in onset and decay, unlike the much faster and larger responses to GABA. However, at very low concentrations (10 μM-50 μM) GABA also produced a small slow hyperpolarization of dorsal roots. Bicuculline (BIC, 50-100 μM) and picrotoxin (PTX, 100 μM) both depressed the hyperpolarizing response of the VR to KJA and GABA. The depolarizing action of KJA on the DR was eliminated by both antagonists revealing a potent hyperpolarization at all agonist concentrations tested (10 μM-0.5 mM). The DR depolarization to high concentration of GABA was antagonized (BIC 50 μM) or reversed (PTX 0.1 mM, BIC 0.1 mM) by the GABA antagonists. The antagonists thus revealed a dual response of the DR to both KJA and GABA by selectively inhibiting the depolarizing component. The dorsal root ganglion, found to be reliably depolarized by GABA (1 mM) was not affected by KJA (1 mM) applied for up to 7 min. These results suggest that KJA activates both PTX-BIC sensitive and insensitive GABA receptors on primary afferents. The latter are possibly responsible for KJA depression of synaptic transmission through a presynaptic action similar to baclofen.

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54.3 STEREOSPECIFICITY OF THE GLIOTOXIC AND ANTI-NEUROTOXIC ACTIONS OF ALPHA AMINOADIPATE. T. de Gubareff,* J.W. Olney & J.F. Collins,* Dept. Psychiatry, Wash. Univ. Sch. Med., St. Louis, Mo. 63110 and Dept. Chemistry, City London Polytech., London, England.

Glutamic (Glu), aspartic (Asp) and α-aminoadipic (αAA) acids are structurally analogous dicarboxylic amino acids which differ only in the length of their carbon chains. Glu and Asp have both excitatory and neurotoxic actions on central neurons; DL-αAA has neither but we have found that it induces a gliotoxic reaction in the arcuate hypothalamic (AH) region of immature mouse brain when administered sc, and when administered together with Glu or its excitotoxic analogs it suppresses their AH neurotoxicity. We have now studied the separate isomers of αAA to determine which is responsible for the gliotoxicity and which for the anti-neurotoxic action of DL-αAA.

Four day old mice were injected sc with a 2% aqueous solution of D or L or DL-αAA at 1 or 2 mg/g body wt and sacrificed 4 hrs later for histological examination of the hypothalamus. We found that the D isomer was without discernable effect at 1 mg/g and induced mild to moderate swelling of glial processes at 2 mg/g. The L-isomer caused more dramatic gliotoxic changes accompanied by an unequivocal *neurotoxic* reaction. Both the gliotoxic and neurotoxic activities of L-αAA were clearly evident at 1 mg/g and substantially more severe at 2 mg/g. DL-αAA at 2 mg/g induced a toxic reaction affecting AH glia but not neurons.

These findings corroborate our prior observation that DL-αAA has gliotoxic but not neurotoxic activity. The new finding that L-αAA, which McLennan & Hall recently described as a neuronal excitant, has significant neurotoxicity, adds new support to the excitotoxic hypothesis (that an excitatory mechanism underlies the neurotoxicity of amino acid excitants). More importantly, the demonstration that L-αAA has significant neurotoxicity but D-αAA does not, in light of the non-neurotoxicity of DL-αAA, signifies that D-αAA is an effective antagonist of the neurotoxicity of L-αAA. Blockade of this type of neurotoxicity by a molecule (D-αAA) known to antagonize the depolarizing action of amino acid excitants, an action thought to be mediated through postsynaptic receptors on the dendrosomal surfaces of the neuron, strongly implicates these receptors and an excitatory (depolarization) mechanism in the neurotoxic activities of amino acid excitants. Conceivably the excitotoxic activity of Glu or Asp may underly the degeneration of neurons in certain human neurological disorders. If so, antagonists that specifically block the excitotoxic activities of these agents at their excitatory receptors might protect such neurons from degeneration. Supported by USPH grants NS-09156, DA-00259, RSA MH-38894 (JWO) and Huntington's Chorea and Wills Foundation grants.

54.4 DO SUBSTANTIA NIGRA PARS RETICULATA NEURONS BECOME SUPERSENSITIVE TO GABAERGIC DRUGS AFTER STRIATAL KAINIC ACID LESIONS? B.L. Waszczak and J.R. Walters. NIH, NINCDS, Bethesda, MD 20205.

Several investigators have reported that destruction of the striatonigral GABA pathway causes increases in specific ^{3}H-GABA binding in the substantia nigra (SN). These findings suggest that supersensitivity develops at GABA sites postsynaptic to the lesioned striatonigral terminals. Recent studies in this laboratory suggest that a group of cells within the SN pars reticulata, which exhibit a marked sensitivity to the inhibitory actions of GABAmimetic agents, may receive a GABAergic input from the striatonigral pathway. The following studies were undertaken, therefore, to determine if SN pars reticulata cells become supersensitive to GABAergic drugs after destruction of the striatonigral projection.

Unilateral injections of kainic acid (KA), 1 μg/0.5 μl, were made into the striata of male rats (250-300 g). Neuronal cell loss in the striatum and globus pallidus was confirmed histologically, and GAD activity in the SN on the lesioned side was reduced to 36% of that on the unlesioned side. Extracellular, single unit responses of SN pars reticulata neurons to increasing i.v. doses of muscimol (MUSC) or iontophoretically-applied GABA were compared in chloral hydrate-anesthetized control and KA-lesioned rats (14-24 days after the lesion). I.V. administration of MUSC inhibited the firing of reticulata cells from both lesioned and unlesioned rats. However, comparison of cumulative log dose-response curves for MUSC revealed no apparent changes in the sensitivity of cells from KA-treated (n=10) and control (n=9) rats. ED_{50} values for inhibition of reticulata cell firing ranged from approximately 3.5 to 5 mg/kg MUSC for both lesioned and unlesioned groups. In separate experiments in which rats received striatal KA injections of twice (2 μg/1 μl) the original dose (n=8), there were again no significant changes in the sensitivity of reticulata cells to i.v. MUSC.

Iontophoresis of GABA (0.001 M in 0.2 M NaCl) onto reticulata cells also inhibited their firing. IT_{50} values (the product of the time required to inhibit firing by 50%, T_{50}, and the ejection current, I, applied) were used as a measure of the sensitivity of reticulata cells to iontophoresed GABA. Examination of IT_{50} values obtained from 10 control rats (14 cells) and 7 KA-lesioned rats (17 cells) revealed that reticulata cells from KA-treated rats tended to be more sensitive to GABA than cells from untreated rats, but the difference in responsiveness between the two groups was of borderline significance ($.05<p<.1$). Since reticulata cells in lesioned animals did not demonstrate supersensitivity to i.v. MUSC, their increased response to iontophoresed GABA and the reorted increases in GABA binding may not be predictive of physiologically important or clinically significant changes in reticulata cell function in diseases where striatonigral GABA pathways degenerate.

54.5 EFFECTS OF pH AND TEMPERATURE ON GABA DEPOLARIZATIONS (GD) RECORDED FROM CAT DORSAL ROOT GANGLIA (DRG). Jun Nakamura*, J.P. Gallagher, and Patricia Shinnick-Gallagher. Dept. of Pharmacol. and Toxicol., Univ. Texas Med. Br., Galveston, TX 77550.

GABA depolarizes cat DRG by causing an efflux of chloride. Chloride ions move across the membrane through a selective anionic channel. We have attempted to modify this chloride efflux by altering the pH of the solution bathing the DRG. Lowering the pH from 7.4 to 6.4 resulted in a 40% increase above control amplitude of the iontophoretically induced GD. In addition to the enhanced response, its half-decay time was prolonged. The increase in amplitude occurred even though, in the low pH solution, most of the cells' resting input resistance was decreased. On the other hand, raising the pH from 7.4 to 8.9 caused a 20% reduction in the amplitude of the GABA depolarization, although the cells' input resistance was usually slightly increased. The reversal potential of the GD remained constant in either an acidic or alkaline medium. Two possible explanations for these pH effects are that the charge density of the anionic channel has been affected and/or the channel's open life-time has been changed. Two agents that may owe part of their facilitatory action to a mechanism that may be explained by that observed in acidic medium include phenobarbital and ammonium.

Cooling the DRG also altered the GD. Rapid lowering of the bath temperature from 37^0C to 27^0C produced three effects: 1) the amplitude of the GD was diminished; 2) the rise-time was lengthened; and 3) the time for ½-decay was prolonged. An explanation for this temperature effect may be inhibition of the metabolically dependent, inwardly directed chloride pump that has been proposed for these neurons. As a result, the normally high level (50 mM) of chloride in these cells cannot be maintained and the driving force for the GD is diminished. In addition, lowering the temperature may also increase the open channel life-time and thereby prolong the ½-decay time. We have attempted to mimic this depression of the GD with several putative chloride pump inhibitors (SITS, fuorsemide, ammonium ion, bumetanide and pyridoxine) but none affect the GD in a manner similar to lowering the temperature. (Support by USPHS Grant #NS 13727.)

54.6 NEUROTOXICITY OF SIMPLE ALIPHATIC DIAMINES. G. M. Strain and W. Flory*. Veterinary Physiology, Pharmacology, and Toxicology, and Dept. of Veterinary Science, LSU Sch. of Vet. Med., Baton Rouge, LA 70803.

Neurotoxicity of some members of Lathyrus pea family (neurolathyrism) has been shown to be caused by three diamine compounds: α,γ-diaminobutyric acid (DABA), β-N-oxalyl-L-α,β-diaminopropionic acid (ODAP), and γ-N-oxalyl-L-α,γ-diamobutyric acid (ODAB) (Liener, 1969, Toxic Constituents of Plant Foodstuffs, Academic Press). Intracerebroventricular (ICV) injections of these compounds produce symptoms of hyperexcitability, tremor, and convulsions with a stereotyped motor pattern. Studies of DABA and structurally related diamines by Chen et al. (Tox.Appl.Pharm.1972 23:334) showed that some, but not all, simple diamine compounds cause typical convulsions after ICV injection. In this study, diamines with the two following general structures were examined for neurotoxicity after ICV injection in the rat:

$$NH_2 - (CH_2)_n - NH_2 \qquad n \geq 2$$

$$NH_2 - (CH_2)_k - \underset{\displaystyle NH_2}{\underset{|}{CH}} - COOH \qquad k \geq 1$$

Tests of nine diamine compounds showed the general structure-activity relationship (SAR) that toxicity occurs when the carbons bound to the amino groups are separated by at least one methyl linkage, i.e., $n \geq 3$ and $k \geq 2$. Further, the toxicity decreased as the number of separating methyl groups increased. This parallels the SAR observed for compounds producing osteolathyrism, the disorder of mesenchymal tissues seen with other species of Lathyrus, where an amino group must be separated from a terminal cyanide group by one or two methyl linkages (Green, J.Neurosurg., 1960, 17:657).

ODAP has been shown to inhibit the high affinity uptake system for glutamate in rat brain and monkey spinal cord synaptosomes (Lakshmanan and Padmanaban, Nature, 1974, 249:469) and Chen et al. showed DABA to increase brain levels of glutamine and GABA, both of which have glutamate as their precursor. Thus the neurotoxic diamines may act by interacting with the glutamate transmitter system.

55.1 REDUCED LEVELS OF ACETYLCHOLINE IN THE MOTOR NERVE TERMINALS OF AGED RATS. C.T. Gibson* and D.O. Smith (SPON: D.D. Gilboe). Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

Synaptic depression at the phrenic nerve-diaphragm neuromuscular junction is more pronounced in aged rats. This could result from a decreased availability of the neurotransmitter, acetylcholine (ACh). To test this possibility, the ACh content in phrenic nerve terminals of aged (28 mos) and young, control (9 mos) male, Fischer 344 rats were assayed and compared.

The total amount of transmitter per nerve terminal was found to be less in the aged animals. This was determined using a radio-chemical assay for ACh, in which the choline product of ACh hydrolysis was incubated with $AT^{32}P$ to form labeled choline phosphate. Endogenous choline was initially phosphorylated using unlabeled ATP, so only the ACh associated with the tissue was labeled. From measurements of the amount of labeled choline phosphate and the number of end plates assayed, the amount of ACh per nerve terminal was estimated. The results of 9 experiments in each age group indicated averages (±S.E.) of 1.33×10^{-4} ($\pm 0.45 \times 10^{-14}$) moles/nerve terminal and 2.59×10^{-14} ($\pm 0.84 \times 10^{-14}$) moles/nerve terminal in the old and the control animals, respectively. This difference is significant statistically ($p < 0.05$). Thus, there is a reduction of about 49% in the ACh levels of phrenic nerve terminals of aged rats.

These estimates of ACh content are consistent with those obtained in other investigations. Whether the difference in total ACh content is significant physiologically, however, cannot be concluded from these data. Experiments are now in progress to determine the rates of release of ACh, the fraction of ACh released, and the levels of choline acetyltransferase and acetylcholinesterase in aged and control rats. With these additional data, the importance of lower ACh levels in aged rats should be easier to judge.

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55.2 THE RELATIONSHIP OF GRADATIONS IN MOTOR AXON ACETYLCHOLINESTERASE REACTION PRODUCT DENSITIES TO REGIONAL PERCENTAGE COMPOSITIONS OF TYPE I AND TYPE II MUSCLE FIBERS. L. T. Malmgren, Otolaryngology Labs, Upstate Medical Center, Syracuse, NY 13210.

Gruber and Zenker (Brain Res., 141:325, 1978) recently reported that, in the rat, motor axons to muscles composed of predominantly fast muscle fibers (high ATPase) stain more densely for acetylcholinesterase (AChE) than motor axons supplying muscles composed of predominantly slow muscle fibers (low ATPase). In the present investigation this relationship has been further examined in the cat.

Nerves to muscles with differing percentage compositions of Type I (low ATPase) and Type II (high ATPase) muscle fibers were processed (10 μ thick cryostat sections) for the histochemical localization of AChE (Karnovsky and Roots, J. Histochem. Cytochem. 12: 219, 1964) using iso-OMPA as an inhibitor of "pseudocholinesterase." The specificity of the histochemical reaction was established using controls. Various incubation times and temperatures were used to determine the optimal incubation conditions for detection of differences in axonal reaction product densities.

After 48 h incubation at 4° C alpha motor axons in nerves to muscles containing mainly Type II muscle fibers (high ATPase), such as the caudofemoralis and the tibialis anterior, were intensely stained for acetylcholinesterase. Alpha motor axons supplying muscles consisting of predominantly Type I fibers (low ATPase), on the other hand, such as the soleus and the quadratus femoris, had no clearly detectable AChE reaction product, and did not differ from sensory axons in the dorsal spinal roots at this incubation time and temperature. These results are similar to those reported by Gruber and Zenker for the rat. However, when the incubation was extended to 72 h at 4° C some of the alpha motor axons to the quadratus femoris had faint AChE while others did not. When the nerve to the soleus was incubated under these conditions, the alpha motor axons remained essentially unstained. These differences in axonal AChE reaction product densities may be related to the finding that while the soleus muscle consists of an essentially homogeneous distribution of Type I muscle fibers, the quadratus femoris muscle includes regions where some Type II fibers are present and other regions where they are lacking. The graded AChE staining intensities characteristic of groups of alpha motor fibers supplying these muscles may reflect the regional percentage composition of muscle fiber types in the area of the motor unit.

55.3 DOES THE Ca^{++} DEPENDENT FRACTION OF K^+ INDUCED CHOLINERGIC TRANSMITTER RELEASE OCCUR FROM THE CYTOPLASMIC OR VESICULAR FRACTION OF MOUSE BRAIN? P.T.Carroll and J.M.Aspry*, Dept. of Pharmacol., Univ. of Rhode Island, Kingston, RI 02881.

Incubation of mouse forebrain minces for 30 min in lithium Krebs medium (low Na^+, high K^+, L.K.) lowers the ACh content of the crude vesicular fraction (P_3) independently of the cytoplasm (S_3) relative to normal Krebs (N.K.) incubation - Carroll & Nelson, 1978. Subsequent incubation of L.K. pretreated minces in N.K. with either ^{14}C choline or the analog ^{14}C homocholine refills the P_3 fraction with newly synthesized ^{14}C ACh or ^{14}C acetylhomocholine (^{14}C Ah), respectively. Refilling of the P_3 fraction occurs independently of the S_3 fraction since higher ratios of ^{14}C ACh or ^{14}C Ah to ACh (0.63 and 7.3) are attained there relative to the S_3 fraction (0.35 and 0.44, respectively) - Nelson et al., 1980. One can utilize the marked difference in the ratio of ^{14}C Ah to ACh in the P_3 fraction relative to the S_3 fraction to ascertain the subcellular origin of the spontaneous or K^+ induced release of cholinergic transmitter by incubating these pretreated minces a third time for 5 min in N.K. or high K^+ and comparing the ratios of ^{14}C Ah to ACh released spontaneously or by high K^+ into the medium with the S_3 and P_3 ratios of ^{14}C Ah to ACh prior to release. The results indicate that these pretreated minces release 2.0 nmol/g of ^{14}C Ah and 4.1 nmol/g of ACh spontaneously yielding a ratio of 0.49 which closely corresponds to that in the S_3 (0.44) prior to release. Neither omission of Ca^{++} nor elevation of Mg^{++} (16mM) alter the spontaneous release of ^{14}C Ah or ACh. Incubation of minces a 3rd time in high K^+ stimulates the release of ^{14}C Ah from 2 to 6 nmol/g but does not alter the release of ACh (4.1 to 4.6 nmol/g). The K^+ induced change in ^{14}C Ah (6-2) relative to ACh (4.6-4.1) released into the medium is 8.0, a value which corresponds to that present in the P_3 fraction (7.3) prior to release. In this case, omission of Ca^{++} or elevation of Mg^{++} significantly reduces the K^+ induced release of ^{14}C Ah but does not alter that of ACh. Pre-incubation of minces in L.K. relative to N.K. alters the ratio of ^{14}C Ah to ACh in the P_3 markedly but not the S_3 and also alters the ratio of ^{14}C Ah to ACh released by high K^+ markedly but does not alter the ratio of ^{14}C Ah to ACh released spontaneously. The present results confirm the prediction made by others (Carroll & Goldberg, 1976; Katz & Miledi, 1977; Gorio et al., 1978) that the spontaneous release of cholinergic transmitter occurs from the cytoplasm independently of Ca^{++} and that the depolarized release of cholinergic transmitter occurs from a vesicle-bound fraction by a Ca^{++} dependent process. (Supported by NSF grant #BNS 78-05160 A01.)

55.4 CHOLINERGIC ACTIONS OF ACETYLPYRROLIDINECHOLINE, A FALSE NEUROTRANSMITTER. Jerry J. Buccafusco and Robert S. Aronstam. Dept. of Pharmacology, Medical College of Georgia, Augusta, GA 30912.

Pyrrolidinecholine is a substrate for high affinity choline transport systems as well as cholineacetyltransferase, and the acetylated derivative, acetylpyrrolidinecholine (AcPyCh) is released by peripheral cholinergic nerve terminals as a "false neurotransmitter". Several studies have indicated that AcPyCh is less potent as an agonist than acetylcholine (ACh) in peripheral muscarinic and nicotinic systems (e.g., Collier et al., Mol. Pharmacol. 12, 340, 1976). In this study the potency of AcPyCh as an agonist for cholinergic receptors was determined.

Intraventricular injection of ACh or AcPyCh (15-120 nmoles) in anesthetized rats produced a dose-related increase in arterial blood pressure (10-30 mm Hg). The effects of both agents were mediated through stimulation of central muscarinic mechanisms insofar as they were blocked by prior injection of atropine (5 nmoles). There were no significant differences in either the magnitude or duration of the increase in blood pressure produced by the two drugs.

AcPyCh inhibited the binding of [^{3}H]3-quinuclidinyl benzilate to muscarinic ACh receptors (mAChR) in membranes prepared from rat cerebral cortex or brain stem (ED50 = 2.6 and 0.14×10^{-6} M, respectively). The binding of AcPyCh did not follow mass action kinetics: Hill coefficients, 0.67±0.02 (cortex) and 0.54±0.04 (brain stem) and Scatchard plots indicate multiple binding components with either tissue. The binding measurements fit a two receptor population model involving high and low affinity AcPyCh binding sites (K_D's = 8×10^{-8} and 7×10^{-6} M). The ratio of high to low affinity receptors was 0.43 in the cortex and 1.04 in the brain stem. Treatment of cortical membranes with 1 mM N-ethyl maleimide increased the affinity of AcPyCh 3-7 fold, apparently through a conversion of low affinity receptors to the high affinity species. In these binding properties, AcPyCh behaves as a typical mAChR agonist with an affinity similar to that of ACh.

AcPyCh inhibited the binding of [^{3}H]ACh, [^{3}H]d-tubocurarine and [^{3}H]nicotine to the nicotinic receptor (nAChR) of Torpedo electroplax with K_i values of 2.5, 3.6 and 2.2×10^{-7} M, respectively. AcPyCh also stimulated the binding of [^{3}H]phencyclidine ([^{3}H]PCP) to the ion channel associated with the nAChR (ED50 = 2.2×10^{-7} M). Preincubation of electroplax membranes "desensitized" the receptor-ion channel complex in a time- and concentration-dependent manner ($t_{\frac{1}{2}}$ = 18 sec with 10^{-6} M AcPyCh), as evidenced by a decrease in the affinity of [^{3}H]PCP binding. These findings indicate that AcPyCh is also a potent agonist of the nAChR possessing an affinity similar to that of ACh.

55.5 IS HOMOCHOLINE A COMPETITIVE ALTERNATE SUBSTRATE TO CHOLINE FOR ACETYLATION BY MEMBRANE-BOUND CHOLINE-O-ACETYLTRANSFERASE IN MOUSE FOREBRAIN? C.G. Benishin* and P.T. Carroll (SPON: R.B. Hill) Dept. of Pharmacol. & Toxicol., Univ. of Rhode Island, Kingston, RI 02881.

The choline (C) analog homocholine (H) is similar to C in many aspects of cholinergic metabolism. Both C and H are transported and acetylated by cholinergic preparations and released from the superior cervical ganglion and forebrain minces (e.g. Collier et al., 1977). Both extracellular C and H can be utilized to refill a crude vesicular fraction (P_3) of mouse forebrain, previously depleted of its acetylcholine (ACh) content, with newly synthesized ACh or acetylhomocholine (AHCh) respectively, independently of the cytoplasm(Nelson et al., 1980). However, soluble (S) choline acetyltransferase (ChAT) which is believed to be present in the cytoplasm and responsible for synthesis of releasable transmitter, does not acetylate H (e.g. Barker & Mittag, 1975). The objective of this investigation was to determine if H, like C, can be acetylated by membrane-bound (MB) ChAT which is non-ionically bound to vesicular and/or neuronal membranes.

Smith and Carroll (1980) have recently reported that two forms of ChAT exist in cholinergic nerve endings:one which is solubilized by high ionic strength washes of the P_3, and the other which remains associated with the P_3. Approximately 80% of the non-soluble ChAT activity is found in a P_4 pellet which is obtained by sucrose density gradient centrifugation. This fraction contains membrane- associated organelles, and non-hydrolyzable ACh (Carroll and Benishin, 1979). The acetylation of both C and H by this fraction is saturable. Also, NVP, a specific inhibitor of ChAT, reduces both C and H acetylation by the P_4. ACh and AHChcompetitively inhibit C acetylation by S and MB ChAT, the latter compound being more potent. To determine if H serves as an alternative substrate to C for MB ChAT, the kinetic parameters of C acetylation were determined in the absence and presence of fixed H concentrations. When combined velocity of acetylation is determined as a function of C concentration, the combined acetylation is unaltered by H at a C concentration of 42uM. According to Cha (1968), this kinetic pattern indicates that the two substrates, C and H, are competitive alternate substrates for the same catalytic site with the V_{max} of C acetylation greater than the V_{max} of H acetylation. These results suggest that MB ChAT acetylates both C and H and that acetylation of cholinergic precursors by S ChAT may not be essential for the synthesis of releasable cholinergic transmitter. (Supported in part by NSF grant #BNS 78-05160 A01)

55.6 EFFECTS OF CHRONIC ADMINISTRATION OF SOMAN ON ACETYLCHOLINE METABOLISM. Tsung-Ming Shih, David E. Lenz* and Donald M. Maxwell*. US Army Biomedical Laboratory, Aberdeen Proving Ground, MD 21010.

We have examined in rats the time course effects of the repeated subcutaneous administration of sub-lethal doses of soman (60 µg/kg), a completely irreversible inhibitor of acetylcholinesterase (AChE). The levels of AChE, acetylcholine (ACh) and choline (Ch) were determined in 4 discrete brain areas (brain stem, cerebellum, mid-brain and cerebral cortex). In addition, behavioral effects, as determined by a drinking behavior test, were monitored. It had been previously reported that rats, chronically treated with diisopropylfluorophosphate (DFP), show depressed whole brain AChE activity, elevated levels of brain ACh, and have been able to adapt to this stress as demonstrated by a behavioral test (J. Pharmacol. Exp. Ther. 192, 73, 1975). Our study was carried out to examine the generality of these results with another organophosphorus compound. In the current study animals were dosed on two schedules: (A) three times a week for 6 weeks, and (B) once a week for 6 weeks. Animals were sacrificed either 3 (A) or 7 (B) days after 2, 4 or 6 weeks of chronic dosing by heart exsanguination for AChE assay using the method of Groff et al. (Clin. Toxicol. 9, 353, 1976) or by focused head microwave irradiation for brain ACh and Ch assay using gas chromatography-mass spectrometry (Anal. Biochem. 55, 438, 1973). During the 6-week period there was no difference in drinking behavior in either treatment regimen A or B between control and dosed rats. Treatment B produced a 25-40% AChE inhibition (after 6 weeks) in brain stem, mid-brain, and cerebral cortex, but no AChE inhibition in cerebellum. The levels of ACh and Ch did not change in any of the brain areas. Treatment A caused a severe inhibition (45-75%) of AChE in all brain areas. The inhibition leveled off after 4 weeks of dosing, except in brain stem where progressive inhibition was found. Even under these conditions of severely depressed AChE activity, the levels of ACh and Ch in the 4 brain areas examined were not altered as compared with controls during the 6-week period. The differences between our observations with soman and previous results with DFP may be due to the differences in the greater specificity of soman vs. DFP for AChE. In spite of reduced AChE, brain area ACh levels remain relatively constant and adapt quickly to the sustained depression of AChE activity by soman.

55.7 EFFECT OF LEAD INGESTION BY RATS ON ACETYLCHOLINE SYNTHESIS IN VITRO. Gerald H. Sterling, Kevin O'Neill*, Mary R. McCafferty* and John J. O'Neill. Depts. of Pharmacology, Hahnemann Medical College & Hospital, Philadelphia PA 19102 and Temple Univ. Sch. Med., Philadelphia, PA 19140

The neurotoxic effects of lead are well established at high dose levels. However, the effects on the CNS of levels that do not cause overt symptoms are only now being ascertained. Several neurochemical studies have shown the inhibition of acetylcholine (ACh) release in the CNS of lead-exposed animals[1-3]. Modak et al.[4] has demonstrated significant changes in ACh, choline acetyltransferase and acetylcholinesterase after chronic lead ingestion. Our experiments were undertaken to examine the effect of chronic ingestion of low doses of lead on the synthetic pathways to the acetyl moiety of ACh. Rats were given lead acetate at 200 and 600 ppm in their drinking water for 20 days. Sodium acetate was used as a control. After the exposure period, the rats were sacrificed. Blood and brain lead levels were measured by anodic stripping voltammetry. Brain cortex slices were incubated with D-(6-^{3}H) glucose and either D-(6-^{14}C) glucose or (3-^{14}C)-β-hydroxybutyrate in Krebs-Ringer bicarbonate buffer containing 105mM potassium, 1mM choline and 0.4mM eserine sulfate. ACh and intermediates were isolated by column chromatography and the percent of ^{3}H and ^{14}C-incorporation and ^{3}H/^{14}C ratios were determined. Blood and brain levels significantly increased as the dose was changed from zero to 200 and to 600 ppm. When incubated with labeled glucose, ACh synthesis was inhibited over the 20 day period at 200 ppm lead but there did not appear to be an inhibition of glycolysis as measured by lactate production. At 600 ppm, there was a considerable decrease in citrate production as well as inhibition of ACh synthesis. The effect of lead was similar when ^{14}C-labeled β-hydroxybutyrate was substituted for the labeled-glucose. Since there was approximately the same amount of ACh inhibition whether the citrate pathway or acetoacetate pathway was measured, it appeared that lead was exerting some generalized effect, probably on energy metabolism. This study was supported in part by EPA #68-03-2381.

[1]Brain Res. 148:451 (1978).
[2]Biochem. Pharmacol. 26:397 (1977).
[3]Res. Commun. chem. path. Pharmacol. 15:483 (1976).
[4]Toxicol. Appl. Pharmacol. 34:340 (1975).

55.8 ACETYLCHOLINE DYNAMICS IN RAT HIPPOCAMPAL SLICES. B.E. Swartz & D.J. Jenden, Brain Research Institute and Department of Pharmacology, UCLA School of Medicine, Los Angeles, California 90024

Using GCMS analysis and stable isotope labelling, we have examined some aspects of ACh and Ch metabolism in the rat hippocampal slice preparation. Initially slices were incubated in oxygenated Krebs-Ringer bicarbonate solution (Kr; pH 7.5) with or without [$^2H_4$]Ch (10 µM) and using either glucose (10.2 mM) or pyruvate (20 mM) as substrate. Prior to incubation, ACh levels are 0.455 ± 0.063 (all values in nmol [mg protein]$^{-1}$ ± S.E.) and Ch levels are 12.120 ± 5.807 (n = 14, both). ACh rapidly increases to a plateau of 1.730 ± 0.164 at 1 hr while Ch decreases to 5.44 ± 0.623 (n = 22). ACh levels are well maintained at 5 hr (1.517 ± 0.105) whereas Ch continues to decline (2.546 ± 0.138; n = 10), although the specific activity of both ACh and Ch continues to rise (0.29 ± 0.02 and 0.32 ± 0.02 at 1 hr, respectively to 0.45 ± 0.11 and 0.55 ± 0.08 at 5 hr). The specific activities of ACh and Ch are highly correlated (r = 0.972, n = 9) in this preparation. Ch levels decline more rapidly during the first 10 min of incubation with pyruvate substituted for glucose or in the absence of [$^2H_4$]Ch. At 3 hr no differences were noted. Slices were prepared from quadrants (2.5 mm each) of left and right hippocampi. In both, ACh levels were greater in ventral (3rd or 4th) than septal (1st) quadrant. [Ll; ACh = 1.011 ± 0.62, Ch = 4.03 ± 0.290; L4; ACh = 1.820 ± 0.269, Ch = 7.302 ± 0.856; n = 6]. To examine the effects of altering exogenous Ch concentration on ACh release, slices were preincubated in Kr and transferred for 20 min to a medium containing 10 µM Tetram (an AChEI) and 10 µM [$^2H_4$]Ch or 50 µM [$^2H_4$]Ch with Tetram. K^+ levels were 5 or 25 mM. Raising exogenous Ch caused an elevation in release rates as shown below:

	[$^2H_4$-Ch]	5 mM K^+(n)	25 mM K^+(n)
Release Rate	10	10±1.2(27)	22±3.3(14)
(pmol mg^{-1}min^{-1})	50	16±1.8(15)*	19±1.2(14)

* $p < 0.01$, 50 µM vs 10 µM

Raising external K^+ eliminates this effect. We conclude that the hippocampal slice may be a useful preparation in which to study the cholinergic terminal but is a heterogenous structure whose subregions may have differentiable properties. (Supported by USPHS grants MH-17691 and MH-15345.)

55.9 MUSCARINIC CHOLINERGIC RECEPTOR BINDING AFTER CHRONIC ADMINISTRATION OF TRICYCLIC ANTIDEPRESSANTS. B.D. Bohman*, M.J. Karbowski* and A.E. Halaris. Dept. of Psychiatry, The Univ. of Chicago, Chicago, IL. 60637.

Muscarinic acetylcholine receptor binding was examined in whole mouse brain after chronic administration of tricyclic antidepressants (TADs) to determine whether tolerance to the antimuscarinic effects of these agents could be accounted for by changes in this parameter.

Tolerance was demonstrated in the following manner. Mice were injected (i.p.) for either 2 or 4 weeks with 10 mg/kg/day of imipramine (IMI) or amitriptyline (AMI). One day following the last injection, they were injected (i.p.) with 0.2 mg/kg oxotremorine along with 5 to 65 mg/kg IMI or AMI and observed over the following 15 minutes for the presence or absence of tremors. The ED_{50}'s for suppression by these TADs of oxotremorine-induced tremors increased, as compared to control animals, by about two-fold after 2 weeks and nearly three-fold after 4 weeks of pre-treatment.

Muscarinic receptor binding was also examined after 2 and 4 weeks of 10 mg/kg/day AMI or IMI. Mice were decapitated one day after the final injection. The brains were quickly homogenized in 10 volumes of ice-cold 0.32M sucrose and centrifuged at 1000g for 10 minutes. The supernatent was re-homogenized; 10 µl aliquots were then incubated for 60 minutes at 37°C with various concentrations of ^{3}H-quinuclidinyl benzilate (QNB) in 2 ml of 0.05M sodium-potassium phosphate buffer. The assay was terminated by rapid vacuum filtration, and the filters were subsequently assayed for radioactivity by liquid scintillation spectrometry. Specific binding was calculated by subtracting non-specific binding (measured in the presence of 1.0 µM atropine) from total ^{3}H-QNB binding in the absence of a receptor blocker.

Bmax was determined both at 2 and 4 weeks by measurement of specific binding in the presence of a saturating concentration (2.0 nM) of ^{3}H-QNB. At 2 weeks, Bmax and Kd were also calculated independently by Scatchard analysis. There was good agreement between the two methods of calculating Bmax. Neither IMI nor AMI produced a significant change ($p > 0.05$) in either Bmax or Kd following 2 or 4 weeks of treatment.

It appears that the development in mice of tolerance to the antimuscarinic effects of these TADs is not due to alteration of either the number or the conformation of central muscarinic receptors.

55.10 CHANGES IN REGIONAL CNS ACETYLCHOLINE AND CHOLINE LEVELS AFTER MICROWAVE SACRIFICE. J.M.Gorell*,R. Bauer*and M.Buckley* (SPON: E.P.Schoener). Depts. of Neurology & Pharmacology, Wayne State Univ. Sch. Med., Detroit, MI 48201.

During studies of brain acetylcholine(ACh) metabolism,we found unexpected changes in regional CNS ACh and choline (Ch) levels 30 min or more after "adequate" microwave (MW) sacrifice.

Adult male Swiss-Webster mice (30-36g.) were treated (9-10A.M.) with head-focussed MW(3.6kW forward, ≤ 0.1kW reflected power at 2450 MHz for 320-340 msec;Metabostat 4104,Gerling-Moore,Inc.,Sta. Clara,CA). Two groups of mice were immediately dissected at room temperature,CNS regions(cerebral cortex,CX;cerebellum,CB;brainstem,BS;striatum,S;hippocampus,H)were placed into liquid N_2 cups, weighed in turn,and homogenized in either formic acid:acetone(ACh or Ch assay;Goldberg & McCaman,1973)or Triton X100(choline acetyl transferase(ChAT) or acetylcholinesterase(AChE)assay;Fonnum,1969). Other mice were MW'ed,but CNS regions were not dissected until 30 or 120 min after treatment;these were analyzed for ACh and Ch. Mice were rejected for chemical study if the deep forebrain temperature 10-15s post-MW was < 85°C. Brain temperatures were the same in all groups;mean values ranged from 87.8-89.6°C.

	Interval Between MW and Homogenization of CNS Regions		
	Immediate	30 Min	120 Min
CX - ACh	22.1±1.2(15)	18.6±1.5(10)	25.9±2.3(11)
CB - "	5.9±0.2(15)	6.6±0.5(11)	11.6±0.8(12)***
BS - "	37.1±1.4(14)	23.4±1.8(10)***	22.4±1.6(11)***
S - "	86.5±4.0(12)	46.7±5.1 (9)***	34.1±2.9(12)***
H - "	28.5±1.1(14)	21.2±1.8(11)**	23.9±1.4(12)*
CX - Ch	27.6±2.2(13)	26.6±2.0 (7)	31.0±1.9(10)
CB - "	28.3±1.2(14)	35.3±2.7 (9)*	49.7±2.5 (8)***
BS - "	30.3±1.3(12)	34.2±3.2(10)	44.7±4.2 (9)**
S - "	36.1±1.9(11)	33.3±2.4 (9)	33.7±2.3 (9)
H - "	25.2±1.3(14)	26.6±2.1 (8)	44.5±8.4 (7)*

Values are the mean(nmol/g) ± SEM of number of mice indicated. * P < .05, ** P < .01, *** P < .001 vs. Immediate group.

Preliminary experiments suggest that too little AChE or ChAT activity persists in mouse CNS regions after MW to account for observed changes in brain ACh levels. Extracts of 5 CNS regions of 8 mice were made immediately post-MW. Measurable AChE(ie. ≥ 84 pmol/mg wet wt/h)was present in just 1 S and 1 CB region, and no ChAT activity(ie. ≥ 11 pmol/mg wet wt/h)was detected.

These changes in regional CNS ACh and Ch levels post-MW cannot be explained at present. However, our data emphasize the need to cool and quickly homogenize MW'ed CNS regions to minimize postmortem artifacts.

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55.11 THE REVERSE REACTION OF BRAIN CHOLINE ACETYLTRANSFERASE: SALT EFFECTS AND PRODUCT INHIBITION, L.L. Hsu* and L.-P. Chao (SPON: H.E. Hirsch). Department of Psychiatry and Behavioral Sciences, The University of Texas Medical Branch, Galveston, Texas 77550 and Department of Neurology, the Reed Center, UCLA L.A. CA 90024.

We have recently reported a simple and sensitive assay for the reverse reaction of choline acetyltransferase (ChAc, EC 2.3.1.6) by separating and measuring the reaction product ^{14}C -acetylcoenzyme A (AcCoA) using a Dowex 50W-8 (Na^+form) cation exchange resin column. Now we report on the effects of salts and product inhibition on the reverse reaction of ChAc which has been purified to homogeneity from bovine brain (Chao and Wolfgram, J. Neurochem. 20: 1075-1081, 1973). An aliquot of brain ChAc was incubated with 5 mM ^{14}C -Acetylcholine (ACh) and 50 µM CoA with and without salt, in 90 µl of 0.05M sodium phosphate buffer (pH 7.0) at 37° C for 30 minutes. At the end of incubation, the reaction was stopped by addition of 0.5 ml of ice-cold distilled water. The reaction mixture was applied to a Dowex 50W-8 (Na^+form) resin column (2.0 x 0.7 cm) to remove the unreacted ^{14}C -ACh. The reaction product, ^{14}C -AcCoA was eluted from the Dowex column with 1.5 ml distilled H_2O and combined with the effluent for measurement of radioactivity. Reaction mixtures containing boiled enzyme served as blanks. For product inhibition studies, the activity of reverse brain ChAc was determined at various concentrations of ^{14}C -ACh and a fixed CoA concentration (5 µM) in the absence or presence of 1 mM choline or 0.1 mM AcCoA.

Our results indicated that NaCl, KCl and KBr at 0.22M greatly stimulated the reverse brain ChAc activity (to 161%, 180%, and 155% of the control respectively). On the contrary, NaF at the same concentration inhibited the reverse ChAc activity (to 70% of control). The stimulating effects of salts may be due to the monovalent cations whereas the inhibitory effects may be due to the halogen anions.

The double reciprocal kinetic plots showed that the reaction product choline inhibited the reverse ChAc activity noncompetively with respect to ACh. Furthermore, the other reaction product AcCoA exhibited a competitive inhibition with respect to ACh, unlike the product inhibition pattern reported for the reverse ChAc from human placenta. More detailed studies of salt effects and reverse ChAc kinetics are under investigations for elucidating its reaction mechanism.

55.12 EFFECT OF DIETARY CHOLINE ON THE KINETICS OF THE HIGH AFFINITY CHOLINE UPTAKE SYSTEM. K.L. Parrish, B.A. Trommer* and L. Wecker, Dept. Pharmacology, Louisiana State Univ. Med. Ctr., New Orleans, LA 70112.

Recently much interest has focused on the use of the neurotransmitter precursor choline in the treatment of various neurological and psychiatric disorders postulated to involve hypocholinergic activity. However, the effects of chronic choline availability on central cholinergic biochemical parameters have not been investigated. Therefore, this study was designed to ascertain the relationship between dietary choline availability and central cholinergic activity as assessed by kinetic characterization of the sodium-dependent high affinity choline uptake system (HACUS). Rats were randomly divided into 3 groups and maintained on: a) choline deficient diet, b) basal choline diet (0.2% choline chloride) or c) supplemented choline diet (2.0% choline chloride). After 28-32 days on the dietary regimen, the rats were decapitated and the striatum and hippocampus removed. The kinetics of the HACUS (Km and Vmax) were determined in P_2 fractions utilizing substrate concentrations of 0.1-1.43uM. Dietary choline availability had no effect on the affinity of the HACUS. In synaptosomes from rats maintained on the choline supplemented diet, no change was noted in the Vmax of the HACUS. In contrast, the Vmax of uptake in synaptosomes from rats maintained on the choline deficient diet was increased to 122% of control (basal diet) in striatum and to 141% of control in hippocampus. Although increased choline availability has been suggested to increase cholinergic activity, present results indicate that dietary (chronic) choline supplementation does not change the HACUS. However, the choline deficient diet does increase the velocity of the HACUS in both brain regions. This finding does not reflect the differential effect of dietary choline on the steady-state concentration of acetylcholine in the striatum versus the hippocampus, since dietary choline deficiency decreased the concentration of acetylcholine in the striata to 85% of control without affecting hippocampal concentrations (Life Sci.25:375, 1979). The suggestion that choline loading increases cholinergic activity has not been demonstrated in the present study. The intriguing finding whereby limited choline availability induces an increase in the Vmax of the HACUS in both brain regions requires further elucidation and is currently being investigated in our laboratory. Of particular interest is the dependence of the phenomenon on the length of time the animal is maintained on the diet. (Supported by NIMH # 33443).

55.13 BLOCKADE OF NICOTINIC CHOLINERGIC RESPONSES BY A TOXIN COMPONENT OF BUNGARUS MULTICINCTUS VENOM. M. Quik*, J.M. Trifaró*. M. Vella Lamarca*, D. Ramirez-González* and B. Collier*. (SPON: B. Esplin). Dept. Pharmacology, McGill Univ., Montreal H3G 1Y6 PQ

The use of α-bungarotoxin (α-BGT) as a ligand for the nicotinic receptor at sites other than the neuromuscular junction is currently under question. Although most reports indicate that α-BGT does not inhibit cholinergic transmission at neuronal synapses, Chiapinelli & Zigmond (1978,PNAS,75,2999) have demonstrated a block but only in chick sympathetic ganglia and only with some preparations of the toxin. Recent results of Ravdin & Berg (1979, PNAS,76,2072) indicate that other α-toxins (fraction III) in Bungarus (B.) multicinctus venom may be responsible for this action. Because of the importance of developing a ligand for neuronal nicotinic sites, the present experiments reinvestigated the effect of B. multicinctus toxins on such receptors. Venom was fractionated into its components by two commonly used procedures. METHOD A was that of Lee et al. (1972, J.Chromatogr.72,71) and Ravdin & Berg (1979). After ion exchange chromatography, an elution profile was obtained which was similar to that previously described. α-Toxin fraction II (α-BGT II), which corresponds to the commonly used α-BGT, was found to inhibit (1) nicotinic cholinergic transmission in rat sympathetic ganglia by 50% at a concentration of 1 µg/ml (2) the carbachol (1 mM) stimulated release of ^{3}H-noradrenaline from cultured bovine adrenal medullary cells by 75% at 0.5 µg/ml and (3) carbachol stimulated uptake of ^{22}Na in the same cells by 59% at 1µg/ml. Fraction III which also contains α-toxins, was without effect at these concentrations. METHOD B used to isolate α-BGT* was similar to that of Berg et al. (1972,PNAS,69,147). Crude venom was first applied to a Sephadex G-50 column and consequently to an ion exchange column. When α-BGT* was isolated in this manner, it did not inhibit the above mentioned responses. However, both α-BGT II and α-BGT* blocked neuromuscular transmission in the rat phrenic nerve-diaphragm preparation. This suggests that some component is present in the α-BGT II preparation which is responsible for the blockade of nicotinic transmission at neuronal synapses. Experiments are currently underway to identify this component.

	Nicotinic response after incubation with	
Preparation	α-BGT II	α-BGT*
Rat sympathetic ganglia	block	no effect
Bovine adrenal medullary cells		
(1) ^{3}H-NA release	block	no effect
(2) ^{22}Na uptake	block	no effect
Rat phrenic-diaphragm	block	block

Supported by the MRC (Canada).

55.14 DEFINITION OF THE CHOLINERGIC LESION IN EARLY THIAMINE DEFICIENCY. L.L. Barclay,* G.E. Gibson, and J.P. Blass. Cornell Univ. Medical College, Burke Rehab. Center, White Plains, NY 10605.

Impaired synthesis of acetylcholine (ACh) by thiamine-deficient brain tissue was reported in 1940, but the functional significance of the cholinergic lesion has been controversial. We recently reported that the cholinesterase inhibitor physostigmine was as effective as the vitamin itself in treating early behavioral effects of pyrithiamine-induced thiamine deficiency (Clin. Res. 28:516A, 1980). These studies have been extended.

On a quantified "string test," 44/69 pyrithiamine-treated rats performed poorly by day 5-6 (score 1.5±0.6); the other 36% performed normally (score 8.2±0.3). None lost weight or showed gross neurological abnormalities. Arecholine (2.0 mg/kg), a direct muscarinic agonist, was as effective in restoring string test performance (to 4.6±0.5) as was physostigmine (to 3.8±0.1) or thiamine (to 4.1±0.2). The effect of physostigmine was blocked by the muscarinic blocker, atropine, but not by methatropine, its peripherally acting analogue. Nicotine had no effect at three different doses. Mecamylamine, a nicotinic ganglionic blocker, did not block the effect of physostigmine.

Biochemically, by day 6-7 of pyrithiamine treatment, levels of acetylcholine were reduced by 38% ($P<0.001$) and incorporation of [$^{2}H_{4}$]choline was reduced by 34% ($P<0.01$) in animals with low string test scores compared to those with normal string test scores. Surprisingly, incorporation of [U-^{14}C]glucose into acetylcholine increased (25%, $P<0.01$) in low compared to normal string test score animals. Specific activities of both precursors were unchanged.

Thiamine deficiency induces an early central muscarinic cholinergic lesion which is functionally significant.

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55.15 ACETYLCHOLINE EFFECTS IN THE LATERAL GENICULATE NUCLEUS REGION OF THE CAT. J.M.Godfraind (SPON: M.Meulders), Lab. Neurophysiol., Univ. Louvain, UCL 5449, B-1200 Brussels, Belgium.

The excitatory and the inhibitory effect of acetylcholine iontophoretically applied, respectively on the lateral geniculate nucleus (LGN) neurones and on the perigeniculate reticular (PGN-R) cells, were examined under different experimental conditions. Results are summarized in the table. These observations show that the excitability of the LGN neurones and that of the PGN-R cells - thought to play the role of inhibitory interneurones - are greatly dependant of the animal preparation.

Experimental conditions	Ach excitation	Ach inhibition
Influence of animal preparation		
1. midpontine pretrigeminal	present	present
2. chloralose 80 mg/kg i.v.	present	absent
3. urethane 1 g/kg i.v.	present	present
4. 1% fluothane in N_2O/O_2	present	present
Effect of iontophoresis of atropine and mecamylamine		
1. atropine	blocked	blocked
2. mecamylamine	blocked	not blocked
Effect of i.v. atropine or dexetimide, under urethane anaesthesia	present	blocked
Effect of iontophoresis of nicotinic blocking agents after pretreatment with i.v. atropine or dexetimide, under urethane anaesthesia		
1. mecamylamine	antagonized	not antagonized
2. DHβE	antagonized	not antagonized
3. d- tubocurarine	antagonized	not antagonized
4. hexamethonium	antagonized	not antagonized
5. decamethonium	non-specific	non-specific

55.16 CHOLINE POST MORTEM INCREASE: EFFECT OF TISSUE AGITATION, pH, AND TEMPERATURE. J.W. Kosh, R.M. Dick*, and J.J. Freeman. Div. of Pharmacology, Coll. of Pharm., Univ. S. Carolina, Columbia, S.C. 29208.

The post mortem production of choline in brain tissues is well known. In the present study we have examined the extent of post mortem choline (Ch) production in brain and other tissues of the mouse. The influence of agitation, pH, and temperature on the post mortem choline production in brain tissues was also examined. All tissue samples were assayed for acetylcholine (ACh) and Ch using gas chromatography (Kosh, et al., J. Chromatog. 163, 206-211[1979]). Brain, eye, heart, lung, liver, ileum, kidney, and skeletal muscle tissues from male albino mice (HA/ICR, 25-35g) were incubated for 15 minutes at 38°C and homogenized in formic acid-acetone prior to assay. The effect of physical agitation (oxygen or nitrogen aeration, vigorous stirring, or sonication) during incubation for 15 minutes at 38°C was determined in brain tissue homogenates. The effect of temperature and pH on the post mortem choline production was determined in brain tissue homogenized in Locke's solution adjusted to a pH of 6.6 or 9.0, followed by incubation for 15 minutes at either 0, 20, 38, 48, or 68°C. All of the tissues studied except skeletal muscle demonstrated a significant post mortem increase in choline. The brain produced the largest percent increase (700%), followed by the kidney (454%), and the lung (383%). The other tissues exhibited a 200-300% increase in choline. Several types of physical agitation significantly changed ACh and Ch values compared to control homogenates which were not agitated. Sonication had the greatest effect by increasing the Ch level from 180 nmoles g^{-1} (control)to 324 nmoles g^{-1}. Aeration with oxygen or nitrogen or mechanical stirring gave similar Ch values (303-305 nmoles g^{-1}). Sonication was the only treatment which reduced ACh levels (from 4.7 to 0.2 nmoles g^{-1}). The production of choline was more pronounced at pH 9.0 than at pH 6.6 at temperatures between 0 and 48°C. Maximal Ch production occurred at 48°C and was inhibited at 68°C at both pH's. In summary the post mortem production of choline in the mouse appears to be enzymatic, occurs in many peripheral tissues and can be stimulated by several types of physical agitation. The enzymes that catabolize phospholipids in the brain to generate choline may also be active in peripheral tissues.

55.17 STIBONIUM-CHOLINE: A POSSIBLE TOOL FOR THE LOCALIZATION OF CHOLINERGIC NEURONS. E.M. Meyer*, D. White*, R.J. Barrnett*, and J. R. Cooper. Yale University School of Medicine, New Haven, CT 06510 .

An analog of choline was synthesized in which the nitrogen was substituted with antimony by reacting trimethylstibene with iodoethanol. This compound, hereafter called Sb-choline, was purified over a weak cation exchange column (CG-50) and by organic cation exchange with tetraphenylboron. It was found to be over 99% pure via NMR, IR, and atomic analyses for C, H, and Sb. The Sb-acetylcholine analog (Sb-ACh) was further synthesized by treating Sb-choline with acetic anhydride in the presence of pyridine. Both Sb-ACh and Sb-choline stained positively with Dragendorfs reagent, and could be separated from their nitrogen containing counterparts by thin layer chromatography or CG-50 columns. Preliminary results show that Sb-choline is acetylated by a crude preparation of rat brain choline acetyltransferase at the same maximal rate as choline. It is also phosphorylated by choline kinase at the same rate as choline, which provides us with an assay for Sb-choline or after hydrolysis, Sb-ACh. As expected, Sb-choline also appeared to be a competitive inhibitor of both enzymes. After neutron activating the Sb-choline (performed by R. Litman, Lowell University Lowell, MA) to form small quantities of ^{122}Sb- and ^{124}Sb-choline, we were able to show that this compound, at concentrations from 10-100 μM, was taken up by synaptosomes at about 80% the rate of ^{3}H-choline, acetylated, and subsequently released by potassium depolarization in a calcium-dependent manner. When cortical slices were incubated with 10 μM ^{122,124}Sb-choline or ^{3}H-choline and subsequently a synaptosomal preparation was made from these slices, it was found that ^{122,124}Sb-ACh and ^{3}H-ACh were localized in both the cytoplasmic and vesicular fractions. However, K^{+}-depolarization preferentially released both compounds from the cytoplasmic pool. Our results suggest that Sb-ACh should be an excellent substrate for the in situ localization of ACh via x-ray microanalysis with which it should be possible to determine whether the transmitter is localized in or released from the cytosol, vesicles, or the terminal plasma membrane. We also plan to use this technique to map cholinergic tracts in the CNS, to which end initial experiments are in progress.

55.18 CHOLINERGIC RECEPTOR ANTAGONISM BY PURIFIED PEPTIDE TOXINS FROM CONUS GEOGRAPHICUS. O. McManus*, C. Gonzalez*, J. Musick. Dept. Physiol., Univ. Utah Col. Med., Salt Lake City, UT 84108.

Two tridecapeptide toxins (G_1 and G_{11}) have been isolated from the crude venom of the marine snail Conus Geographics, and their composition has been reported (Gray et al., Fed. Proc., 61:2171, 1977; Cruz et al., Arch. Bioch. Biophys., 190:2, 1978). Since the lethal effect of these toxins (LD_{50} = 12 μg/KG in mice) is probably due to flaccid paralysis of skeletal muscle, we have investigated the site of paralytic action by equimolar mixtures of G_1 and G_{11}. The G_1-G_{11} mixture produced a reversible blockage of the contraction of neurally stimulated mouse diaphragm muscle, but did not affect the contraction of directly stimulated muscle. Complete blockage of neurally induced contractions was seen with a G_1-G_{11} concentration of 4.6×10^{-7} within 22 minutes. The time course of onset of toxin effects on muscle contraction was dependent on toxin concentration, but was generally slower than curare, and comparable to α-Bungarotoxin (α-BT), indicating that diffusion into the muscle was a limiting factor. Blockage of neurally evoked contractions was not due to impairment of impulse conduction in motor axons because paralytic concentrations had no effect on the amplitude of compound nerve action potentials recorded from ventral roots of the bullfrog sciatic nerve (McManus and Musick, Soc. Neurosci. Abs., 5:1644, 1979). These results suggested that paralysis occurred by impairment of neuromuscular transmission. The G_1-G_{11} mixture reduced the amplitude of miniature endplate potentials in the mouse diaphragm (McManus and Musick, 1979) and in the frog sartorius. Endplate potentials were reduced in the frog sartorius, apparently by a postsynaptic action, because a 22% decrease in EPP amplitude ($p < .001$) could be observed without a significant reduction in EPP quantal content ($p > .4$). Possible antagonism of the acetylcholine receptor (AChR) was investigated by measuring the inhibition of I^{125}-αBT binding to endplate regions of the mouse diaphragm. The G_1-G_{11} mixture inhibited I^{125}-α-BT binding in a qualitatively similar fashion as did d-tubocurarine, but G_1-G_{11} concentrations tenfold less than curare produced comparable inhibition. These results suggest that part of the paralytic effect of G_1-G_{11} is due to antagonism of acetylcholine binding to its receptor, and that G_1 and/or G_{11} is a tenfold more potent antagonist of endplate acetylcholine receptor than curare. Supported by NIH grants NS07938 and NS12635 from the USPHS, Biomedical Research Support Grant 5-S07RR05428 and Univ. of Utah Graduate Research Fellowship.

55.19 REGULATION OF Ach RECEPTORS ON CULTURED RAT MYOTUBES BY Ca^{2+}. James McManaman* and S.H. Appel. Department of Neurology, Baylor College of Medicine, Houston, Texas 77030.

Muscle activity is known to regulate the levels of certain muscle specific proteins. In particular the number of Ach receptors has been shown to be increased by denervation, or agents that interfere with electrical excitation, and to be decreased by electrical stimulation. Similar results have been shown for myotubes in culture. As yet the factor(s) controlling the activity related changes in Ach receptor levels have not been defined. However, the involvement of Ca^{2+} in the regulation of Ach receptors is suggested by the well known changes in intracellular Ca^{2+} concentrations during muscle contraction and the involvement of Ca^{2+} in the expression of muscle specific proteins during development. We have investigated the role of Ca^{2+} in the regulation of Ach receptors on cultured myotubes by incubating myotubes in culture medium containing 1/10 the normal concentration of Ca^{2+} (Ca^{2+} deficient medium).

The number of Ach receptors on the surface of rat myotubes cultured in Ca^{2+} deficient medium was decreased to approximately 30% of control, as measured by ^{125}I-α bungarotoxin binding. In contrast the level of creatine phosphokinase was decreased only 10% and the incorporation of ^{35}S-methionine into protein was unaffected by culturing myotubes in Ca^{2+} deficient medium. The decreased number of Ach receptors was not due to a loss of myotubes since the DNA content per plate was identical to control. In addition the integrity of the myotube membrane was unaffected by incubation in Ca^{2+} deficient medium as indicated by normal resting membrane potentials and action potential amplitudes. Incubating myotubes in Ca^{2+} deficient medium did not affect the rate of degradation of Ach receptors but decreased the rate of insertion of new Ach receptors into myotube membranes, and decreased the level of intracellular Ach receptors. The rate of insertion of new Ach receptors into myotube membranes was not affected by culturing in Ca^{2+} deficient medium for the first 4 to 6 hours, thereafter the rate of insertion decreased significantly.

The decreased rate of insertion was increased to control levels by the addition of Ca^{2+} to the Ca^{2+} deficient medium. This effect was blocked by inhibitors of protein synthesis. These results are direct evidence for the involvement of Ca^{2+} in the regulation of Ach receptors and suggest that Ca^{2+} influences the synthesis of Ach receptors rather than transport of pre-existing Ach receptors to the surface. (Supported by The Muscular Dystrophy Association and a Dementia Training Grant #AG00061-01 470-G07865 from the National Institute of Aging)

55.20 CYCLIC AMP-INDUCED INCREASES IN ACETYLCHOLINE RECEPTOR REQUIRES PROTEIN AND RNA SYNTHESIS. James C. Blosser, James L. McManaman* and Stanley H. Appel. Department of Neurology, Baylor College of Medicine, Houston, Texas 77030

The synthesis and turnover of acetylcholine receptor (AchR) in muscle is regulated by both muscle activity and neurotrophic agents. However, little is known about the intracellular factors and mechanisms which ultimately mediate the changes in receptor content. Recently, we demonstrated that cAMP increases the levels and rate of insertion of AChR into plasma membrane of cultured embryonic chick myotubes (J. Biol. Chem. 255:1235 (1980)). To determine whether the effect of cAMP is due to an actual increase in synthesis of receptor the effects of inhibitors of protein and RNA synthesis have been examined. Insertion rate of AChR into external membrane is assessed by preblocking existing receptors with α-bungarotoxin and then measuring the appearance of new receptors at various times thereafter with ^{125}I-α-bungarotoxin. Intracellular cAMP levels were increased by treatment with 10^{-3}M cholera toxin. Cycloheximide at concentrations which inhibited protein synthesis by over 90% (3 μg/ml) was found to completely block the increased insertion rate of AChR induced by cholera toxin. Actinomycin D (0.03 μg/ml) also completely blocked receptor insertion in control and cholera toxin-treated cells under conditions in which [^{3}H]uridine incorporation into total RNA was inhibited by over 90%. Neither DNA content nor cAMP levels were decreased by actinomycin D suggesting that cell viability was not altered. However, because of known side effects of this agent, cells were also treated with an inhibitor of RNA polymerase II, α-amanitin. Both the control and cAMP-induced increase in AChR insertion rate were decreased by 80% without measurable effects on cAMP levels or total RNA synthesis. The extent to which the cAMP-induced increase in AChR requires de novo synthesis is being assessed by labeled amino acid incorporation into receptor. These results suggest that cAMP induces an increase in AChR through a transcriptional mechanism. (Supported by the Muscular Dystrophy Association and NSF Grant BNS-7914115)

55.21 SURFACE SPECIALIZATIONS ON CULTURED MYOTUBES FROM EMBRYONIC CHICK THIGH CONTAIN A HIGH DENSITY OF ACETYLCHOLINE RECEPTORS. Thomas G. Burrage and Thomas L. Lentz. Section of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

Surface specializations (patches) on cultured myotubes from thighs of 11 day old chick embryos incubated for 5 days were characterized by heavy metal stains and the binding of horseradish peroxidase-labeled α-bungarotoxin (HRP-α-BTX) for localization of acetylcholine receptors (AChR). The specializations consisted of slightly raised surface ridges (0.1 to 0.5 μm in width) and wider regions (2-5 μm) sometimes composed of the smaller ridges. The specialized zones were characterized by an external coating of amorphous to fine textured material, considerably more extensive than the sparse developing basement lamina occurring elsewhere on the myotube surface. On the internal surface and coextensive with the external layer, dense material was applied immediately adjacent to the plasma membrane. The extraneous coating of these patches stained with ethanolic phosphotungstic acid, bismuth iodide, and ruthenium red, and most likely contains complex carbohydrates. The specialized regions contain a high density of nicotinic AChR, as shown by the binding of HRP-α-BTX. Reaction product occurred in the plasma membrane of the patch. The larger reactive regions correspond to the "hot spots" observed at the light microscopic level. A lower density of HRP-α-BTX binding was seen occasionally in the membrane adjacent to the patch regions. This low density staining may represent diffuse receptors present in sufficient concentration to be detectable with this technique. Coated pits were often associated with patch regions. These stained with HRP-α-BTX and contained ruthenium red-staining material, indicating the pits may be involved in the transport of receptor-bearing membrane and/or extraneous coat material to the myotube surface. Caveolae of the developing sarcotubular system near surface patches were also stained with HRP-α-BTX. The specialized patch regions were widely separated on these uninnervated surfaces with only 1 to 3 usually occurring over the entire profile of the cell in section. Patches were most abundant on the basal surfaces of cells and where cells contacted the substrate or other cells. Microtubules and microfilaments occurred in close proximity to the patch regions but were found elsewhere in the cell as well. These studies show that external and internal specializations are associated with a high density of AChR in the absence of innervation. Constituents of these specializations may be involved in the localization or positioning of a high density of AChR at these sites. (Supported by NIH Grant F32 NS06144-02 and NSF Grant BNS 79-12898).

55.22 DEVELOPMENT OF ALPHA-BUNGAROTOXIN BINDING SITES IN THE CEREBRAL CORTEX OF NORMAL AND REELER MOUSE. P. F. Strang-Brown and V. Wootten*. Dept. of Anatomy, Univ. of Cincinnati College of Medicine, and Institute for Developmental Research, Children's Hospital, Cincinnati, Ohio 45267.

Biochemical studies have demonstrated changes in alpha-bungarotoxin binding during the development of the cerebral cortex in the rat and the guinea pig (Salvaterra and Moore, Biochem., Biophys. Res. Comm. 55:1311, 1973; Wade and Timiras, Brain Res. 181:381, 1980). Toxin binding in the cerebral cortex has been shown to peak between days 12 and 20 postnatal.

This study used the in vitro method of Segal et al., (Brain Res., 148:105, 1978) to show the ontogenetic changes in the morphological distribution of αBTX binding sites in the somatosensory cortex of normal and reeler mouse (strain C57BL/6J). Somatosensory cortices from animals of the following postnatal ages: 0,3,7,10,14,16,18, and 20 days were analyzed. In the cortex of normal mouse, the toxin binding increased from day 0 to a peak value on day 16. Days 18 and 20 showed similar values to day 16. In the cortex of the reeler mouse, the toxin binding showed peaks on days 3 and 20. Morphological differences in the toxin binding peaks between the normal and reeler mouse suggests an alteration in the process of receptor development and synaptogenesis in the reeler mouse.

55.23 EFFECT OF IN VITRO AND IN VIVO KETAMINE ON CHOLINE UPTAKE IN MOUSE STRIATUM. Christina VanderWende, Barbara Masley* and Marie T. Spoerlein. Rutgers Univ. Coll. of Pharmacy, Box 789, Piscataway, N.J. 08854.

A detailed study of the effect of ketamine on the cholinergic functions of different areas of the brain has not been done. Therefore, we chose to start such studies with the effect of ketamine on striatal cholinergic function using high affinity sodium dependent choline uptake (HANDCU) as a measure of this activity (Simon et al., J. Neurochem. 26: 909-922, 1976).

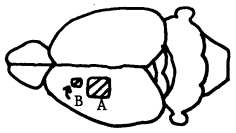
Male albino Carworth CF-1 or male albino Swiss Webster mice were used for these studies. For the in vitro experiments, ketamine was added directly into the incubation medium to give final concentrations ranging from 10^{-4} to 10^{-9} M. For the in vivo studies, ketamine was administered intraperitoneally 4 min. prior to sacrificing the animals. Animals were decapitated; the brains removed; and the striatum dissected over a bed of crushed ice. The striata were homogenized in 2.0 ml of 0.32 M sucrose and centrifuged for 10 min. at 1,000 g. The low speed supernatant was recentrifuged at 17,000 g to give the P_2 pellet. The P_2 pellet was resuspended in 1.2 ml 0.32 M sucrose and incubated with 54 uM choline and 0.49 uM ^{3}H-choline both in the presence and absence of Na^+. After incubating for 3 min. at 31°, the tubes were plunged into an ice bath, then centrifuged 15 min. at 6,000 g. The pellet was surface washed, digested with Protosol and counted in 10 ml Aquasol containing 0.5 ml acetic acid. Protein was determined by the Bio-Rad procedure.

In vitro ketamine caused a slight but significant increase in HANDCU (8-13%) at concentrations between 10^{-6} and 10^{-9}M where the increase was repeatedly greater at the lower concentration. At high concentrations (10^{-5}-10^{-4}) there was no change in HANDCU compared to controls. No explanation for these results is yet available.

In the in vivo experiments, ketamine resulted in significant increase in HANDCU at doses causing loss of the righting reflex and at toxic doses. Interestingly, higher toxic doses caused less of an increase than lower non-toxic doses.

56.1 A SECOND FORELIMB MOTOR AREA EXISTS IN RAT FRONTAL CORTEX. E.J. Neafsey. Dept. Anat. Loyola Univ. Med. Cent., Maywood, IL 60153.

Movements evoked by intracortical stimulation(2.5 msec pulses,500-5 μamps,350 Hz,300msec trains) in the frontal cortex of adult Long-Evans rats were studied. The rats were anesthetized with Ketamine (100mg/kg IP, with supplemental doses as needed). Glass insulated tungsten microelectrodes(50-150 μ exposed at tip) were inserted tangential to the cortical surface, beginning laterally and ending medially. The depth of the tip below the surface medially was 1.4-1.9mm.In each rat 15 to 20 penetrations spaced .25mm apart were made from near the frontal pole to just behind bregma. Along the 3-5mm course of each track, stimulation was delivered every .25mm. If movement occurred at 500μamp, its threshold(i.e.,current reliably evoking movement) was determined.The mean threshold was 240μamps(SD=150). For the forelimb two separate regions that produced movement were found. The first(2.5x2.5mm) was located where other workers(Hall and Lindholm,Brain Res.66:23-38,1974) have described the rat forelimb area and is labeled A in the figure below. The movements evoked included shoulder retraction, elbow flexion and extension, pronation and supination of the forearm, wrist extension, and,infrequently, digit extension. The second area (1x1mm;labeled B in figure below) was located rostrally .5 to 1.5mm from the first area. A zone of neck and/or vibrissae movements always separated the two forelimb areas. The movements evoked from the rostral area included elbow flexion, wrist extension, and digit extension and flexion. Digit movements were reliably evoked here but were uncommon in the more caudal motor region. Following HRP injection into the rat cervical enlargement, a separate cluster of labeled cortical cells has been found in the approximate location of this rostral forelimb motor area (Wise et al, Neuroscience 4:65-78,1979), providing an anatomical basis for its existence. This region appears to be concerned with control of wrist and digit movement.



56.2 CORTICAL MECHANISMS OF TWO-DIMENSIONAL AIMING ARM MOVEMENTS. I. AIMING AT DIFFERENT TARGET LOCATIONS. A.P. Georgopoulos, J.F. Kalaska* and J.T. Massey*. Dept. of Physiology, The Johns Hopkins School of Medicine, Baltimore, Md. 21205.

Arm movements aiming at targets bring the hand to final spatial coordinates that coincide with those of the target. How commands for such movements are formed in the brain using spatial (target location) information are largely unknown. We are investigating this problem using an apparatus in which a handle can be moved freely in the two (X-Y) dimensions across a plane. Monkeys were trained to move the handle and capture (within a circle attached to it) lighted targets in a reaction time task (J.T. Massey et al., Fed. Proc., 1980). This apparatus provides a high degree of spatial correspondence between movements and targets without dissociation between display and manipulandum. Of 9 targets, one is at the center and 8 around it on a circle of 8 cm radius. The animal first captures the center light and holds there for a variable length of time, then a peripheral light comes on and the animal has to move the handle to capture the target to receive a liquid reward. Since the 8 targets are arranged equidistantly around the circumference of the circle, the directions of the movements made from the center to the targets cover the whole circle with a resolution of 45°.

The cortical mechanisms of formation and execution of these aiming movements were explored using single cell recordings in motor and adjacent premotor areas of the frontal cortex in two monkeys. We studied 211 cells (isolated in 40 penetrations) that discharged in relation to spontaneous arm movements. Characteristics of responses in the task were as follows: (a) The latency (mostly within the reaction time), magnitude, direction of change (increase or decrease of discharge) and time course of the response varied with the sector of the circle within which the aiming (center-out) movement was made; for many cells the changes occurred continuously and gradually within that sector as movements of different radial directions were produced. The size of the sector was large for many cells; for some others it was narrow. Maxima and minima of activation (or inhibition) occurred at different radial directions within the sector for different cells. Therefore, the process of composition of commands for spatially oriented movements engages the activity of partially overlapping (in the directional aspect) cell populations. (b) Several movement-related cells showed a short-latency, stimulus-locked response when target lights appeared in certain locations. This may reflect a selection mechanism by which movement-related precentral populations are activated in correspondence with the location of the target, probably leading to the generation of movements aimed at that target. (Supported by USPHS Grant 5-RO-EY03167-02).

56.3 CORTICAL MECHANISMS OF TWO-DIMENSIONAL AIMING ARM MOVEMENTS. II. CHANGE OF TARGET LOCATION. J.F. Kalaska*, J.T. Massey* and A.P. Georgopoulos. Dept. of Physiology, The Johns Hopkins School of Medicine, Baltimore, Md. 21205.

Changes in the location of the target while an aiming movement is being generated (e.g., during the reaction time) result in orderly modification of the motor output (A.P. Georgopoulos et al. Fed. Proc., 1980): the hand moves towards the first target for a period of time and then changes direction and moves to the second target. The duration of the first movement is a linear function of the time for which the first target was on. We are studying this problem using an apparatus which allows free movement of a handle in the two (X-Y) dimensions of a plane. Monkeys were trained to move the handle and capture (within a circle attached to it) lighted targets in a reaction time task: they moved from the center to peripheral target lights located on a circle of 8 cm radius. The location of the target was changed from 50 to 400 msec after the appearance of the first target. Under these conditions the ensuing arm movements change direction at various times, as described above; by contrast, saccadic eye movements (monitored using implanted EOG electrodes) were all-or-none to the first target (and from there to the 2nd target). This dissociation of eye and hand movements indicates that the process which generates aiming movements differs for the two systems. For the arm, this process begins when the target appears and can be modified by subsequent changes in the spatial (target location) information.

We studied the effects of change in target location on the activity of single cells in motor and adjacent premotor areas of the frontal cortex in two monkeys. 80 cells (isolated in 40 penetrations) were studied in the above task; they discharged in relation to spontaneous arm movements. We chose target locations (and, therefore, directions of aiming movements) for which cells showed different patterns of response (see A.P. Georgopoulos et al., this Meeting). The activity of the cells was modified in an orderly fashion by the change in target location for the whole range of times used; that is, for changes in target location occurring from 50 to 400 msec after the appearance of the first target. The time from the first neuronal change (after the 1st target) to the second change (after the 2nd target) was a function of the time for which the 1st target was on. Therefore, neurons in motor and premotor areas show continuous modifiability of their discharge under conditions of changing spatial (target) information, in accordance with the motor behavioral data mentioned above. We postulate that these populations are involved in a continuous utilization and translation of this information to appropriately organized (in the spatial domain) motor output. (Supported by USPHS Grant 5-RO-EY03167-02).

56.4 CORRELATION OF TASK-RELATED CORTICAL NEURON ACTIVITY WITH EMG RESPONSES PRODUCED BY MICROSTIMULATION AT THE RECORDING SITE. E. M. Schmidt and J. S. McIntosh. Lab of Neural Control, NINCDS, NIH, Bethesda, MD 20205

The relationship between task-related activity of precentral cortical neurons and EMG responses produced by intracortical microstimulation (ICMS) at the recording site was examined in two Macaca mulatta monkeys. They were trained to perform a wrist flexion-extension task to several different target positions under different load conditions that were applied by a torque motor. Chronic EMG recording electrodes were implanted in up to six forearm muscles, along with an electrode in the mesencephalic pyramidal tract (PT) for cell identification. A microdrive base was implanted over the arm region of the contralateral precentral motor cortex. Glass coated Pt-Ir microelectrodes were used for recording and ICMS. Stimulus trains consisted of 17 pulses (0.2ms biphasic) at 400 Hz applied at specific times during the flexion-extension movements. Stimulus current was $\leq$ 20 μA. For simplicity in presentation, cortical cell firing patterns are divided into three groups depending on their correlation with muscle activity. Extensor (E) cells were more active during the extension phase of the task, flexor (F) cells were more active during flexion, while (E-F) cells either increased or decreased activity with both movements. Task related neuron activity was evaluated at 123 sites where ICMS produced either excitation or inhibition in forearm muscles. The total number of cells of each type is listed in the table along with the positively identified PT cells, which are enclosed in parentheses.

TASK RELATED CELL FIRING PATTERNS	E	F	E-F
ICMS → FLEXOR EXCITATION	18(2)	32(4)	7(2)
ICMS → EXTENSOR EXCITATION	18(9)	7(3)	4(2)
ICMS → FLEXOR INHIBITION	16(4)	10(3)	7(3)
ICMS → FLEXOR INHIB. AND EXTENSOR EXCIT.	3(3)	1(1)	

The majority of F cells are correlated with ICMS-produced flexor excitation while the majority of E cells are correlated with ICMS-produced extensor excitation and/or flexor inhibition. At sites where flexor inhibition was produced by ICMS, the majority of cells were type E. A large number of cells do not fit with the expected ICMS response pattern, suggesting an overlap of cortical outflow to different muscles. This is consistent with our previous results (see Neurosci. Abst. 5:1300,1979) where there was considerable overlap of individual forearm muscle representations in the cortex as revealed by ICMS.

56.5 STRUCTURAL AND FUNCTIONAL ANALYSES OF THE ORGANIZATION OF THE MOTOR CORTEX IN MACACA FASCICULARIS. M. Wiesendanger and B.J. Sessle. Institut de Physiologie, Univ. de Fribourg, Switzerland, and Faculty of Dentistry, Univ. of Toronto, Canada.

The details of the fine organization of the motor cortex and its anterior and posterior borders continue to be a matter of debate. We have examined these details in three monkeys with a combination of techniques including cortical microstimulation in the unanaesthetized animal, electrophysiological identification of corticospinal neurons and forelimb muscle and cutaneous afferent inputs to the pre- and postcentral cortex under barbiturate anaesthesia, and HRP labeling of corticospinal neurons by retrograde labeling from the cervical spinal cord; data was subsequently related to cortical cytoarchitecture in the same animals. Approximately 5000 intracortical loci were tested with microstimulation. "Positive" microstimulation effects (<30 μA) could only be demonstrated in areas 4 and in a posterior strip of area 6 (lateral). In area 4, microstimulation thresholds of 1-5 μA were common for evoking EMG responses and movements in distal forelimb muscles. Similar threshold values, although not as common, were also noted for proximal forelimb, trunk and hindlimb muscles. The fingers were represented nearest the central sulcus, with horseshoe-shaped bands of cortex representing progressively more proximal muscles around this central "finger core", in agreement with Kwan et al. (J. Neurophysiol. 41, 1120, 1978). Within these gross patterns, we also found multiple foci evoking a particular movement, indicating that multiple intracortical microzones may exist for each movement.

Electrophysiological identification and HRP labeling revealed that corticospinal neurons were concentrated in area 4, although scattered nests were found postcentrally and in posterior area 6. Under anaesthesia, low-threshold proprioceptive input was abundant in area 3a, but single unit responses were not recorded in area 4 from which positive microstimulation could be demonstrated. These findings indicate that the "excitable cortex" as defined by positive microstimulation effects is limited to regions showing dense concentrations of corticospinal neurons. The negative microstimulation effects from area 3a may be related to the scattering of corticospinal neurons or to the involvement of these neurons not in direct motor control but possibly in corticofugal modulation of somatosensory transmission.

(Supported by the Swiss National Science Foundation and the Canadian Medical Research Council).

56.6 MULTIPLE REPRESENTATION OF FACE, JAW AND TONGUE MOVEMENTS IN MACACA FASCICULARIS AS REVEALED BY CORTICAL MICROSTIMULATION. M. Sirisko*, G.E. Lucier, M. Wiesendanger and B.J. Sessle. Faculty of Dentistry, Univ. of Toronto, Canada M5G 1G6, and (M.W.) Institut de Physiologie, Univ. de Fribourg, Switzerland.

The cerebral cortical motor representation of the face, jaw and tongue has largely been defined by ablation and surface stimulation studies. Despite the recent use of microstimulation methods to provide greater definition of the motor representation of the forelimb in particular, little attempt has yet been made to use similar methods to examine the finer details of face, jaw and tongue motor organization. Thus, the present study was initiated in five unanaesthetized monkeys to characterize this motor organization by examining the simple twitch movements and EMG responses evoked by cortical microstimulation. Stimulus intensities were usually less than 30 μA, and intracortical sites were stimulated every 250 microns or less of microelectrode vertical depth which averaged around 3 mm in precentral cortex and 5 mm in pericentral cortex; histological reconstructions of microelectrode tracks (which were usually 1 mm apart) were subsequently made. The face, jaw and tongue motor representation occurred in the precentral cortex at sites that were more lateral than the forelimb and trunk representations (which were also mapped in the same animals), although a small overlap occurred especially between face and forelimb representations. The face representation tended to form a horseshoe around a central, laterally facing, core of tongue and jaw muscle movements. Contralateral movements predominated, but ipsilateral movements were seen at some cortical sites lateral to the contralateral representation. Thresholds of 1-5 μA were frequently effective in evoking discrete twitches limited usually to a few muscle fibres within an individual muscle. EMG responses at suprathreshold latencies of 7-20 msec were noted. In addition, we found multiple microzones of cortical tissue representing a particular face, jaw or tongue muscle movement. Even within a 2x2 mm square of tissue in which more than 60 microelectrode penetrations were made and sites tested 1.5-2 mm below the cortical surface, a particular movement could be represented more than once. Because of such multiple representation, three clearly distinct areas each representing the face, jaw or tongue were not seen. In accord with recent findings by ourselves and others for the forelimb, this study suggests that there are multiple intracortical microzones representing a particular face, jaw or tongue movement, and that each microzone might be considered part of a radial microcolumn of motor cortex.

Supported by the Canadian Medical Research Council and the Swiss National Science Foundation.

56.7 ACTIVITY OF INDIVIDUAL NEURONS IN MONKEY SOMATOSENSORY CORTEX (SI) REFLECTS BOTH LIMB POSITION AND TENSION. Hugo Solis*, Von Ayre Jennings*, and Yvon Lamour (SPON: M. Abdelmoumene). Lab. of Neurophysiology, NIMH, Bethesda, MD 20205.

Vallbo has shown that frequencies of muscle spindle afferent discharge exhibit marked changes when a subject reverses the direction in which isometric force is being exerted, and Matthews has shown that spindle afferent inputs modify sense of limb position. Given these two observations, it seemed to us that a reversal in direction of isometric contraction might modify discharge frequencies of SI neurons in regions classically thought to subserve position sense. This hypothesis was examined by determining the interaction between limb position and muscle tension in SI neurons of a monkey performing active isometric contractions.

Single unit activity was recorded from SI in both hemispheres of a monkey (Macaca mulatta) performing supinating or pronating isometric contractions (.1 Nm) against a handle in two positions (80°, 100°) using the paradigm of Jennings et al. (this vol.). Of 203 task-related SI neurons, 56 displayed resting discharge frequencies which were greater at one or the other handle position. For a 20° shift in position (force held constant) the mean change in discharge frequency was 9.8 Hz. Each of these position sensitive cells was reciprocally related to the direction of exerted force, i.e., they were more active for one force direction and less active for the other. These changes in force (position held constant) resulted in a mean alteration in discharge frequency of 17.2 Hz. Furthermore, the position and force sensitivities of most cells were congruent: cells more active in a supinated position were also more active with supinator force and vice versa. Thus, 91% of the supination position sensitive neurons were more active with supinating force and 79% of the pronation position sensitive cells were more active during pronating force. Most neurons of this type (i.e., congruent for tension and position) were found in two discrete SI zones, one identified as area 3a (15 of 42 task-related units) and the other as area 2 (24 of 61). Other parietal fields appeared to have fewer (area 1, 4 of 44; area 5, 5 of 17) or no (area 3b, 0 of 39) cells of this type.

These results indicate that position sensitive SI neurons also reflect muscle tension, and that inputs to SI from muscle receptors or central motor systems such as the motor cortex may play a role in the mechanisms by which limb position is signaled by SI neurons.

56.8 PREMOTOR NEURONS IN THE POSTARCUATE CORTEX OF THE MONKEY. Von Ayre Jennings*, Yvon Lamour and Hugo Solis* (SPON: M. Herkenham). Lab. of Neurophysiology, NIMH, Bethesda, MD 20205.

The cortical region posterior to the arcuate sulcus has been shown by others to receive inputs from visual association cortex (area 7) and to project to precentral motor cortex (MI), suggesting that it is involved in the visual guidance of limb movement. With this in mind, we compared the activity of neurons in the region posterior to the genu of the arcuate sulcus (PAr) with MI neuronal activity during active arm movements triggered by visual cues.

Single unit activity was recorded in a monkey (Macaca mulatta) performing pronating or supinating arm movements on the basis of a visual display. The task involved two modes: isotonic displacements (20°) and isometric contractions (.1 Nm). In the isotonic mode the visual display was controlled by arm position while in the isometric mode the display was controlled by force. The task was one involving compensatory tracking, with reward being given for the display being held on center. Shifts in the monkey's arm position or force were triggered by a shift in the position of the illuminated lamp, thus requiring the monkey to make an isotonic or isometric contraction in order to return the "on" lamp to the center position.

31 PAr cells were found to form a distinct functional group. After a visual cue (VC), these neurons, termed VC cells, were characterized by a change in discharge frequency which was time-locked to VC onset. In contrast, the onset of activity in 313 extensively studied MI neurons was better correlated with movement onset than with the visual cue. Furthermore, in response to cues triggering either isotonic movements or isometric contractions, the onset of VC activity preceeded that of MI activity by an average of 67 msec. This early activity was always specific for one particular cue direction. For example, VC neurons activated by a cue for an isotonic supinating displacement were also activated by the cue for exertion of an isometric supinating force and showed decreased or no change in activity following a cue for a pronating movement or contraction.

These results provide evidence for a functionally distinct premotor zone. During a visually-triggered task, PAr neurons are characterized by directionally specific and short latency stimulus-bound activity. Since a relation to eye movements or contractions of proximal muscles was ruled out by appropriate tests, the results suggest that PAr neurons may be links in a pathway by which central motor commands reach MI following the occurrence of visual stimuli which require a motor response.

56.9 FUNCTIONAL CHARACTERISTICS AND SEGREGATION OF CUTANEOUS AND NON-CUTANEOUS NEURONS IN MONKEY PRECENTRAL MOTOR CORTEX (MI). Yvon Lamour, Von Ayre Jennings*, and Hugo Solis*. Lab. of Neurophysiology, NIMH, Bethesda, MD 20205.

The present report contrasts the behavior of MI neurons which receive cutaneous versus non-cutaneous inputs in a monkey that grasped a handle and performed pronating or supinating isotonic movements (20°) or isometric contractions (.1 Nm) in a compensatory tracking task. Reward required that the central lamp of a visual display be illuminated, and the display was controlled by force feedback in the isometric mode and position feedback in the isotonic mode. Stimuli were delivered to the handle during steady holding via a servo-controlled torque motor and consisted of changes in handle position (position pulses, 50 msec, 10° or ramp displacements, 150 msec, 20°).

Peripheral receptive fields could be determined for 154 MI neurons; 53 were classified as receiving cutaneous inputs and 101 as receiving non-cutaneous inputs. Cutaneous units had receptive fields located on the fingers, hand, or arm and were concentrated in the bank of the central sulcus (MI/c) while neurons responding to non-cutaneous inputs were concentrated in more rostral regions (MI/r). During steady holding, MI/c units showed little sensitivity to the direction of handle pulses or ramps in contrast to MI/r units which were usually directionally sensitive. Similarly, cutaneous neurons in MI/c were activated during both directions of active isotonic movement or isometric contraction but displayed little sustained activity related to steady force or position. MI/r cells, on the other hand, were usually reciprocally related to the direction of movement and isometric force and, in many cases, their activity was also related to maintained position and force. Finally, following the visual cue triggering an active displacement or contraction, the change in discharge frequency of cutaneous MI/c neurons lagged that of MI/r cells by an average of 61 msec and preceeded the first change in position or force by an average of only 42 msec.

These results confirm previous demonstrations of submodality segregation of cutaneous and non-cutaneous inputs to separate MI regions. More importantly, the results show MI/c neurons to be activated later in visually triggered motor behavior and less related to limb position and exerted force than cells in MI/r. This is consistent with the suggestion of Strick and Preston that MI consists of at least two functional motor areas. The present observations indicate that MI/r plays a role in initiation of motor activity and the maintenance of limb position and force, while MI/c is concerned with the control of movements under tactile guidance.

56.10 THE ACTIVITY OF CLOSELY SPACED MOTOR CORTICAL PYRAMIDAL TRACT PROJECTING NEURONS DURING LOCOMOTION. C. Palmer, W.B. Marks, M.J. Bak and G. Pedersen*. Lab of Neural Control, NINCDS, Bethesda, M.D. 20205.

An attempt has been made to examine the functional significance of the afferent-efferent columnar organization of the feline motor cortex in the awake, freely moving animal. In 4 cats groups of closely spaced (75μ-200μ) microelectrodes were chronically implanted in the motor cortex (Palmer et al., Neurosci. Abstr. 5:1281, 1979). Pyramidal tract (PT) neurons were identified by antidromic invasion from the medullary pyramids, obeying these criteria: constant latency, following stimulation at 100Hz, and cancellation of the antidromic response by collision with an orthodromic action potential. Muscles were chronically implanted with EMG electrodes and length gauges across the elbow joints.In one cat, 28 PT forelimb motor cortical neurons were recorded during locomotion, (latencies to pyramidal stimulation 0.94 ms to 2.94 ms) from 4 closely spaced electrodes. Two of them (A,B,)were 200μ apart. The somatosensory receptive fields were determined for each neuron with natural stimulation in the awake animal: 20 (71%) had forepaw cutaneous receptive fields, 3 (11%) had afferent input from 'deep' receptors (elbow and toe joints) and 5 (18%) had undetectable fields. Microstimulation was carried out at each electrode using 50 ms trains of .2 ms pulses at 400 Hz . Thresholds ranged from 10 to 30μamp. 23 (82%) of the PT neurons were modulated during locomotion: 11 decreased their firing rate during the end of swing and the begining of stance while the remainder increased their firing rate in relation to the movement. Of these, 9 units had bursts of activity during the flexion part of swing, 2 units had two bursts of activity during the begining and end of swing and 1 unit during the end of swing. 18 of these PT neurons were recorded at electrodes A and B thus yielding information about cortical function in relation to columnar organization. At A 10μamp stimulation gave short latency (20 ms) EMG responses in Pronator teres (PrT), in electrode B 200μaway, stimulation (10 amp) activated PrT and Extensor Carpi Radialis (ECR) while inhibiting Flexor Digitorum Profundus (FDP) with latencies of 20 to 30 ms.In locomotion PrT and ECR are active during swing while FDP is active in stance. At electrode A 8 of the 11 PT units recorded had increased activity during the swing phase of locomotion. At electrode B, with a more complex microstimulation response (ie the activation of flexor muscles and the inhibition of an antagonist) 7 of the 8 PT units had decreased activity as described above. It appears from this small sample of data that the motor modulation of PT neurons may be organized in a 'columnar' fashion.

56.11 AVERAGED MOTOR POTENTIALS ASSOCIATED WITH VOLUNTARY HAND MOVEMENTS WITHIN THE HIPPOCAMPAL FORMATION OF THE MONKEY. Joseph C. Arezzo* and Herbert G. Vaughan, Jr. Dept. of Neuroscience, Albert Einstein Coll. of Med., Bronx, N.Y. 10461.

In rodents and dogs there are characteristic alterations in the frequency on the EEG within the hippocampal formation coincident with motor behavior described as voluntary, as opposed to consummatory or reflexive. The electrical rhythms in the hippocampus of primates, including man, differ substantially from those in lower mammals and their relationship to movement is not apparent. Hippocampal involvement in motor control in the primate is, however, suggested by lesion and stimulation studies and by the recent anatomical demonstration of a direct projection from the subiculum to portions of the frontal cortex in the monkey (Rosen and Van Hoesen, 1977).

Motor potentials (MP) and multiple unit activity (MUA) associated with stereotyped self-paced wrist extension movements were recorded within the hippocampal formation of three monkeys. The rectified EMG was used to synchronize the averaging process. Potentials were recorded from a series of movable depth electrodes introduced vertically through a 10 x 20mm matrix to allow for the analysis of the intracranial field potential gradients.

MPs within the hippocampal formation consist of a large amplitude (300 uv) complex series of deflections which begin between 150 and 200 msec prior to the EMG onset and continue for a period of more than 1 sec after the movement. The field potential gradients are characteristic of an open field generator and the polarity of several components invert across the pyramidal layer of CA3. Volume conducted MPs can be traced to the dorsolateral surface of the brain where they interact with locally generated activity. There are no slow potential changes in the hippocampal MP equivalent to the N1 component (readiness potential) seen in the cortex, however the onset of antecedent phasic activity in the hippocampus precedes that seen in the precentral gyrus. MUA is recorded concurrent with the antecedent portions of the hippocampal MP and continues for approximately 150 msec after termination of contraction. The late positive MP component is not associated with changes in MUA.

The EEG recorded in the vicinity of the hippocampal formation is dominated by large amplitude low frequency activity (2-3 Hz) on which is superimposed low voltage fast activity. During a voluntary movement the low frequency rhythm is suppressed and the amplitude of the EEG is reduced. These changes precede the onset of the EMG by a variable period which can be as much as 500 msec. (Supported by grant MH 06723 from the USPHS).

56.12 SPIKE-TRIGGERED AVERAGES, ARTIFACTS, AND ARTIFICES. C.C. Boylls, Jr. Dept. Physiology, Medical School, Univ. Bristol, Bristol BS8 1TD, U.K.

The correlation technique variously known as spike-triggered or post-spike averaging is often employed to detect postsynaptic influences (upon EMG's, PSP's, or other spike trains) of the triggering-spike source (neuron or nerve fiber). It appears to be commonly accepted (e.g., ref. 1) that the method both resurrects postsynaptic effects from noisy backgrounds and allows accurate measurement of latencies. Certain authors (2) have additionally assumed that signs (facilitation, depression) and durations of effects can be correctly estimated.

In the present work it first is shown that even for simple linear, time-invariant postsynaptic processes, spike-triggered averaging per se cannot yield reliable indications of latencies, signs, or durations of effects, and can actually introduce, rather than reduce, noise in such measurements. These difficulties, which can be severe, arise from the nonuniform representation of frequencies within typical triggering spike trains. Without prior knowledge of the postsynaptic process, accurate results are assured only if the triggering train has "white noise" frequency characteristics. As demonstrated in the remainder of this study, however, it may be possible in many instances to "whiten" the frequency representation of triggering spike trains by computing the spectral densities of such trains and subsequently employing them to weight the results of spike-triggered averages (an application of the Wiener-Lee theorem). The considerable improvements so obtained in spike-triggered estimates of (linear) postsynaptic processes are illustrated. Extensions to the nonlinear situation are under inyestigation.

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2 Kirkwood, P.A., Sears, T.A., Nature 252 (1974) 243-244.

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56.13 SOME DIENCEPHALIC PROJECTIONS OF THE FRONTAL LOBE IN CAT. E. Ramon-Moliner. Dept. of Anatomy. Sherbrooke University, School of Medicine, Sherbrooke, Quebec J1H 5N4. Canada.

In a previous communication (Abst. Soc. Neurosci. 5:119, 1979) the diencephalic projections of fibers leaving the pericruciate gyrus in the cat were determined by means of multiple injections of ^{35}S methionine in that gyrus. The present work is aimed at establishing the main differences between the projections reported at that time and the projections from those regions of the frontal cortex which do not include the pericruciate gyrus. ^{35}S methionine was injected (20-30 uC per injection) in the following sites: (1) the rostral gyrus proreus; (2) the caudal gyrus proreus; (3) the anterior extremity of the anterior suprasylvian gyrus (rostral to the gyrus coronalis); (4) the portion of the anterior suprasylvian gyrus lateral to the gyrus coronalis. All these sites shared with the pericruciate gyrus a varying degree of projection to the caudate nucleus and the thalamus. However, there were major differences with the pericruciate projections. The gyrus proreus projected to the medial portion of the head of the caudate nucleus, to medially located thalamic centers (ventralis medialis, centralis lateralis, reuniens, and dorsalis medialis). In addition, its rostral portion projected to the nucleus parafascicularis and the dorsolateral central gray matter of the mesencephalon, whereas its caudal portion projected to the lateral preoptic area, the hypothalamus and the ventral tegmental area. A projection to the rostral gyrus cinguli was also found. The anterolateral frontal lobe projected to the lateral portion of the head of the caudate nucleus although more scarcely than the pericruciate gyrus. It also projected to the claustrum and to the putamen. The thalamic projections included the lateral portion of the n. ventralis anterior, the lateral dorsalis medialis, the centralis medialis and part of the zona incerta. The cortical region lateral to the gyrus coronalis (anterior suprasylvian gyrus) projected to lateral thalamic regions: the lateral portions of the nuclei ventralis lateralis and ventralis posterolateralis. It also projected to the nucleus interstitialis. The thalamic projection of this cortical area shared with the pericruciate gyrus the presence within the lateral complex of a surprisingly well outlined ovoid field devoid of cortical afferents.

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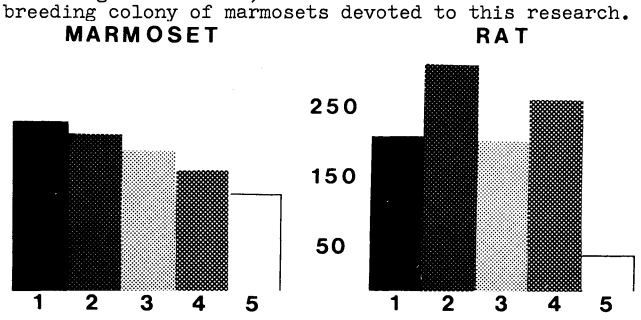
56.14 MARMOSET MOTOR CORTEX-A CYTOLOGICAL STUDY. T.J.TOBIAS. Univer. Paris VI, 45 rue St. Peres, Paris 75006 France.

In contrast to other monkeys, the marmoset possesses an agyric motor cortex. A recent study (Tobias, T.J. "Towards Analysis of Marmoset Motor Cortex" in press,) documents the existence of a single representation of distal forelimb musculature as described for Macaque by Kwan et al. (J. Physiologie 74:231, 1978.) Marmoset ability to perform an asymetrically reinforced, single digit flexion-extension, delayed alternation paradigm suggests that, in spite of relative cortical simplicity the South American simian is capable of motor behaviour comparable in complexity to tasks solved by Macaque. In brief, marmoset is ideally suited for intracellular studies of primate motor cortex. But, since Callitrichidae are endangered/vulnerable, it is preferrable to employ any other lissencephalic 300g mammal capable of independent digit movement. By comparison with the rat, however, it is clear that ethological differences between primates and rodents appear as well in motor cortex organization. Observations include: more myelin in monkey grey matter, even in layer I--which is 10% thinner than in rat; 25% thicker grey matter in monkey, containing, however, fewer cells than the rat; Marmoset possesses more large (35μ) cells in layer V, and has a much thicker layer VI, with large bundles of myelinated axons coursing superficially and laterally in both laminae. Graphs represent sum of neurons (vertical) from 3 animals within a 300μ strip from hand area in each of 5 zones (horizontal) of 500μ, beginning with Pia. These data are thus disharmonious with the concept of substituting a nonsimian, and indicate the need for a breeding colony of marmosets devoted to this research.



56.15 GABA NEURONS OF MONKEY MOTOR AND SENSORY CORTEX: AN IMMUNOCYTOCHEMICAL STUDY. C.R. Houser, J.E. Vaughn, E.G. Jones and S.H.C. Hendry. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010 and Department of Anatomy and Neurobiology, Washington University, St. Louis, MO 63110.

GABA appears to be an important inhibitory neurotransmitter in the neocortex and is believed to be used by certain local circuit neurons. A knowledge of the location and morphological characteristics of these neurons is particularly germane in higher mammals where local circuit neurons are most numerous and presumably play a significant role in cortical function. We have identified GABA neurons in the primary motor and somatosensory cortical areas of monkeys (M. fascicularis) using an immunocytochemical method for the localization of the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD). Prior to immunocytochemistry, colchicine treatment was used to increase the GAD content of neuronal somata.

GAD-positive staining of somata and more proximal dendrites has been obtained for a large number of non-pyramidal neurons distributed in all cortical layers within both motor and sensory areas. The GABA somata in layer I are small (~10 μm in diameter) and show a limited amount of dendritic staining. GABA cell bodies in all other layers are morphologically heterogeneous. They range in size from small to large and exhibit a variety of proximal dendritic patterns, including apparent multipolar, bipolar and bitufted forms. A characteristic type of GABA neuron found within layers III-V is of large diameter (20-30 μm) and exhibits a multipolar dendritic pattern. The characteristics of this cell type correspond closely to those of cortical basket cells as described by previous investigators. These earlier studies have shown that basket cells form axonal arborizations around the somata of pyramidal neurons. In the present material, numerous GAD-positive puncta corresponding to GABAergic axon terminals are distributed over the somata of large and giant pyramidal cells. Thus, previous observations in conjunction with our own suggest that basket cells provide a GABAergic innervation of pyramidal cell bodies and, therefore, would be capable of exerting a powerful inhibitory control over projection neurons of motor and sensory cortex. The results of this study indicate that GABA neurons are numerous within primate sensorimotor cortex and appear to be of several morphological types. While the findings for motor and sensory areas are qualitatively similar, current efforts are directed toward determining if there are quantitative differences in the laminar distribution of GABA neurons between these two functionally distinct areas and toward a further definition of the various GABAergic cell types. (Supported by grants NS12116 and NS10526.)

56.16 SUPRASYLVIAN GYRUS PROJECTIONS TO MOTOR AREA IN THE CAT CEREBRAL CORTEX STUDIED BY RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE R. S. Babb, K. D. Larsen, R. S. Waters and H. Asanuma. Rockefeller University, New York, N.Y. 10021

The distribution of horseradish peroxidase (HRP) labelled neurons in the anterior suprasylvian gyrus (area 5) was investigated as part of a broader study of the afferents of the motor cortex (area 4) in the cat cerebral cortex.

Nine adult cats were used in this study. In each animal two, three or four injections of 50% HRP (Sigma VI) in normal saline, each from 0.02 to 0.05 μL, were placed close together and about 1 mm deep bilaterally or unilaterally in the lateral pericruciate region of cortical area 4γ, a region from which movements of the front paw can be elicited by stimulation. After a survival time of about 44 hours the brain of each animal was perfused and processed with tetramethyl benzidine according to the method of Mesulam (J. Histochem. Cytochem. 24: 1273, 1976).

The results can be summarized as follows: (1) All the injections gave rise to cell bodies of neurons in the ipsilateral anterior suprasylvian gyrus (area 5) labelled with the blue HRP reaction product. (2) Two clearly separated regions, one in the depth of the ansate sulcus with cytoarchitecture corresponding to sub-area 5a defined by Hassler and Muhs-Clement, and the other on the surface of the suprasylvian gyrus corresponding to sub-area 5b, were both found to contain labelled cell bodies. (3) No labelled cell bodies were found in the region between these two labelled zones described above. (4) The labelled cell bodies were typically medium-sized, pyramidally-shaped and situated in lamina III in loose clusters with densities of about 16 and 7 cells per mm^2 in sub-areas 5a and 5b respectively. (5) Close to the sites of injection in the underlying white matter, compact fascicles of HRP filled fibers were observed. (6) The somata were only moderately labelled compared to darkly labelled cell bodies observed in the thalamus. (7) In addition to area 5, areas 17, 18 and 19 of visual cortex were examined and found to contain no labelled cells.

From these observations it is concluded that projections from area 5 to the ipsilateral area 4γ originate from neurons with medium-sized pyramidal-shaped cell bodies situated in lamina III. The cell bodies are loosely clustered in two groups, one situated in sub-area 5a and the other in sub-area 5b, and both groups sending axons to converge on the lateral pericruciate region of area 4γ.

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56.17 ABLATION OF FELINE SENSORIMOTOR CORTEX DECREASES GLUTAMATE UPTAKE IN THE PROJECTION AREAS OF CORTICOSPINAL TRACT. A.B. Young, M.B. Bromberg, and J.B. Penney, Jr.* Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

Despite recent advances in defining the neurotransmitters of specific pathways, the neurotransmitter(s) of the corticospinal tract is (are) still unknown. We have recently provided strong evidence in the rat that cortical inputs to ventrolateral nucleus (VL) of thalamus and red nucleus (RN) are glutamatergic (Bromberg, et al: Neurology 30: 396, 1980). Since these projections are thought to be collaterals of the corticospinal tract, we have investigated the problem further in cats.

In a series of cats, we have performed unilateral extensive ablations of pericruciate (sensorimotor cortex) under Ketamine anesthesia. Two weeks after the operation, animals are again anesthetized as above, and their brains exposed. The cats are then sacrificed by spinalization. The brains and spinal cords are put in ice cold 0.32 M sucrose and 1 mm punches taken from ventrolateral nucleus of the thalamus, caudate nucleus, red nucleus, pons, medullary reticular formation, dorsal column nuclei, and from Rexed layers IV-VIII of cervical, thoracic and lumbar spinal cord. Punches were homogenized in 0.32 M sucrose with a Potter-Elvehjem homogenizer and spun at 20,000 x g for 20 minutes. The resultant pellets were resuspended in sucrose and uptake performed as previously described (Young, et al: Brain Res. 73: 1, 1974) at a concentration of 1×10^{-7}M.

Glutamate high affinity synaptosomal uptake is decreased ipsilateral to the lesion as far as the level of the dorsal column nuclei after which uptake is decreased contralateral to the lesion. The decrease in uptake reflects a decrease in the number of glutamate transport sites not in the affinity of the site. This loss of transport sites is most likely due to loss of presynaptic glutamatergic corticospinal axon terminals. Supported by USPHS grants NS00464-01, NS00420-01 and NS15140-02, The United Cerebral Palsy Research Foundation and Michigan Memorial Phoenix Project grant 567.

57.1 SPECIES VARIATION IN THE DOPAMINE CONTENT OF CEREBRAL BLOOD VESSELS. J.F. Reinhard, Jr. and M.A. Moskowitz. Lab. of Neural and Endocrine Regulation, MIT, Cambridge, MA 02139.

A role for dopamine (DA) in the pharmacological regulation of cerebral blood flow has been shown by in vitro studies that measure the ability of this molecule to contract or relax cerebral blood vessels, or by in vivo studies which record changes in cerebral blood flow after DA administration. The notion that dopamine participates in the physiological regulation of this tissue would be supported by finding relatively large amounts of dopamine in the walls of cerebral blood vessels. Thus we have measured the content of catecholamines within large and small blood vessels from rat, cat, ferret, dog, cow, and human brains.

The middle, anterior cerebral, and basilar arteries were dissected free of the surrounding pial tissue. Microvessels were isolated using a density gradient method, and choroid plexus was isolated from the lateral ventricles. The tissues were homogenized in 0.1 M perchloric acid and the catecholamines were isolated on small alumina columns. Catecholamines were further separated and quantitated using high pressure-liquid chromatography with electrochemical detection.

Noradrenaline (NA) was detected in all of the vessel preparations studied. In cerebral vessels from rat, dog, and man, DA levels were 10-15% of the concentrations of NA. Feline and bovine vessels, however, contained relatively high levels of DA which were equal to 30 and 100% of the vessel's NA, respectively. The proportion of DA to NA in these tissues is considerably higher than we have observed for tissues receiving exclusively a noradrenergic innervation (i.e. pineal), suggesting that DA might be present outside of noradrenergic nerve terminals (e.g. in mast or endothelial cells, or in nonadrenergic nerve terminals). Thus in cat and cow brains, dopamine is contained within cerebral blood vessels where it might act to modify cerebral blood flow.

57.2 EFFECTS OF NICOTINE ON CARDIAC AND PULMONARY ARTERIES OF THE RAT. J.M. Sullivan*, N.A. Connors*, M.W. Rana*, and C.L. Lawson* (SPON: L. Massopust). Dept. of Anatomy, St. Louis University School of Medicine, St. Louis, MO 63104.

We have reported (1980 AAA Abstracts, Pp. 184-185) that nicotine increased cardiac arterial medial mass in the rat. The purpose of this study was to demonstrate the morphological effects of nicotine on pulmonary arteries as compared to the nicotinic effects on rat cardiac arteries. In general, the cardiovascular and pulmonary vascular responses to nicotine parallel those that accompany stimulation of the sympathetic nervous system. The stimulation of sympathetic ganglia and the adrenal medulla, together with the release of catecholamines from sympathetic nerve endings account for the similarity. Forty-four Sprague-Dawley rats (22 males & 22 females) were divided into four groups. Group I received 0.5 mg/kg of nicotine in one injection per day. Group II received 1.0 mg/kg in two injections per day. Group III received 1.5 mg/kg in three injections per day. Group IV received 1.0 ml of normal saline per day and served as the control. After sixty days, thirty-six animals were anesthetized with pentobarbital (50-60 mg/kg, i.p.) perfused with 10% buffered neutral formalin, and serial sections of hearts and lungs were processed for light microscopic histological studies. Sections of the hearts and lungs of the other eight animals were freeze-dried and processed for fluorescence histochemical localization of catecholamines. Cryostat sections (10 μm in thickness) were processed and examined for fluorescence. By means of the point-counting technique of Chalkley under a constant magnification of 400X, relative cross-sectional areas of intimal nuclei, media, and medial nuclei were determined for arteries in each heart and lung. The general histological stain used was H&E, however, in some cases trichrome stains were used to assure that neither adventitia nor intima was being included in measurements of arterial media. The numbers of intimal and medial nuclei were also counted in each artery. Light microscopic studies of the cardiac and pulmonary arteries were compared. Fluorescence histochemistry indicated an increase in catecholamine fluorescence. (Supported by Life Insurance Medical Research Fund #G-70-8).

57.3 EVIDENCE FOR A CENTRALLY MEDIATED SYMPATHOLYTIC ACTION OF POST-SYNAPTIC α-ADRENERGIC RECEPTOR ANTAGONISTS. Robert B. McCall and Stephen J. Humphrey.* The Upjohn Co., Kalamazoo, MI 49001.

Prazosin is a unique α-adrenergic receptor antagonist which possesses a high degree of selectivity for postsynaptic α-receptors. Unlike classic α-receptor antagonists, prazosin decreases mean arterial pressure (MAP) without causing an accompanying increase in heart rate (HR) or plasma renin activity (PRA). The present study was designed to determine if the lack of reflex increase in HR and PRA results from a central sympatholytic action of prazosin. Intravenous administration of prazosin (50 μg/kg) to anesthetized, baroreceptor denervated cats resulted in a rapid fall in MAP (-42%) and no significant change in HR. Prazosin administration was also associated with a prolonged inhibition of sympathetic nervous discharge (SND) recorded from the external carotid or splanchnic nerves. SND was significantly reduced within 5 minutes of the administration of prazosin and remained depressed throughout the 2-hour observation period. SND was maximally inhibited (-41%) 1 hour after the drug was given. Finally, prazosin significantly attenuated the pressor effect of intravenous norepinephrine. In contrast, administration of vehicle to control animals failed to alter MAP, HR, or SND. Prazosin also reduced MAP (-39%) and SND (-39%) in baroreceptor intact cats and failed to significantly alter HR. In contrast, phentolamine (1.0 mg/kg, i.v.; an α-antagonist which fails to enter the brain) decreased MAP (-30%) but increased SND (+74%) in baroreceptor intact cats. Phentolamine significantly increased HR when compared to prazosin treated animals. In a final set of experiments, the effects of WB-4101 (0.5 mg/kg, i.v.; an α-antagonist which possesses a high degree of selectivity for postsynaptic α-receptors) on MAP, HR, and SND were studied. Like prazosin, WB-4101 produced a significant decrease in MAP (-63%) and SND (-45%) in baroreceptor denervated cats. WB-4101 also produced a significant bradycardia. These data indicate that the sympatholytic action of prazosin and WB-4101 results from a centrally mediated reduction in SND as well as a peripheral blockade of α-adrenergic receptors. The relative importance of either sympatholytic component in the hypotensive action of these agents was not determined. However, the centrally mediated reduction in SND may explain the lack of tachycardia and PRA increase during the hypotension produced by prazosin. Finally, since prazosin and WB-4101 have been shown to selectively block postsynaptic α-adrenoreceptors in the central nervous system, these data suggest that central noradrenergic neurons normally facilitate transmission in central sympathetic pathways.

57.4 COMPARISON OF THE EFFECTS OF HUMAN-RAT (Ile^5 A II) AND BOVINE (Val^5 A II) ANGIOTENSINS ON PLASMA CATECHOLAMINES IN PITHED RATS. Robert C. Speth, Mahesh C. Khosla*, Michelle M. Spech*, and Carlos M. Ferrario. Research Division, Cleveland Clinic Foundation, Cleveland, OH 44106.

With a view towards finding a difference in the ability of human-rat angiotensin (Ile^5 A II) and bovine angiotensin (Val^5 A II) to release catecholamines, equipressor doses of these angiotensins were infused into two separate groups of pithed rats. A third group of pithed rats received isotonic saline vehicle only. Rats (270±15 g) were anesthetized with methoxyflurane, tracheotomized, pithed by insertion of a steel rod through the orbit and into the spinal cord and respired mechanically. The femoral artery was cannulated for blood pressure and heart rate monitoring, the femoral vein was cannulated for drug infusion and the carotid artery was cannulated for blood sampling. One hour after pithing, rats were infused with either saline, Ile^5 A II (72 ng/min) or Val^5 A II (72 ng/min) at a rate of 0.2 ml/min until blood pressure stabilized at an elevated level, at which time a sample of blood was removed from the carotid artery. Plasma catecholamines were determined by radioenzymatic assay using catechol-O-methyl transferase and ^{3}H-S adenosyl methionine. Both Ile^5 A II and Val^5 A II increased mean arterial pressure (MAP) by 84% and 80%, respectively, from the average baseline value of 55 mm Hg. Saline infusion produced an insignificant, 5% increase in the MAP. None of the infusions significantly altered heart rate from the basal value of 316 beats/min, indicating a complete loss of central nervous reflex heart rate control after pithing. Plasma dopamine concentration did not differ significantly among the three groups. Saline infused animals had a mean concentration of 175±35 pg of dopamine HCl/ml of plasma. The mean plasma epinephrine concentrations in all three groups were extremely low (< 30 pg/ml) which was below the reliable sensitivity of this assay. The mean plasma norepinephrine (NE) concentration was significantly elevated in Ile^5 A II infused rats by 81% and 58% over saline and Val^5 A II infused rats, respectively. Mean plasma NE concentration in Val^5 A II infused rats did not differ significantly from that of saline infused rats, that value being 292±37 pg/ml. These results indicate that human-rat angiotensin elevates plasma NE concentration but that bovine angiotensin does not. These differential effects on plasma NE concentration may indicate a difference in the mechanisms whereby these angiotensins produce their pressor effects.

57.5 MECHANISM BY WHICH AMPHETAMINE CAUSES MYDRIASIS IN THE RAT. Harry Klemfuss* and Lewis S. Seiden (SPON; James B. Lucot) University of Chicago, Chicago, IL 60637.

The mydriatic action of amphetamine is commonly considered one of the sympathomimetic effects of this drug. We have examined how amphetamine causes pupillary dilation in unrestrained, conscious rats by using a photographic technique. This response is not mediated by sympathetic pathways since the mydriatic effect of 1 mg/kg d-amphetamine is not altered by chronic sympathetic denervation or by subsequent adrenalectomy. Eyedrops containing physostigmine prevent the mydriasis, indicating that parasympathetic pathways are involved. To investigate the role of catecholamines in amphetamine mydriasis, we attempted to prevent it by pretreating rats with specific receptor blockers. Yohimbine, which preferentially blocks presynaptic α-noradrenergic receptors, partially antagonized the mydriasis. Blockade of both pre- and post-synaptic receptors with phenoxybenzamine eliminated the mydriasis completely, which suggests that both kinds of receptors may be involved. Dopaminergic receptors do not seem important in this response, since neither pimozide nor sulpiride, which block different subpopulations of dopamine receptors, had any effect on the mydriasis. Haloperidol caused a slight pupillary constriction at doses of 0.4 or 2 mg/kg, but only the higher dose prevented amphetamine mydriasis. Since the lower dose effectively blocks dopamine receptors while high doses of haloperidol also affect adrenergic systems, it appears that noradrenergic but not dopaminergic antagonism is responsible for haloperidol's effect. Additional evidence for the importance of α-noradrenergic receptors comes from our observation that the α-noradrenergic agonist clonidine also causes a dose-related mydriasis in conscious rats. We propose that amphetamine mydriasis in the rat is mediated primarily be central α-noradrenergic systems on parasympathetic pathways, since α-noradrenergic but not dopaminergic agents block the mydriasis, and physostigmine but not sympathectomy prevents amphetamine mydriasis. (Supported by PHS MH 99919; MH 14274).

57.6 EFFECTS OF DRUGS INTERFERING WITH METABOLISM OF OCTOPAMINE IN SPONTANEOUS HYPERTENSIVE RATS. D. Casset-Senon*, B. Delbarre, G. Delbarre . Lab. Chir. Exp., Fac. Méd., 37032 TOURS CEDEX FRANCE.

We have demonstrated that p.octopamine injected in lateral ventricle and cisterna magna of spontaneous hypertensive rats (SHR) induces dose-dependant falls of blood pressure (BP) (IRCS, Med. Sci., 1980, 8, 23).

We have investigated in SHR the cardiovascular action of octopamine precursors and the action of drugs interfering with the metabolism of this amine. The administration of pargyline (20 mg/kg i.p.) which increases brain octopamine levels, results in a significant reduction of BP in SHR. This decrease is greater after administration of tyrosine (30 mg/kg i.p.) and tyramine (5 mg/kg i.p.). Similarly drugs known to inhibit activity of phenylethanolamine N-methyltransferase (PNMT) and to increase brain octopamine level such as SKF 64139 (7,8 dichloro-1,2,3,4-tetrahydroisoquinoline hydrochloride) and DCMB (2,3-dichloro-α-methyl-benzylamine hydrochloride), decrease BP in SHR.

In unanaesthetized cats, p.octopamine (30 µg/kg), injected in lateral ventricle, decreases significantly BP. This effect is not antagonized by piperoxane (4 mg/kg i.p.), yohimbine (0.2 mg/kg i.p.) and cyproheptadine (0.2 mg/kg i.p.).

These results provide experimental evidence that octopamine may be involved in central blood pressure regulation. Moreover the receptors of octopamine seem independant of those of noradrenaline, adrenaline and serotonin.

57.7 MECHANISMS INVOLVED IN CENTRAL EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$. G. Feuerstein*, C. J. Helke, J. Saavedra, D. M. Jacobowitz and I. J. Kopin. (Spon: A. M. Laties). Laboratory of Clinical Science, NIMH, Bethesda, MD 20205.

Injection of prostaglandin (PG) $F_{2\alpha}$ into the lateral ventricle (i.c.v. 10 µg/rat in 5 µl) of lightly anesthetized rats (0.7% halothane in oxygen) elicited increases of: blood pressure (BP) + 40 mmHg, heart rate (HR) + 150 beats/min, respiratory rate (RR) + 34 resp/min and rectal temperature (RT) up to 39.8°C. Intravenous injection of the same dose of $PGF_{2\alpha}$ had only a transient (2-3 min, 10-20 mmHg increase) effect on BP. The increases of BP and HR following $PGF_{2\alpha}$-i.c.v., were rapid but tapered off within the experimental period (80 min), whereas the RR and RT responses developed gradually but were sustained throughout the entire experiment. In $PGF_{2\alpha}$-i.c.v. rats, plasma norepinephrine (NE), radioenzymatically assayed, increased 4 fold - up to 930 pg/ml ($p<0.001$, $n = 10$), closely correlating with the BP and HR responses. Plasma epinephrine did not change following $PGF_{2\alpha}$-i.c.v. NE content in brainstem nuclei: A_1 and A_2 + nucleus tractus solitarius (NTS), were reduced by 45% and 31% respectively ($p<0.05$); in the A_2+NTS region there was also a 56% decrease in dopamine concentration ($p<0.05$). NE, epinephrine and dopamine levels in the dorsomedial and the ventromedial hypothalmic nuclei were not affected by $PGF_{2\alpha}$-i.c.v. In hexamethonium (10 mg/kg, i.v.) pretreated rats, BP and HR were still increased over control values by $PGF_{2\alpha}$-i.c.v., at a time when complete blockade of $PGF_{2\alpha}$-induced NE release prevailed. The RR and RT responses to $PGF_{2\alpha}$ were also attenuated by hexamethonium. The residual BP and HR responses to $PGF_{2\alpha}$-i.c.v. in the hexamethonium-treated rats persisted even in bilaterally nephrectomized (acute) rats or hypophysectomized (transpharyngeal, 3 days prior to the experiments) rats.

In conclusion, the data presented demonstrates that $PGF_{2\alpha}$, by a central action, activates the peripheral sympathetic nervous system, as well as central adrenergic pathways. Furthermore, other factors (non-renal and non-pituitary) are also involved in the central effects of $PGF_{2\alpha}$. The discrete sites for the central actions of $PGF_{2\alpha}$-i.c.v. are still unknown but may involve the brainstem nuclei, in which changes in catecholamine levels were found (A_1 and A_2 + NTS), presumably as regards to the cardiovascular responses.

57.8 STIMULATION OF R2 CHEMORECEPTORS ELICITS BILATERAL RENO-RENAL REFLEXES IN THE RAT. Paula R. Rogenes* (SPON: Robert A. Mueller). Dept. Physiol., U.N.C., Chapel Hill, N.C. 27514.

Electrical stimulation of renal afferent fibers results in either a decrease (in rabbits and dogs) or an increase (in cats) in efferent renal nerve activity (ERNA). Recent work in this laboratory has demonstrated the existence of two populations of chemoceptive receptors within the rat kidney termed renal "R1" and "R2" chemoreceptors (Recordati, et al., Circ. Res. 43:534, 1978, and 46:395, 1980). The purpose of the present study was to determine whether the R2 receptors participate in the afferent limb of a reno-renal reflex arc.

Sprague-Dawley rats were anesthetized with sodium pentobarbital. Postganglionic ERNA was recorded from a fine filament dissected from a single nerve bundle which had been cut close to the hilus of the left kidney. Multiunit ERNA was quantified with a window discriminator frequency meter. In some cases the responses of single units could be identified with the aid of a computer sorting program. R2 receptors were stimulated by backflow of nondiuretic urine into the renal pelvis of either kidney. Observations are reported only from animals in which blood pressure remained stable throughout the procedure.

ERNA was counted for three consecutive periods lasting one minute each and taken before, during, and after backflow of nondiuretic urine. An increase of multiunit ERNA over resting discharge rates was observed during backflow of urine into the contralateral as well as into the ipsilateral renal pelvis (mean increase of 21.5% and 20.9% respectively). There was no difference between ERNA before and after backflow of urine. Single unit analyses indicated that stimulation of ipsilateral and/or contralateral R2 receptors resulted in an increased firing frequency (range 50-180% over control) in some units. Other units showed no change or, less frequently, a reduction in activity during R2 receptor stimulation.

Thus, backflow of nondiuretic urine into either the ipsilateral or contralateral renal pelvis had an overall excitatory effect on ERNA in anesthetized rats. These data support the possibility that acute obstruction of the lower urinary tract results in altered ERNA bilaterally which could mediate adjustments in renal hemodynamics and excretory function. Observations of varying responses among single units give evidence that renal nerves contain a mixture of efferent fibers which may reflect functional heterogeneity. (Supported by NIH Training Grant T32 AM 07047 and NINCDS Grant NS 14899).

57.9 Kainic acid administration to mesolimbic sites: electrocorticographic and cardiovascular effects. L. S. Clark, A. Y. Deutch, and L. J. Peacock. Department of Psychology, University of Georgia, Athens, GA 30602.

We have presented data indicating that kainic acid administration to the lateral ventricle of barbiturate-anesthetized rats (4.7 nM in 1.0 ul buffered saline) elicits a rapid onset tachycardia. Because data from our own and other laboratories suggest that KA is a powerful epileptogenic agent at certain central loci, and that profound autonomic alterations accompany seizure activity, we have atttempted to 1)discern the temporal relationship between the onset of tachycardia and the appearance of epileptiform spikes following KA administration, and 2)to characterize the electrocorticographic patterns of seizure discharge resulting from administration of the neurotoxin to selected central sites.

KA administration reliably results in a short latency tachycardia following administration to the mesolimbic terminal fields nucleus accumbens septi (ACB) and nucleus interstitialis stria terminalis (NIST). Recording between a rostral skull screw and a caudal depth electrode (anterior thalamus) ipsilateral to the site of KA injection, a delay of approximately six minutes is observed between the onset of tachycardia (defined as a minimum of a 20% decrease in interbeat interval from baseline) and the first appearance of spikes. The records of both the lateral ventricle (IVT) and NIST groups show a desynchronization at approximately five minutes after the initiation of infusion (i.e., well after tachycardia onset) with spikes not appearing until approximately nine minutes. Vehicle administration also results in ECoG desynchronization; however, the electrographic activity rapidly returns to baseline appearances and does not evolve into seizure discharge. ACB injected subjects initially showed a pattern similar to that observed in animals receiving KA to the NIST and IVT; however, the tachycardia subsided at about nine minutes only to reoccur with a time course paralleling the increase in spike density and amplitude. Of these three groups the NIST animals showed the most profound spiking with spike amplitudes approaching 500 uV.

Using the same procedures, the effects of KA administration to the mesolimbic terminal field central amygdaloid nucleus or to site of origin of mesolimbic fibers, the ventral tegmental area (VTA) were as follows. No tachycardia was elicited by KA injection to either site. KA infusion to the VTA, however, did evoke spiking with a relatively long latency onset (12 minutes); amygdaloid administration was without effect. Thus, the initial tachycardia observed in response to KA administration can be differentiated from seizure-induced autonomic effects.

57.10 REGIONAL BRAIN CYCLIC NUCLEOTIDE AND HORMONAL RESPONSE IN GENETICALLY HYPERTENSIVE RATS (SHR) TO AMINERGIC AND CHOLINERGIC STIMULATION. D.R. Collins*, J.L. Meyerhoff, G.J. Kant, L.L. Pennington* and R.H. Lenox (SPON: H.C. Holloway). Dept. Med. Neurosci., Walter Reed Army Inst. Rsch., Washington, DC 20012 and Dept. of Psychiatry, Univ. of Vermont, Burlington, VT 05405

Spontaneously hypertensive rats (SHR) have been shown to be hyperactive and hyper-responsive to stressful stimuli as compared to their Wistar/Kyoto normotensive (WKY) controls. Previous work from our laboratory has demonstrated a relationship between motor activity, various stressors and the regional pattern of cyclic nucleotide response in the brain (Meyerhoff, et al.,Life Sci.,24, 1979; Lenox et al., Neuroendocrinology, In Press). We have also carefully examined in vivo regional brain cyclic nucleotide and hormone response to both aminergic and cholinergic stimulation (Bates et al., Neuroscience Abstracts, 5, 1979; Lenox et al.,Life Sci., In Press). Based upon these results, we have proceeded to examine the regional cyclic nucleotide and hormone response in SHR animals exposed to amphetamine or oxotremorine.

Female genetically hypertensive rats (SHR Okamoto/NIH/Vermont) and age-matched group of WKY normotensive controls, weighing between 250-300 gm were maintained in a light-cycled chamber. Stage of estrus was determined by vaginal smears. Animals received an intraperitoneal injection of either oxotremorine (2 mg/kg), d-amphetamine sulfate (5mg/kg) or saline 10 min. prior to sacrifice by exposure to high power microwave irradiation. Animals receiving the cholinergic agonist were pretreated (30 min.) with methylatropine (0.5mg/kg) to avoid excess peripheral cholinergic stimulation. Motor activity of all animals was measured for the 30 min. prior to sacrifice. Following sacrifice trunk blood was collected for hormone assays and 13 brain regions were dissected for radioimmunoassay of cyclic AMP and cyclic GMP.

Preliminary results in eight regions of the brain indicate similar concentrations of cyclic AMP in both SHR and WKY except for the pineal (SHR) where levels of cyclic AMP were increased twofold. This elevated pineal cyclic AMP might reflect heightened sympathetic activity observed in the SHR by other laboratories. SHR receiving oxotremorine had elevated cyclic AMP in the amygdala and both groups had striking increases in pituitary cyclic AMP confirming results previously reported by our laboratory. Regional cyclic AMP in both SHR and WKY responded similarly following amphetamine treatment. Mean serum prolactin indicate lower levels in the SHR, with both groups showing a significant reduction following amphetamine administration. These data thus far indicate that cyclic AMP and pituitary hormone response may serve to further differentiate the central nervous system of the SHR.

57.11 DIFFERENTIAL DEPLETION OF CNS CATECHOLAMINES: EFFECTS ON BLOOD PRESSURE AND DRINKING BEHAVIOR. F. J. Gordon, A. K. Johnson, & M. J. Brody* Depts. of Psychology & Pharmacology, and The Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

Previous studies from this laboratory indicated that depletion of CNS catecholamines produced by lateral ventricular (LVT) injection of 6-hydroxydopamine (6-OHDA) resulted in deficits both for drinking behavior and the central regulation of arterial pressure (Gordon, et al., Brain Research, 178:161-173, 1979). The purpose of the present study was to identify which catecholamine-containing neurons (norepinephrine (NE) or dopamine (DA)) and which CNS region(s) innervated by them might participate in the pressor and drinking responses produced by central drug stimulation.

Forebrain NE was reduced in rats by injecting 4 µg 6-OHDA into the ascending noradrenergic bundles (NAB). Spinal cord NE was depleted by intracisternal (IC) injection of 50 µg 6-OHDA. Moderate depletion of forebrain DA was produced by IC injection of 200 µg 6-OHDA + pargyline i.p. (IC + P), and severe DA depletion by bilateral injection of 4 µg 6-OHDA into the substantia nigra (SN) of desipramine pretreated rats. Pressor responses to various doses of LVT angiotensin II (AII), carbachol (CARB), and hyperosmolar NaCl (HYP); and drinking responses to LVT AII and CARB were examined.

NAB 6-OHDA reduced telencephalic and hypothalamic NE by more than 80% without significantly affecting brain DA or spinal cord NE. IC 6-OHDA depleted spinal cord NE by 80% and forebrain NE by 20-25% without reducing brain DA. IC + P 6-OHDA resulted in a 61% fall in telencephalic DA, and 28% and 94% depletion of forebrain and cord NE respectively. SN 6-OHDA reduced telencephalic DA by 86% and NE by 29% without affecting NE in other CNS regions.

IC + P 6-OHDA reduced LVT AII drinking without affecting LVT CARB drinking. SN 6-OHDA attenuated drinking to both LVT AII and CARB. Pressor responses to LVT AII, CARB, and HYP were largely unaffected by these 6-OHDA treatments. Almost complete destruction of brain and/or spinal cord NE (NAB, NAB + IC, IC + P 6-OHDA) failed to alter centrally mediated drinking and pressor responses.

These data indicate that the integrity of brain DA neurons are required for the behavioral but not hypertensive responses produced by central drug stimulation. Furthermore, it appears that the pressor action of centrally injected drugs can be reduced only if CNS NE is depleted by injecting 6-OHDA LVT (Gordon, et al., 1979). This observation raises the possibility that the antihypertensive action of 6-OHDA when injected LVT may be unrelated to its specific catecholaminergic neurotoxicity. Another possibility is that catecholaminergic neurons intrinsic to the hypothalamus, which might be reached only if 6-OHDA is injected LVT, may constitute a functionally critical pool of neurons responsible for the initiation of pressor responses produced by central drug stimulation. (USPHS NIH 1-RO1-HL24102 & HLP-14388; NIMH 1-KO2-MH00064)

57.12 HARMALINE INDUCED AUTONOMIC CHANGES FROM THE CEREBELLUM IN CONSCIOUS DOGS. K. J. Dormer and E. H. Brown*, Dept. of Physiology and Biophysics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73034.

Harmaline is a drug that produces motor tremor through specific activation of cells in the inferior olive which then cause repetitive firing in climbing fibers, thus activating Purkinje cells and cerebellar nuclei including the fastigial nucleus (FN). At doses above and below the tremorogenic threshold autonomic changes are also observed, including tachycardia and hypertension. In an attempt to confirm the autonomic role of the FN in response to muscle afferent activation, harmaline was given to chronically instrumented dogs to observe heart rate (HR), left ventricular pressure (LVP) and dP/dt, or HR and aortic pressure (AP) changes. Dogs were implanted with solid-state pressure transducers into the left ventricle or descending aorta, using sterile technique. After recovery from the thoracotomy, electrodes were implanted into the rostral FN which produced tachycardia and hypertension when stimulated electrically. Harmaline was injected in doses of 1.5 and 0.7 mg/kg for the LVP (n=2) and AP (n=2) dogs respectfully, and cardiovascular parameters recorded for 10 minutes following injection. Lesions were then placed in the rostral regions of FN, using a radiofrequency lesion generator (30 VRF). After 5 - 10 days recovery from lesioning, the harmaline injections and recordings were repeated. HR was increased 33% by the larger dose and negligibly by the smaller. Post lesion, the HR was not increased at all. LVP increased from 124 to 153 mmHg or 23% prelesion, and decreased by 4% after FN lesioning. Myocardial contractility as indexed by dP/dt increased by 48% before lesioning and 20% postlesioning. Of the dogs implanted for AP, only one received FN lesioning and AP increased 22% before lesioning, and decreased by 4% following lesioning. Mild tremors were also observed at the 1.5 mg/kg doses prior to lesioning but were not evident postlesioning. Thus partial removal of the FN reduces the autonomic changes associated with harmaline activation of the inferior olive and FN. This provides further evidence (see Dormer and Brown Fed. Proc. 39(3):842,1980) that muscle afferents to the cerebellum are responsible for some of the cardiovascular changes observed during exercise and that this response may be mediated by the rostral FN. This work was supported by a Young Investigators Award HL 22747.

57.13 EFFECTS OF INTRAVENTRICULAR ADMINISTRATION OF BEPRIDIL ON CARDIOVASCULAR HEMODYNAMICS OF ANESTHETIZED AND CONSCIOUS DOGS.
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The effects of intraventricular administration (IVT) of Bepridil were studied in anesthetized and conscious adult mongrel dogs (14-20 kg.). Each animal was anesthetized with sodium pentobarbital (35 mg/kg i.v.) and a stainless steel cannula was implanted into the left lateral ventricle. Animals used for the unanesthetized studies were allowed to recover for 5-7 days. The anesthetized dogs were instrumented to determine cardiac output (CO), heart rate (HR) and mean blood pressure (BP). They were injected IVT with Vehicle alone (V) or Bepridil solution (B). In these animals V produced little or no changes in HR or BP. However, B produced dose related (50-400 µg) significant ($p<.05$) increases in BP, CO and total peripheral resistance (PR) with peak responses occuring 15-20 min. after IVT injection. As the 400 µg dose of B produced the largest changes in cardiovascular dynamics its effects were investigated in conscious animals. The conscious dogs were injected IVT with (V) or Bepridil (B), while heart rate and blood pressure were measured at 5 min. intervals 15-20 min. before and up to 60 mins. after injection. In the conscious animals the V produced no significant change in resting heart rate or systolic blood pressure at any time after administration. However, B at high doses (400-800 µg) produced significant ($p<.01$) increases in systolic blood pressure (SBP) (12-40 mmHg), with a peak increase occuring at 15-20 mins. after IVT administration. Heart rate remained unchanged.

These results domonstrate that central administration of B produces an increase in blood pressure, an action which is opposite to the systemic effect of the compound.

57.14 HYPOTENSIVE EFFECTS AND PREGANGLIONIC SPLANCHNIC NERVE ACTIVITY OF E. COLI ENDOTOXIN IN THE CAT.
S. Koyama*, H. L. Santiesteban*, W. S. Ammons* and J. W. Manning. Dept. of Physiology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Within one minute after intravenous injection of 1 mg/kg E. Coli endotoxin (Difco. 0111:B4) in alpha chloralose anesthetized cats, mean blood pressure was 10% above control pressure. This was followed within 5 min. by a rapid fall to 75% of control pressure. There followed a temporary recovery of mean blood pressure to 80% of control. Finally there was a secondary decay in mean blood pressure, so that after 60 min. mean blood pressure was depressed to 55% of control pressure. The aortic pulse pressure initially decreased to 80% of control, but then recovered to 95% of control. Finally there was a secondary decay in pulse pressure. Preganglionic splanchnic nerve (PSN) activity decreased to 80% of control level before the rapid fall of mean blood pressure. There followed a temporary recovery of PSN activity coincidence with the maximal fall in mean blood pressure. During the ensuing secondary decay in mean blood pressure, PSN activity gradually decreased to 50% of control level at 60 min.after injection of E. Coli endotoxin. At 30 min. after intracisternal injection of phentolamine (500µg/kg), E.Coli endotoxin was injected intravenously. Hypotension induced by intravenous injection of endotixin was abolished by the intracisternal pretreatment with phentolamine. Pulse pressure was decreased to 85% of control at 5 min.after intravenous administration of endotoxin. There followed an increase of pulse pressure to 15% above control which persisted during experimental periods (60 min.). The changes in PSN activity with intravenously injected endotoxin was abolished by the intracisternal pretreatment with phentolamine. In conclusion, we confirmed the previous report (Fed.Proc. 39:840,1980) that the hypotensive effects of E.Coli endotoxin may be mediated by a central autonomic blood pressure regulatory circuits, by stimulation of central alpha adrenergic receptors which lead to inhibition of brain stem sympathetic pathways that participates in baroreflex adjustments.

57.15 MORPHOMETRIC CHANGES IN LUMBAR COLONIC NERVES OF RABBITS AFTER TREATMENT WITH NERVE GROWTH FACTOR - ANTISERUM.
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In late embryonic development the administration of antiserum to nerve growth factor (A-NGF) causes a degeneration of adrenergic nerves. We studied the effect of A-NGF on morphometry of lumbar colonic nerves (LCN) which originate in the inferior mesenteric ganglion (IMG) and innervate the descending colon. The unmyelinated fiber (UF) diameters in these nerves ranged from 0.1 µm to 1.4 µm. The number and density of fibers per nerve varied greatly among control animals.

A combined pre- and postnatal treatment with A-NGF was performed in two ways: 1) 5 pups were injected (0.05 ml/g body weight) one week before delivery through the exposed uterus and then for five days after birth; 2) one adult mother was immunized with NGF and then mated. Serum A-NGF levels were determined by measuring the amount required to precipitate 50% of the ^{125}I-labeled NGF in a double antibody precipitation radioimmunoassay procedure. At the age of two months the rabbits were perfused and the LCN prepared for EM evaluation.

There were no gross differences in the size of the IMG between those of the A-NGF and control animals. The mean of the median diameters of the UF of the LCN in 7 treated (no difference between the two types of treatment) animals ($\bar{x}$ = 0.39 µm ± 0.04 µm) was significantly ($p < 0.01$) smaller than the mean in 6 control animals ($\bar{x}$ = 0.51 µm ± 0.03 µm). Inspection of the fiber size distribution indicates that the frequency of small fibers was increased whereas the frequency of large fibers remained the same. There was no detectable change in overall fiber density between these two groups, which might be due to the great variability seen in control animals.

The ability of LCN stimulation to inhibit colonic motility was evaluated in vitro by measuring propulsive contractions with an intraluminal cannula and stimulating the LCN with bipolar electrodes. In three treated rabbits (2-treatment no. 1, 1-treatment no. 2) the response to LCN stimulation was not changed.

These findings show that A-NGF treatment which does not eliminate sympathetic inhibition of the colon results in a shift of the fiber size distribution to smaller diameter categories due to an increased number of small fibers.

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57.16 SODIUM DEPLETION ALTERS THE CENTRAL RESPONSE TO ANGIOTENSIN II AND NALOXONE.
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The central actions of angiotensin II (AII) at the level of the area postrema require the participation of the opiate system in the brain (Szilagyi & Ferrario, Neurosci. Abs. 5, 51, 1979). As previously reported naloxone depressed and morphine potentiated the pressor responses to AII administered via the vertebral arteries; those to intravenous infusions of the peptide were unaffected. The intricacies of this interrelationship between AII and the opiate system are not clear as yet.

Since sodium depletion in dogs is accompanied by elevated levels of circulating AII and a blunted pressor response to intravenously administered AII, the effects of morphine blockade on the central response to the peptide were examined. Fifteen male mongrel dogs (20-25 kg) were used. Five dogs were maintained on a normal sodium diet (40 mEq Na+/day) while the remaining 10 were placed on a low sodium diet (4 mEq Na+/day) for 21 days and given 40 mg furosemide (im) for 2 days prior to the experiment. All dogs were anesthetized with α-chloralose (66 mg/kg iv) after premedication with morphine (2 mg/kg im). Angiotensin II (1-20 ng/kg/min) was infused intravenously and via the vertebral arteries before and again after injection of the morphine antagonist naloxone (0.8 mg iv) and the pressor responses recorded. Dose-response curves obtained in both conditions were compared.

In normal dogs, treatment with naloxone resulted in a significant parallel shift of the vertebral AII dose-response curve to the right of control. That obtained with intravenous AII was unchanged after blockade of morphine. Animals which were maintained on a sodium depleted diet not only possessed a diminished intravenous response to AII (as previously published), but also exhibited a significantly diminished response to vertebrally infused AII so that intravenously and vertebrally elicited responses were identical, a finding reported for the first time. The vertebral dose-response curve was shifted parallel and to the right of control. Introduction of naloxone in this preparation did not alter the intravenous response and only slightly shifted the vertebral dose-response curve.

The data indicate that 1) sodium depletion abolished the greater responsiveness of the area postrema to vertebrally versus intravenously infused angiotensin II and 2) the influence of the opiate system on the response appears to be diminished in the sodium depleted state.

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57.17 PRESSOR RESPONSE TO NALOXONE IN HYPOTENSIVE CATS AFTER LESION OF THE CAUDAL RAPHE NUCLEI. Paul S. Blum and James A. Spath, Jr.*, (Spon: N. Moskowitz). Dept. of Physiol., Thomas Jefferson Univ., Philadelphia, PA 19107.

Anesthetized cats were subjected to a discrete lesion of the caudal raphe nuclei, spinal transection at C1, or sham-operation. These cats then were bled to a mean arterial blood pressure (MABP) of 40 mm Hg and maintained at that pressure by adjusting volume with a servo-controlled pump. After 60 minutes, the servo system was discontinued, and 2 mg/kg naloxone was administered intravenously followed by a continuous infusion of 2 $mg \cdot kg^{-1} \cdot hr^{-1}$. The peak increase in the MABP after administration of naloxone was 0.8 ± 4 mm Hg in the sham-operated group (n=6), 17 ± 5 mm Hg in the group with caudal raphe lesion (n=4) and 21.7 ± 7 mm Hg in the group with spinal cord transection (n=3). An analysis of varience showed that there was a significant difference in the mean increase in blood pressure when the sham group was compared to the two lesioned groups ($p<0.01$). There was no significant difference in the blood pressure response comparing the two types of lesions. The increase in blood pressure was transient and MABP returned to 40 mm Hg or less in all hemorrhaged cats within 30 minutes of naloxone administration, despite continued infusion of the drug. These data indicate that naloxone administration produced a significant increase in MABP after either spinal transection or lesion of the caudal raphe nuclei in hypotensive cats but not in cats with intact neural pathways. Moreover, these results suggest that endogenous opiates, in conjunction with descending neural pathways that originate in the caudal raphe nuclei, contribute to the pathophysiology of hemorrhagic hypotension. Interruption of these central pathways allows effects of opiate receptor blockade with naloxone to be manifest. Supported by HL-20283.

57.18 EFFECTS OF ETHANOL, DIAZEPAM AND PENTOBARBITAL ON RESTRAINT STRESS AND PLASMA CATECHOLAMINES IN RATS. Kathryn H. DeTurck* and Wolfgang H. Vogel, Dept. Pharmacol., Thomas Jefferson Univ., Philadelphia, PA 19107.

Groups of 4-6 male Wistar rats, fitted with indwelling jugular catheters, were given ip injections of ethanol (500 mg/kg), valium (5 mg/kg) and pentobarbital (5 mg/kg), and were allowed to rest 15, 30 and 10 min, respectively. About half of the animals remained in the home cage as controls, while the others were then subjected to 30 minutes of immobilization. Blood samples were taken from both groups at 0 min (before restraint), at 1, 5, 15 and 30 min during restraint and at 60 min (30 min after release from restraint). Plasma norepinephrine (NE) and epinephrine (E) were determined by the radioenzymatic assay from Upjohn.

The ip injections of the 3 drugs had no significant effect on baseline NE and E concentrations, as first measured after a sufficient rest period to allow for absorption. However, when the treated rats were then taped to the workbench, the substantial elevations in plasma NE and E, characteristic of such extreme stress, were considerably reduced. Analysis of area under the curve data showed a significant ($p < 0.01$) reduction in both NE and E increases with prior administration of ethanol. These animals demonstrated less struggling and tape biting than the untreated controls, although their amount of vocalization was increased. Diazepam also caused a decrease ($p < 0.01$) in plasma NE levels, but had no significant effect on increased E levels. This corresponded with a marked decrease in all outward signs of distress, including struggling, tape biting and vocalization. The administered dose of pentobarbital had no significant effect on either NE or E increases, although peak values for both were somewhat decreased. The rats appeared as agitated as the control animals, with the incidence of tape biting somewhat increased.

57.19 ALCOHOL, DIAZEPAM, AND MORPHINE SULFATE REDUCES BOMBESIN-INDUCED ELEVATION OF GASTRIC PRESSURE AND MOTILITY IN THE RAT. W. G. Young. Dept. of Neurosciences, University of California, San Diego, La Jolla, California 92093.

Bombesin (BBS) increases gastric pressure (GP) and motility (M) in the awake rat. 9 rats, Sprague-Dawley males (300-400 g), were surgically implanted with indwelling catheters (Dow Corning Silastic tubing #602-305)(See: Deutsch, J. A. and H. S. Koopmans. Science, 179:1242, 1973) and their gastric activity was monitored by a pressure transducer. All rats demonstrated elevated GP and M when injected intraperitoneally (IP) with 2 - 16 µg/kg of synthetic BBS. The effects lasted for several hours and were significantly distinct from pre-injection resting waveforms. The BBS activity consisted of a sharp rise in GP from the resting level to 8 - 10 cm of water pressure higher after injection. This was followed by greatly enhanced contractions in M with amplitudes ranging from 36 - 40 cm of water and a frequency of about 4 per minute. Injections of the vehicle could not elicit this activity. Alcohol at 0.25 g/kg (IP) was able to reduce the BBS effects on GP and M. Morphine sulfate at 10 mg/kg (IP) had similar effects to alcohol. Diazepam (Valium, Roche Laboratories) at 5 mg/kg completely inhibited both BBS-induced GP and M. Lower doses of diazepam down to 250 µg/kg blocked the effects only partially, and a good dose-response was not obtained. BBS effects on GP and M could not be blocked with the anticholinergic methscopolamine (Pamine bromide, Upjohn Company) at 1.6 mg/kg. The present study does not determine whether these effects result from central or peripheral actions.

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57.20 CLONIDINE-ADENYLATE CYCLASE INTERACTIONS IN SYMPATHETIC PREGANGLIONIC NEURONS. Donald N. Franz*, Robert G. Peterson*, and Parley W. Madsen*. (SPON: S.S. Stensaas). Dept. Pharmacology, Univ. Utah, Salt Lake City, Utah 84132.

Previous studies indicate that bulbospinal norepinephrine (NE) pathways to sympathetic preganglionic neurons (SPGNs) are excitatory (Life Sci. 14: 793, 1974; Psychopharmacology 62: 9, 1979). Other studies show that clonidine depresses activation of SPGNs by both segmental and bulbospinal pathways; this depression is selective, independent of central monoamine stores, and readily reversible to tolazoline (Clin. Exp. Hypertension 1: 115, 1978). Recent evidence that alpha$_2$-receptor activation by clonidine in some cellular systems suppresses adenylate cyclase (D. U'Prichard, Clonidine Symposium, FASEB, 1980) prompted the present analysis of possible interactions between clonidine and adenylate cyclase in SPGNs.

In unanesthetized, spinal cats, sympathetic discharges recorded from upper thoracic preganglionic rami were evoked by stimulating intraspinal (IS) excitatory pathways in the cervical cord or segmental spinal reflex (SR) pathways. Aminophylline (10-50 mg/kg) or isobutyl-methylxanthine (IBMX, 0.5-2 mg/kg) rapidly and markedly enhanced transmission through the IS pathways, the largest doses to averages of 175% and 240% of control values, respectively, within 10 min. Enhancement and rate of onset were dose-dependent. Spinal sympathetic reflexes (SR) were gradually enhanced after a delay of 20-30 min. Spinal somatic reflexes were not affected. These results suggest that cyclic AMP increases excitability of SPGNs in response to activation of adenylate cyclase by bulbospinal pathways. Preservation of basal levels of cyclic AMP may account for enhancement of SR.

Clonidine HCl (10-50 µg/kg) markedly depressed transmission through both pathways with the IS pathway being 5X more sensitive than the SR. The IS pathway could be depressed completely, but the SR pathway could not be depressed beyond 60%, even by much larger doses. Clonidine pretreatment prevented or markedly reduced the enhancement of transmission through either pathway by aminophylline or IBMX, depending on dose. The results suggest that adenylate cyclase activity in SPGNs is regulated by positively and negatively coupled receptors and support the proposal that clonidine inhibits SPGNs by activation of the negatively coupled receptors. (Supported by NIH grants HL-24085 and GM-07579 and the Montana Heart Association.)

57.21 CONTRASTING EFFECTS OF CLONIDINE AND 5-HTP ON SPINAL SYMPATHETIC PATHWAYS. Parley W. Madsen*, Bradford D. Hare*, Chaichan Sangdee*, and Donald N. Franz*. (SPON: L.M. Partlow). Dept. Pharmacology, Univ. Utah, Salt Lake City, Utah 84132.

Although the antihypertensive effect of clonidine is generally ascribed to actions on brainstem cardiovascular centers, we have found that it produces marked depressant effects on spinal sympathetic neurons (Clin. Exp. Hypertension, 1: 115, 1978) which are at least equal to that on higher centers (Fed. Proc., 39: 409, 1980). Similarities between the depressant effects of clonidine and 5-HT precursors on spinal sympathetic pathways originally suggested that clonidine might act on inhibitory 5-HT receptors. The weight of contrary evidence prompted further analysis of these similarities.

In unanesthetized, spinal cats, the effects of clonidine were compared with those of 5-HTP on transmission through two spinal sympathetic pathways, segmental spinal reflex (SR) pathways and descending intraspinal (IS) excitatory pathways. Evoked sympathetic discharges were recorded from upper thoracic preganglionic rami and were analyzed on-line by signal averaging.

Clonidine HCl (5-50 μg/kg) produced a parallel, dose-dependent depression of transmission through each pathway. However, the IS pathway was 5 times more sensitive to clonidine (ED50 = 6 μg/kg) than the SR pathway (ED50 = 30 μg/kg). Furthermore, whereas 50 μg/kg of clonidine depressed transmission in the IS pathway by nearly 100%, the SR pathway could not be depressed more than 60% by doses of 50-200 μg/kg. In contrast, D,L-5HTP was more effective in depressing the SR pathway than the IS pathway (ED50's = 32 and 44 mg/kg, respectively), 50 mg/kg depressing the SR by almost 100% and the IS by only 60%. Tolazine rapidly reversed the effects of clonidine on both pathways in a dose ratio of about 100:1; reversal of 5-HTP-induced depression by larger doses was slow and inconsistent. These contrasting results indicate that clonidine and 5-HTP depress the excitability of sympathetic preganglionic neurons by different mechanisms, probably involving alpha$_2$- and 5-HT receptors, respectively. Each mechanism may contribute independently to regulation of the sympathetic outflow. (Supported by NIH grants HL-24085 and GM-07579.)

57.22 **EFFECTS OF CLONIDINE ON SPONTANEOUS ACTIVITY IN A SYMPATHETIC-CHOLINERGIC SYSTEM.** Patricia J. Bernthal* and Michael C. Koss (SPON: J. I. Moore). Dept. of Pharmacology, Univ. of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190.

Because of its sympathetic-cholinergic nature, we have used the electrodermal response (EDR) of the sudomotor system as a model for studying the effects of pharmacologic agents on central sympathetic outflow (Davison & Koss, Neuropharmacology, 1976, 15:197-201; Koss & Davison, Naunyn-Schmied. Arch. Pharmacol. 1976, 295:153-158). Clonidine, which is considered to inhibit tonic sympathetic activity by a central action (Haeusler, Naunyn-Schmied. Arch. Pharmacol. 1973, 278:231-246) depresses the amplitude of centrally, peripherally, or reflexly evoked EDRs, an action which is reversed or prevented by yohimbine (Koss & Davison, Eur. J. Pharmacol.,1976, 37:71-78; Bernthal & Koss, Eur. J. Pharmacol., 1979, 60:23-29). In the present study we compared the effects of clonidine and yohimbine on spontaneous EDR activity in the unanesthetized cat with those previously observed using evoked EDRs in an effort to see if earlier findings could be verified using tonic sympathetic activity.

Because spontaneous electrodermal activity is so irregular and difficult to quantify, the electrical potentials were processed over 60-sec periods through an integrator (Grass 7P10B). Cats were decerebrated under ether as previously described (Bernthal & Koss, Brain Res. Bull., 1978, 3, 437-441), and allowed to recover for approximately 90 min before any pharmacologic manipulations were begun. Some cats were subsequently spinalized at the cerivcal level (C_1). The amplitude of spontaneous EDR activity in the unanesthetized decerebrate preparation was diminished by less than 10% over a three-hour period. Clonidine (3 or 10 ug/kg, iv) rapidly depressed the amplitude of spontaneous EDR responses in unanesthetized decerebrate or decerebrate-spinal preparations, exerting a maximal effect in less than five min. Spontaneous EDR activity was depressed by approximately 50% for the 90 min observation period. Subsequent administration of yohimbine (0.5 mg/kg, iv) reversed the clonidine-induced inhibition, while prior administration of yohimbine prevented clonidine's effect. Administration of yohimbine potentiated the amplitude of spontaneous EDR activity in the unanesthetized decerebrate preparation, but not in spinalized cats. These preliminary results indicate that the effects of clonidine on spontaneous EDRs are analagous to those observed in the tonic activity of other sympathetic systems. In addition, this study provides further evidence for a spinal cord site of action for clonidine.

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57.23 PARTICIPATION OF ADRENOCEPTORS IN THE MEDIAL MEDULLARY RETICULAR FORMATION IN CLONIDINE-INDUCED HYPOTENSION AND BRADYCARDIA. Yih-Huey Chen and Samuel H.H. Chan. Department of Life Sciences, Indiana State University, Terre Haute, IN 47809.

One of the proposed antihypertensive mechanisms for clonidine suggests that the imidazoline compound activates postsynaptic α-adrenoceptors located in the medulla oblongata. Previous experiments from our laboratory implicated the medial medullary reticular formation (MMRF) in rats and cats as a possible site for clonidine to exert hypotension and bradycardia (Chan and Koo, Neuropharmacol. 17:367, 1978; Chen and Chan, Neuropharmacol. in press). The present study was undertaken to further investigate the possible participation of α-adrenoceptors in the MMRF in clonidine-elicited cardiovascular suppressions.

Male Charles River rats anesthetized with pentobarbital sodium (50 mg/kg, i.p.) were used. Tracheotomy and cannulation of the left femoral artery and vein were routinely performed to facilitate ventilation, measure arterial blood pressure and heart rate, and inject drugs. Microinjection of adrenoceptor blocking agents into bilateral MMRF (1 µl in vol.) was delivered via a stereotaxically positioned 27-gauge syringe needle attached to a microinjection device. The effect of such pretreatment on the action of an intravenous administration of clonidine (10 µg/kg) was studied 30 min later.

Microinjection of phentolamine (40 µg) into bilateral MMRF appreciably blocked the hypotensive effect of clonidine, together with a significant reduction in its cardioinhibitory action. In contrast, the vasodepression produced by clonidine was not discernibly antagonized by phenoxybenzamine (50 µg), although there was a significant decrease in its elicited bradycardia. Pretreatment of the MMRF with propranolol (70 µg) did not prevent the clonidine-induced hypotension. There was, however, also a significant reduction in the clonidine-promoted cardioinhibition.

Phentolamine is an equally potent blocker for both presynaptic and postsynaptic α-adrenoceptors, phenoxybenzamine is a preferential postsynaptic α-adrenoceptor antagonist, and propranolol is a β-adrenoceptor blocking agent. Thus, the differential antagonistic effect of phentolamine, phenoxybenzamine and propranolol on clonidine-induced vasodepression may implicate that the availablity of presynaptic α-adrenoceptors in the MMRF is essential for the promotion of hypotension by clonidine. At the same time, the presence of presynaptic and postsynaptic α-adrenoceptors and β-adrenoceptors in the MMRF may be required for the imidazoline compound to exert its bradycardiac actions.

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57.24 **ERYTHROCYTES: IN VIVO SITE OF CATECHOLAMINE TRANSPORT AND METABOLISM IN MAN & RAT.** **N. Alexander, M.T. Velasquez* and N.D. Vlachakis*. Clin. Pharm. Sect., Dept. of Med., Sch. of Med., Los Angeles, CA 90033**

Weil-Malherbe & Bone (1953) showed with flourometric assays that human blood cells contain norepinephrine (NE) and epinephrine (E). Blakeley & Nicol (1978) found that in vitro rabbit erythrocytes take up ^{3}H-NE and that its 0-methylated metabolite,^{3}H-normetanephrine (NM), accumulated inside cells within 5 minutes. We have measured, by radioenzymatic, thin-layer chromatographic methods, the concentration of NE, E, dopamine (DA), NM and octopamine (OA) in plasma and red blood cell (RBC) lysate of normal human subjects and rats. Mean ± SEM values for plasma (pg/ml) vs. RBC lysate (pg/gm of packed rbc) in 7 seated male subjects were: NE, 426±53 vs 436±51; E, 59±5 vs 164±32 ($p<.01$); DA, 87±11 vs 146±46; NM, 1725±239 vs 4070±690 ($p<.01$) and OA, 2090±340 vs 13500±1280 ($p<.001$). The NM/NE ratio in plasma vs the ratio in lysate was 4.2±.067 vs 9.4±1.1 ($p<.001$). Values for plasma vs RBC lysate from 12 rats in home cages were: NE, 463±56 vs 663±79 ($p<.05$); E, 320±79 vs 550±134; DA, 322±83 vs 1528±406 ($p<.01$); NM, 940±158 vs 3510±560 ($p<.001$); OA, 532±83 vs 3710±410 ($p<.001$). After 5 minutes of restraint, plasma NE, E, NM and OA increased significantly whereas only NE increased significantly in lysate. The NM/NE ratio in plasma and lysate from rats in home cages was 2.3±0.5 vs 5.5±0.9 ($p<.005$), respectively. These ratios were similar in the samples collected during restraint. These results show that both human and rat erythrocytes contain NE,E, DA, NM and OA in concentrations similar to or higher than those of plasma implicating erythrocytes as a functionally significant site of transport for,circulating catecholamines. Since erythrocytes also contain catechol-0-methyltransferase (COMT), the higher NM/NE ratio found in RBC lysate suggests that some NM may have been formed from NE inside red cells.

58.1 FACILITATION OF PASSIVE AVOIDANCE BEHAVIOR BY POST-TRIAL ADMINISTRATION OF NALOXONE AND ETHANOL IN MICE. E.G. Zimmermann, D.A. Gorelick and D.L. Colbern*. Depts. of Neuroscience, Psychiatry, Pharmacology and Anatomy, UCLA School of Medicine, Los Angeles, CA 90024.

Post-trial administration of ethanol (ETOH) was recently observed to facilitate retention of passive avoidance behavior in mice. Since the opiate antagonist, naloxone (NAL), has been shown to interfere with certain effects of ETOH, its effect on ETOH-facilitated avoidance behavior was examined.

Adult male Swiss-Webster mice were subjected to a one-trial, step-through passive avoidance task using a shock intensity of 0.1 mA. Immediately following the training trial, 15% ETOH (2g/kg) or saline was injected ip along with either NAL (1, 2 or 5 mg/kg) or saline. NAL alone at all dose levels, like ETOH, produced maximum facilitation of avoidance behavior (approx. 300 sec) when tested 24 hr later. Similarly, NAL plus ETOH resulted in maximum or near maximum improvement in retention at all dose levels. The conditions of the experiment precluded detection of any possible additive effects.

To explore possible additive effects of NAL and ETOH, the doses of NAL were halved. In view of NAL's short duration of action and the uncertain time-course of facilitation, the dose of NAL was repeated 30 min post-trial. Administered in this fashion, NAL again caused marked improvement in avoidance behavior except for the 1 mg/kg dose. NAL plus ETOH also caused near maximal facilitation although the highest dose of NAL attenuated the effect of ETOH ($p < 0.05$).

These results show that post-trial administration of NAL, facilitates retention of passive avoidance behavior, in agreement with a recent report by Messing, et al. (Behav. & Neural Biol. 27:266, 1079). This paradigm failed to detect any antagonism of ETOH effects by NAL except possibly at the highest dose level. The results obtained using two injections suggest that dose and duration of NAL are important variables in determining the extent to which avoidance behavior is facilitated. Further studies are underway to determine the mechanism(s) by which NAL and ETOH improve performance in this task.

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58.2 EFFECTS OF ACUTE AND CHRONIC ETHANOL TREATMENT ON HABITUATION OF THE DR-VR REFLEX IN ISOLATED FROG SPINAL CORD. P. C. RINALDI,* L. Y. NISHIMURA* and R. F. THOMPSON (SPON: R. Messing). Dept. Psychobiology, Univ. of California, Irvine, California 92717.

The effects of ETOH on synaptic plasticity have been examined in very few instances. The isolated frog spinal cord preparation provides the opportunity to examine ETOH effects on habituation in a model system and to determine the particular synaptic mechanisms disrupted by it. Isolated hemisected spinal cords of Rana catesbiana were continuously perfused in buffered Ringers and maintained at 15°C±1. A dorsal and ventral root were draped across electrodes for stimulating and recording respectively. The reflex itself was found to be facilitated at low levels and depressed at the high levels of ETOH. Habituation--decrement of the polysynaptic ventral root response to repeated dorsal root stimulation --and recovery were assessed during controls in normal Ringers. Following this, ETOH was added to the perfusate and habituation repeated at varying time intervals. ETOH concentrations ranged from .025% (20mg/100ml) to 0.5% (400mg/100ml). Similar experiments were conducted with spinal cords from chronically treated frogs. These frogs were maintained for one to two weeks in their home environment with ETOH added to their living water. Frogs maintained thus had blood alcohol levels ranging from 30 to 400mg per 100ml, depending on ETOH concentration in the home water. In general, the degree of habituation appears to be reduced at most ETOH concentrations with some suggestion of biphasic effects at lower concentrations.

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58.3 MODULATION OF MEMORY STORAGE BY OPIATE AGONISTS AND ANTAGONISTS. R.A. Jensen, R.B. Messing, B.J. Vasquez, J.L. Martinez, Jr., K.C. Liang*, C.B. Brewton* and J.L. McGaugh. Department of Psychobiology, University of California at Irvine, Irvine, CA 92717.

Previous research has shown that both naloxone and morphine influence memory storage processes. In these experiments, rats were trained in a one-trial inhibitory avoidance step-through task. They were shocked (0.50 or 0.75 mA; 0.5 sec) upon entering a dark compartment, and injected with drugs following the training trial. Retention was measured one day after training by obtaining the latency of each rat to reenter the dark compartment. Naloxone facilitates retention performance of rats in this task if it is peripherally administered (i.p.) after training, while morphine has amnestic effects. These results are dependent both upon schedule of drug administration and dose. Naloxone enhanced memory when administered in equally divided doses (totaling 1 mg/kg) immediately after training and again 30 min later. Morphine, on the other hand, produced memory impairment when given in a single injection (3 mg/kg) immediately after training.

We now report data indicating that the injection schedule is an important determinant of the direction of the effect of both opiate agonists and antagonists. Both naloxone (1 mg/kg) and naltrexone (0.3 mg/kg) facilitate retention performance when given in divided doses, but an impairment in performance results when naloxone is injected in a single dose 30 min following training. Moreover, if morphine (1 mg/kg) is administered in divided doses immediately and 30 min after training, it enhances retention performance in a manner similar to naloxone and naltrexone, but in contrast to its amnestic effect when it is injected in one dose immediately after training. Thus, if morphine and naloxone are given in single doses, their memory modulatory effects are different from each other; if however, morphine, naloxone, or naltrexone are given in divided doses 30 min apart, then all of the drugs enhance memory. It is therefore apparent that opiate modulation of memory storage processes is dependent upon subtle differences in drug distribution and availability through time.

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58.4 THE ROLE OF OPIOID PEPTIDES IN MEMORY AND LEARNING. I. Izquierdo, D.O. Souza*, R.D. Dias*, M.L. Perry*, M. A. Carrasco*, E. Elisabetsky*, A.C. Paiva*, Dept.Bioquímica, UFRGS centro, 90000 Porto Alegre, RS, Brasil.

Beta endorphin, leu-, met-, and des-Tyr-met-enkephalin (1-10 µg/Kg ip., post-training) cause amnesia for shuttle avoidance learning and for habituation of a rearing response to a tone in rats. Naloxone (0.4-0.8 mg/Kg) has an opposite effect of its own, and antagonizes that of the opiates. At least one opioid peptide, beta-endorphin, is released from the rat brain at a rate of 30-50 ng/25 min during training (but not test) sessions of the two tasks. The release appears to result from non-associative influences of the stimuli used for training, since it is also obtained after sessions of pseudoconditioning or of footshocks alone. The amount released is compatible with the amnesic ED50 of the substance (1 µg/Kg ≅ 140 ng/rat, of which <20% reaches the brain within 120 min from injection).

These findings suggest that there is a physiologic amnesic mechanism mediated by beta-endorphin and, perhaps, by other opioid peptides as well.

In addition, shuttle avoidance and habituation learning appear to be dependent on a state caused by the beta-endorphin that is released, and this appears to normally be released in sufficient amount for that purpose. Naloxone (0.4-1.6 mg/Kg, ip.) given prior to training depresses acquisition of both tasks; beta-endorphin (2 µg/Kg) has no effect. When given prior to test sessions (in which there is no release of the peptide), naloxone has no effect, and beta-endorphin enhances retrieval. These effects occur independently of the influences of both drugs on consolidation. However, it seems possible that the state-dependency may be due to amnesic effects of beta-endorphin on adventitious and interfering forms of learning.

The role of opiates in learning and memory might be more important than, and is independent of, any that they may have in the modulation of pain. First, amnesic ED50s are 100 times lower than analgesic ED50s of opioid peptides; second, their effects on learning and memory are independent of pain; third, beta-endorphin is released both by painful and painless stimuli.

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58.5 EFFECTS OF OPIATE ADMINISTRATION INTO THE AMYGDALA ON PAVLOVIAN HEART RATE CONDITIONING IN THE RABBIT. M. Gallagher, B. S. Kapp, C. L. McNall,* and J. P. Pascoe.* Dept. Psychol., Univ. of Vermont, Burlington, VT 05405.

Previous research conducted in our laboratory has demonstrated that neural systems within the central nucleus region of the amygdala contribute to the acquisition of classically conditioned heart rate responding in the rabbit (Kapp et al., Physiol. Behav., 23, 1109-1117, 1979; Gallagher et al., Pharmac. Biochem. Behav., in press.) Since opioid peptides are highly concentrated within the central nucleus, the present investigation was aimed at determining the effects of intracranial opiate administration into the central nucleus on the acquisition of conditioned heart rate responding in the rabbit. New Zealand rabbits were surgically prepared with cannulae positioned at the dorsal surface of the central nucleus. Two weeks following surgery all animals were trained using a standard Pavlovian conditioning procedure. Drug injections were delivered bilaterally in a 1.0 µl volume immediately prior to the conditioning session.

Compared to unoperated and vehicle injected control groups, central nucleus administration of the opiate agonist levorphanol (5.0 nmole) significantly impaired, whereas naloxone administration (2.5 nmole) significantly facilitated the acquisition of conditioned heart rate responding. These effects of opiate administration occurred in the absence of any significant drug effects on either baseline heart rate or on the decelerative heart rate orienting response. The effects of opiates on conditioned heart rate responding appear to be mediated by opiate receptor mechanisms because the effect obtained with levorphanol exhibited stereospecificity. Finally, data obtained from animals with cannula placements at sites around the central nucleus support the interpretation that the effects of opiates on heart rate conditioning are due to changes in opiate activity within the central nucleus region.

Recent neuroanatomical investigations have identified that the central nucleus possesses reciprocal connections with cardiovascular regulatory systems in the medulla. A role for opioid peptides in regulating these projection systems is suggested by the presence of high concentrations of opioid peptides within both the central nucleus and these medullary regions, including the nucleus solitarius and dorsal motor nucleus of the vagus. Our results support a function for an opiate sensitive system within the central nucleus of the amygdala in cardiovascular responding during Pavlovian conditioning. These results are also consistent with our earlier observations implicating an amygdala opioid peptide system in learning and memory processes (Gallagher & Kapp, Life Sci., 23, 1973-1978, 1978).

58.6 CENTRAL SCOPOLAMINE ADMINISTRATION DIFFERENTIALLY AFFECTS THE CARDIAC COMPONENT OF THE ORIENTING REFLEX AND PAVLOVIAN CONDITIONING. D. A. Powell, Shirley Buchanan, and Linda Hernandez, Neuroscience Laboratory, Wm. Jennings Bryan Dorn Veterans' Hospital, and the University of South Carolina., Columbia, South Carolina 29201

Previous experiments demonstrated that lesions which destroyed the lateral septal nucleus or dorsal hippocampus produced exaggerated Pavlovian conditioned heart rate (HR) responses in rabbits, whereas lesions of midline neocortex and ventral hippocampus attentuated conditioned HR changes. In the present experiments, these findings were extended by the intracerebral administration of the cholinergic antagonist scopolamine into these areas.

New Zealand albino rabbits were prepared with bilateral guide cannulas implanted in the (a) dorsal hippocampus, (b) lateral septum, (c) lateral ventricles, (d) caudate neucleus, or (e) neocortex overlying the septum and hippocampus. The animals were allowed to recover from surgery for one week; at this time orienting was assessed by the presentation of 20 unpaired tones (1216 Hz, 75 dB SPL, 4 sec. duration) with an intertrial interval of 45 sec. Three days later classical conditioning began. The first three days of conditioning were drug free to allow the HR CR to stabilize; three days of drug testing followed. During all 6 sessions, two tones (1216 Hz and 304 Hz/75 dB SPL, 4 sec. duration), were employed as CS+ or CS-. The CS+ was consistently paired with a paraorbital 3 mA, .5 sec., AC shock, while CS- was never paired with shock. Twenty CS+ and 20 CS- trials were randomly administered during each session with a 45 second ITI. During both orienting and conditioning, baseline HR occurring prior to the tones was compared with the HR change occurring during and after the tone in one second blocks. Prior to the beginning of the drug sessions half of the animals in each group received central injections of artificial CSF, while the remaining Ss received one of three doses, scopolamine hydrochloride dissolved in CSF solution administered in a counter balanced order over three days. These doses included 20, 40, or 80 micrograms per 1.0 µliter injection administered bilaterally over a 6 min period. The injections were made immediately prior to the beginning of an experimental session with a microburet connected to an internal cannula which was left in place for a 2 min period following the injection.

The results showed that the magnitude of the initial HR response during orienting was increased dramatically in animals in which scopolamine was injected into the dorsal hippocampus, or septum compared to control Ss with vehicle injections or Ss with scopolamine injections in cortex, caudate nucleus or ventricles. Although HR habituation was normal in drugged septal animals, it was somewhat attenuated in animals with hippocampal injections. Scopolamine attenuated the magnitude of the HR CR in septal and ventricular injected Ss compared to hippocampal injected and control Ss. The HR discrimination was abolished in ventricular injected Ss but not in the septal and hippocampal injected Ss, compared to control Ss. These findings suggest a differential effect of central cholinergic systems on the cardiac component of the orienting reflex and the cardiac component of the Pavlovian conditioned response.

58.7 SCOPOLAMINE MICROINJECTIONS INTO THE CAUDATE NUCLEUS AND INSTRUMENTAL CONDITIONING. DOSE-RESPONSE INTERACTIONS. M.P. Valle* and R.A. Prado-Alcala (SPON : J.A. Roig). Psychophysiol. Lab. Anahuac Univ. and Physiol. Dept., Natl. Univ. of Mexico, Mexico 20, D.F., Mexico.

Microinjections of cholinomimetic drugs or of anticholinergic drugs into the caudate nucleus (CN) produce improvement or impairment, respectively, of both acquisition and performance of instrumental conditioning. However, learning studies on the effects of different doses of these agents, applied to the CN, have only been done with respect to avoidance tasks.

In the present study, independent groups of Wistar rats were trained to press a lever in order to be rewarded with water (continuous reinforcement schedule), and then tested on their performance under the effects of one of several doses of scopolamine (0, 10, 15 or 30 µg), dissolved in isotonic saline, and microinjected 6 min before testing into the dorsal aspect of the anterior CN. Two control groups were also studied: non-injected and microinjected with 30 µg of scopolamine into the parietal cortex.

A highly reliable treatment effect was found. There were no significant differences between the non-injected, cortical, 0 µg and 10 µg groups. In turn, each of these groups differed from the 15 µg and 30 µg groups. As expected, a significant correlation between doses and degree of performance impairment was also found.

These data further support the notion that cholinergic activity of the caudate nucleus plays a critical role in the maintenance of instrumental performance.

A fellowship to R.A. Prado-Alcala from CONACYT-MEXICO is gratefully acknowledged.

58.8 DIFFERENT NEUROTRANSMITTER SYSTEMS OF THE AMYGDALA MEDIATE TASTE AVERSION LEARNING, RECOVERY FROM NEOPHOBIA, AND PASSIVE AVOIDANCE. M. E. Ellis* and R. P. Kesner. Dept. of Psychol., Univ. of Utah, Salt Lake City, Utah, 84112.

Many studies have implicated the limbic system, particularly the amygdala, as an important neurosubstrate for the processing of information concerning novel tastes, food toxicosis, and avoidance behavior in general. Recent research has suggested that dose-related, time-dependent pharmacological manipulations of either of two specific neurotransmitter systems of the amygdala, acetylcholine (Todd and Kesner, JCPP, 92, 1978) and norepinephrine (Ellis and Kesner, Neurosci. Absts., 4, 1978), results in retention deficits for passive avoidance training. In separate experiments the possible involvement of the cholinergic and noradrenergic systems of the amygdala in taste aversion learning and in recovery from neophobia was investigated. After adaptation to a 23 3/4 hr water deprivation schedule rats in the recovery from neophobia experiment were presented with only grape juice for a 15 min period followed 30 min later with a bilateral injection of 1 µg/µl of norepinephrine (NE) 10 µg/µl of physostigmine (PHY, an anticholinesterase) or 1 µl of special buffered saline (SBS) via stainless steel cannulas chronically implanted into the amygdala. An additional group received 1 µg NE at a 3 hr post-grape juice delay. Rats in the taste aversion experiment were presented with grape juice followed 30 min later by an injection of apomorphine (15 mg/kg, i.p.). At the onset of apomorphine-induced illness symptoms (about 5 min), the animals received a bilateral injection of 1 or 10 µg NE, 10 µg PHY or SBS via cannulas directly into the amygdala. An additional group was given 10 µg PHY at a 12 hr post-illness delay. All rats were offered grape juice again 72 hr (Test 2) following the initial taste experience (Test 1).

Recovery from neophobia was indexed by increased consumption on Test 2 compared to Test 1. In the recovery from neophobia experiment, the NE group did not increase consumption on Test 2 compared to the NE delay, PHY, or SBS groups. Taste aversion was indexed by decreased consumption of grape juice during Test 2 compared to Test 1. In the taste aversion experiment, the PHY-group did not decrease consumption on Test 2 compared to the PHY delay, NE, or SBS groups. Hence, while both the noradrenergic and cholinergic systems may be involved in passive avoidance behavior, acetylcholine appears to mediate taste aversion, but not recovery from neophobia; norepinephrine subserves processes associated with recovery from neophobia, but not taste aversion.

58.9 MICROINJECTIONS OF D-AMPHETAMINE IN THE NUCLEUS ACCUMBENS DISRUPT THE RAT'S ABILITY TO LEARN TO IGNORE AN IRRELEVANT STIMULUS. D.M. Staton* and P. R. Solomon* (SPON: R. Nixon). Dept. of Psychology, Williams College, Williamstown, MA 01267.

One of the most direct paradigms to measure an animal's ability to ignore an irrelevant stimulus is that of latent inhibition. Latent inhibition (LI) refers to the finding that nonreinforced preexposure to a stimulus retards conditioning to that stimulus when it is subsequently paired with a reinforcing or punishing event. Data from a variety of preparations indicate that this retardation of conditioning is due to the animal learning to ignore the preexposed stimulus since it does not signal a motivationally significant event.

In the present study we investigated the effects of repeated microinjections of d-amphetamine (d-amp) into either the nucleus accumbens (NA) or the caudate-putamen (CPU) on LI in the rat. We also examined the drug's effects on stereotypy and locomotor activity. Rats received 5 daily bilateral microinjections of .5µl (20µg/µl) of d-amp or the equivalent amount of saline. Half the animals in each condition received d-amp in the NA and the remaining half received the drug in the CPU. Ten minutes after the injection on Days 1 and 3, each animal was observed for a 32 minute period during which time locomotor activity and stereotypy were monitored. In agreement with previous studies, we found that d-amp in the NA increased locomotor activity while having no effect on stereotypy whereas d-amp in the CPU increased stereotypy but had no effect on locomotor activity. Saline injections had no effect on either behavior.

Testing for latent inhibition took place in a two-way avoidance paradigm immediately following the microinjection on Day 5. In Stage 1, half the animals in each of the four treatment conditions (d-amp-NA, d-amp-CPU, Sal-NA, Sal-CPU) were placed in the avoidance apparatus and received 30 tone-alone presentations. The remaining animals explored the apparatus for a corresponding amount of time. Stage 2 consisted of acquisition of the conditioned avoidance response during which the tone signaled the onset of the footshock. The results indicated that animals in both saline conditions and animals in Group d-amp-CPU demonstrated the LI effect with rats in the 30 preexposure group conditioning significantly slower than 0 preexposed controls. Animals in the d-amp-NA group, however, showed no such effect. Preexposed animals conditioned at about the same rate as nonpreexposed controls. Microinjection of d-amp in the NA but not the CPU disrupted the animal's ability to learn to ignore the irrelevant tone in Stage 1. The results are consistent with an accumulating body of literature implicating the mesolimbic system in learning to ignore irrelevant stimuli. (Supported by NIMH Grant MH-33381-01 and NSF Grant BNS 77-14871.)

58.10 APOMORPHINE REVERSES LEARNING DEFICITS IN DOPAMINE DEPLETED RAT PUPS. R. S. Wool*, D. A. Weldon, M. H. Teicher and B. A. Shaywitz. Dept. of Psychology, Hamilton College, Clinton, NY 13323 and Dept. of Ped. and Neuro., Yale Univ. Sch. Med., New Haven, CT 06510.

Neonatal rat pups that are dopamine depleted show a syndrome of transient hyperactivity and of learning deficits in both aversive and appetitive tasks. These results indicate that brain dopamine depletion is a useful model for childhood Attention Deficit Disorder (ADD). The purpose of this study was to examine the effects of the dopamine receptor agonist apomorphine on learning performance of dopamine depleted rats.

Dopamine depletion was produced by administration of desmethylimipramine (20 mg/kg, i.p.) followed by intracisternal 6-OHDA (.1 µg in 20 µL of saline) to 5 day old Long Evans rats. At 6 days of age dopamine depleted and control rats were anesthetized with ether and fitted with intratongue cannulae (Hall, Science, 1979, 205, 206). One day later animals were conditioned to presentation of licorice odor paired with intraoral Enfamil for 20 sec at each of 5 1-min conditioning cycles. On the next day, animals were tested in a rectangular apparatus with a wire screen floor; licorice extract was placed under one half of the apparatus. Learning of the classically conditioned association was measured by the percentage of a 5 min test period spent over the conditioned stimulus (not counting time spent straddling the border of the two halves of the apparatus). Prior to conditioning and testing, animals were injected with either apomorphine (.5 mg/kg, s.c.) or an equal volume of vehicle (saline).

In animals that received saline prior to both conditioning and testing, control pups spent 83% of their time over the conditioned stimulus compared to performance of only 36% by dopamine depleted animals. In control animals, apomorphine administration prior to training, testing, or both phases produced reductions in the amount of time spent over the licorice side. In dopamine depleted animals, conditioned performance was greater for pups receiving apomorphine prior to conditioning in comparison to those pups receiving saline ($\bar{X}_{Ap}$ = 63%, $\bar{X}_{Sal}$ = 35%). Apomorphine administration prior to testing did not affect performance.

These results show that the learning deficits produced by dopamine depletion can be reversed by a manipulation increasing dopamine receptor activity prior to conditioning. In addition, they demonstrate that the effects of dopamine depletion in rat pups can be generalized to a different strain of rat (Long Evans) than had been studied previously (Sprague-Dawley).

58.11 AMNESIA IS INDUCED IN CHICKS BY CERTAIN PROLINE OLIGOPEPTIDES. Joel L. Davis and Arthur Cherkin. Psychobiology Research Laboratory and GRECC, VA Medical Center, Sepulveda, CA 91343.

During a structure-function study of the amnestic potency of L-proline (L-PRO) we found L-prolyl-L-proline (L-PRO-L-PRO) to be even more potent (Van Harreveld, Cherkin and Davis, Pharm. Biochem. Behav., in press). We therefore started a systematic study of small proline peptides (see Table). We report here the amnestic properties of L-PRO-L-PRO and L-prolyl-glycyl-glycyl (L-PRO-GLY-GLY). We injected chicks intracerebrally with 10, 5 or 2 µl/hemisphere of 300mM peptide, 1 min after one-trial training to suppress the peck response to a bead. Suppression was conditioned by coating the bead with an aversive liquid (methyl anthranilate). Retention was tested 24 hr later using the uncoated bead; impaired memory is indicated by increased peck scores (mean sq. rt. of pecks in 10 sec) and reduced avoidance scores (percentage of chicks that avoid pecking for 10 sec).

The results demonstrate that L-PRO-L-PRO is amnestic at the same dose as L-PRO (6.0 µmols). At a dose of 3.0 µmols, L-PRO-L-PRO is not amnestic (nor is L-PRO). L-PRO-GLY-GLY is amnestic at doses of 6.0 or 3.0 µmols but not 1.2 µmols. Amnesia produced by L-PRO-GLY-GLY and L-PRO-L-PRO is retrograde because no effect was observed when injection was delayed for 1 or 4 hr after training.

The results with glycyl-L-proline (GLY-L-PRO), L-prolyl-glycine (L-PRO-GLY) and L-prolyl-L-leucyl-glycine-amide (L-PRO-L-LEU-GLY-NH_2) are in the direction of enhanced memory retention; we are exploring this possibility with a paradigm which is more sensitive to enhancement effects.

Peptide	Dose (µmols/chick)	N	Peck Score ($\sqrt{p}$±S.D.)	Avoidance Score (%)
L-PRO-L-PRO	3.0	20	0.60±1.17	70.0
L-PRO-L-PRO	6.0	167	2.03±1.44*	24.6*
L-PRO-GLY-GLY	1.2	30	1.10±1.50	53.3
L-PRO-GLY-GLY	3.0	30	1.71±1.50*	40.0
L-PRO-GLY-GLY	6.0	29	1.78±1.22*	20.7*
L-PRO-GLY	6.0	30	0.39±0.75	73.3
GLY-L-PRO	6.0	30	0.47±1.10	83.3*
L-PRO-L-LEU-GLY-NH_2	6.0	29	0.54±0.86	65.5
L-PRO†	6.0	304	1.59±1.49*	34.5*
D-PRO (control)†	6.0	296	0.77±1.19	56.1
No injection†	0	50	0.72±1.09	60.0

* Differ significantly from D-PRO ($p<0.01$; t-test for peck score; χ^2 test for avoidance score).

† Data from Van Harreveld, Cherkin and Davis (in press).

58.12 AN EVALUATION OF DRUG-INDUCED LEARNING FACILITATION ON A REPEATED ACQUISITION BASELINE. D. G. Spencer,Jr.* and G. A. Heise* (SPON: D. Schroeder). Dept of Psych., Indiana University, Bloomington, Indiana 47405.

Rats were trained on a two stimulus (panel light and tone) go-no go discrete trial discrimination task. Trials were generated by responses on one lever on a fixed interval 30 sec. schedule. Responses on another lever during the trial were counted as discriminative responses. Incorrect responding on a trial produced a correction trial next, which consisted of the same stimulus. Each session was terminated upon meeting a performance criterion and on the next session, the response requirements for the two stimuli were reversed (i.e., tone-go, panel light-no go became tone-no go, panel light-go, and vice-versa). Rats were maintained on this baseline until performance stabilized, at which point they were injected i.p. 30 min. before every third session with either normal saline, d-amphetamine (0.5 or 1.0 mg/kg), piracetam (100 or 200 mg/kg), or scopolamine (0.5 mg/kg). Scopolamine decreased overall responding and d-amphetamine increased overall responding, but neither drug significantly affected the course of discrimination acquisition. Piracetam did not change discrimination acquisition or responsivity but did facilitate go-stimulus correction trial performance and reduce inappropriate responding. It was concluded that piracetam and d-amphetamine improved performance in ways unrelated to the moment-to-moment learning process.

58.13 NEONATAL LEAD EXPOSURE TO RHESUS MONKEYS CAUSES A LONG-TERM DEFICIT ON A TASK FOR SPATIAL MEMORY. Edward D. Levin* and Robert E. Bowman. Primate Lab, Dept. of Psychology, U of Wisconsin, 22N. Charter St. Madison, WI 53706.

Lead is preferentially deposited in the hippocampus of the rat (Fjerdingstad et al 74, Br Res, V80, p350). Neonatal exposure of the rat to lead causes malformed synapses in the mossy fiber system of the hippocampus (Campbell et al 79, Neurosciences Abs. 1979, #493). Behavioral parallels have been noted in the syndrome resulting from neonatal lead exposure and from hippocampal damage in the rat (reviewed by Petit & Alfano 79, Pharm, Biochem & Behavior, V11, p165). In the monkey Bushnell & Bowman 79 (J Tox & Environ Health, V5, p1015) have found that neonatal lead exposure results in a long-lasting deficit in spatial reversal learning, a task which depends on proper hippocampal functioning in the monkey (Mahut 72, Neuropsychologia, V10, p65). Gaffan 74 (JCPP, V86, p1100) found impaired memory performance in monkeys with fornix transections. This experiment tests for deficits in spatial memory resulting from neonatal lead exposure.

Ten rhesus monkeys were given either 0.0, 0.29, or 0.88 mg/kg/day of lead acetate in their water for the first year after birth, resulting in blood lead concentrations of about 4, 50, or 80 g/dl respectively. When the monkeys were five years old they were tested on the Hamilton Search Task. This task uses six gray boxes in a row on a Wisconsin General Testing Apparatus (WGTA) tray. The monkey was allowed to open one of the self-closing boxes each trial with a 20 second ITI. All boxes were baited at the begining of each session and not rebaited thereafter. Thus for good performance they had to remember which boxes they had already opened. Each session was run until all the boxes had been opened or 50 trials had occurred. The total number of trials were counted until the monkey had a session where it opened six different boxes on the first six trials. A significant deficit was found with lead exposure (curvilinear regression of trials to criterion with neonatal blood lead r=0.650, p .05) There was no deficit seen with an easier criterion of five different boxes on the first five trials of a session. These results suggest that there is no deficit in learning to perform the task of opening different boxes per se but there is a deficit when the monkey's memory is severely tested. Lead seems to cause a deficit in spatial memory in monkeys neonatally exposed. This deficit is seen at least four years after lead exposure has ended.

58.14 LOW LEVEL CHRONIC LEAD (Pb) EXPOSURE INDUCES LEARNING DEFICITS IN YOUNG RAT PUPS. D. H. Taylor*, C. M. Brubaker* (SPON: R. Sherman), Miami Univ., Oxford, OH 45056 and R. J. Bull, Health Effects Research Lab., U.S. Environmental Protection Agency, Cincinnati, OH.

Eleven day old Sprague-Dawley (CD Strain) rat pups whose mothers were maintained on a 200 mg/l dosage of lead (Pb) acetate in their drinking water exhibited differences in a learning paradigm as compared to controls. No significant differences were noted between the control pups and the experimental pups with respect to acquisition rates but there were significant differences between the two groups with respect to extinction rates. Similar results were obtained in test of rat pups whose dams had been maintained on a 400 mg/l dosage of lead (Pb) acetate. These data indicate that low level lead (Pb) exposure can induce significant behavioral deficits in young rat pups.

58.15 FACILITATION OR DISRUPTION OF DELAYED SPATIAL ALTERATION PERFORMANCE IN RATS FOLLOWING HYPOXIA VS ANOXIA. John G. Marriott and Ronald E. Voightman*. Pharmacology Dept. Warner-Lambert Research Division, Ann Arbor, MI 48105

Although many theories of impaired cognitive function in humans have postulated important roles for hypoxia, as might occur with perinatal asphyxia and chronic cerebral insufficiency, few studies have been reported on the acute, proactive effects of hypoxia or anoxia upon complex behavior. Since the acute effects of hypoxia and anoxia may give insights into the mechanisms responsible for more permanent changes in behavior, we studied these effects upon a complex behavior, delayed spatial alternation (DSA), in highly trained rats.

Male, hooded rats of the long evans strain, which had been trained for a minimum of 3 months, were subjected to experimental hypoxia or anoxia immediately prior to testing on the DSA task. Hypoxia was induced by placing the rats in an airtight chamber for 30 min and replacing the air with a mixture of 5.11% oxygen in nitrogen at a flow rate of 7 liters/min. The animals were taken from the chamber at the end of the 30 min treatment and placed in a two-lever operant chamber for the behavioral test session which lasted 100 min. Other groups of animals were placed in an open cyclinder containing carbon dioxide gas for 10 sec to produce anoxia. These animals were revived and allowed 3 min to recover prior to being placed in the operant chamber. Performance on the task was assessed in terms of percent correct responses at each of the retention intervals tested (15, 30, 60, & 90 sec), number of trials completed, and latency to initiation of responding.

Animals treated with 10 sec of anoxia were found to be significantly impaired in all of the measures taken relative to control (untreated) performance. In contrast, animals treated with 30 min of hypoxic conditions actually improved over control values on the percent correct responses measure. Following hypoxia, animals completed fewer trials than during control session. Although the latency to begin responding was significantly delayed, the rate of responding once initiated was not different from normal. Facilitation of performance following hypoxia was apparent for the duration of the test session once the animals began responding.

These results indicate that the behavioral consequences of oxygen deprivation depend upon the nature of the treatment employed. Anoxia and hypoxia apparently have very different effects upon brain function and these effects persist for some time following treatment. These differences may provide insights into the mechanisms responsible for more permanent changes in behavior seen many hours or days following treatment.

59.1 SPROUTING AND SYNAPSE FORMATION PRODUCED BY COLCHICINE.
S. Rotshenker. Dept. of Anatomy & Embryology, Hebrew University-Hadassah Med. Sch., Jerusalem, ISRAEL.

In previous studies (Rotshenker, S.,J. Physiol.,292:535,1979; Rotshenker, S. and Reichert, F., J. Comp. Neurol., in press), we have shown that axotomy of the nerve to one cutaneous-pectoris muscle of the frog induced the intact motor neurons that innervated the intact muscle on the opposite side to sprout and form additional synapses with already innervated muscle fibers. As a consequence, muscle fibers innervated by a single motor neuron became polyneuronally innervated. It was further suggested that the axotomy initiated a signal for sprouting and synapse formation that was transferred transneuronally across the spinal cord from the injured nerve cells to the responding motor neurons. Axotomy could produce this signal by interfering with some trophic interactions between the denervated muscle and injured nerve. It was of interest to examine whether colchicine that blocks axonal transport, and thereby may interfere with such trophic interactions, will imitate axotomy in producing sprouting and synapse formation. Colchicine (0.01 M) was applied topically (5 min.) on nerves innervating left muscles at the axilla. The drug did not produce conduction block, degeneration or denervation. Left and right cutaneous-pectoris muscles were examined electrophysiologically to determine the proportion of muscle fibers innervated by two motor neurons. In intact muscles of normal frogs about 16% of the muscle fibers are polyneuronally innervated. Following colchicine application to left nerves the incidence of polyneuronal innervation increased in right intact muscles 2.5 fold to 38±4% (S.E.M.) starting about 2.5 weeks after the operation. This alteration in the pattern of motor innervation is similar in magnitude and timing to that obtained in intact right muscles following contralateral axotomy at the axilla. Sprouting and synapse formation also occurred in left muscles whose nerves were treated with colchicine. However, in left muscles new synapses were detected as early as 2 days after the operation and the incidence of polyneuronal innervation reached 29±2% (S.E.M.). No alteration in the pattern of motor innervation was detected in either left or right muscles after the application of Ringer to the nerve or colchicine at a distance of 1mm away from it.

59.2 IMPULSE ACTIVITY EVOKES COLLATERAL SPROUTING OF INTACT NERVES INTO AVAILABLE TARGET TISSUE.
B. Nixon+, P. Jackson, A. Diamond*, A. Foerster and J. Diamond. Department of Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5.

We have become interested in the possibility that the collateral sprouting of intact axons may be influenced by impulse activity in them. We showed previously (Jackson and Diamond, Soc. for Neurosci. Abstr. #2135, 1979) in adult rats that repeated testing, to determine whether collateral sprouting of nociceptive afferents had occurred into adjacent denervated skin, apparently was itself responsible for the marked recovery of function in these regions; operated animals that were not examined in the interim period showed no significant recovery 24 days after denervation, although by 40 days some recovery was apparent. We suggested that activity in the remaining nociceptive afferents, elicited by the test stimuli (skin pinching), might have evoked their sprouting into the denervated skin. In order to distinguish between impulse activity per se and other influences possibly attributable to the potentially or frankly damaging pinch stimulus, we have now examined the effects of direct electrical excitation of an intact dorsal cutaneous nerve in the rat after total denervation of skin surrounding its peripheral field; two minutes of excitation (20 Hz) suprathreshold for Group III and C fibres, on days 0, 4 and 8, resulted in a complete return of sensitivity to the denervated skin by 16 days. Section of the electrically excited nerve abolished this sensitivity. We have used silver staining to detect the presence of nerve axons in skin. No axons were found in unresponsive skin that had been denervated for 16 days; however denervated skin that had regained apparently normal sensitivity over the 16 days during which the remaining intact nerve was stimulated showed numerous axons within the dermis. We conclude that the activation of impulses in the nerve, plus the presence of adjacent denervated skin, evoked collateral sprouting of the intact high-threshold mechanosensory axons. Interestingly the low-threshold ("touch" sensitive) nerves, which do not sprout collaterals in the adult rat (Jackson and Diamond, Soc. for Neurosci. Abstr. #1515, 1978) are not induced to sprout into adjacent denervated skin when they are activated continuously by physiological stimulation for as long as 40 days.

We wonder if this dramatic effect of impulse activity on collateral sprouting of the high-threshold nerves is due to their becoming more sensitive to a sprouting influence emanating from denervated skin.

+ M.D.A.C. Predoctoral Fellow.
This work was supported by the M.S. Society of Canada.

59.3 LOCALIZATION OF NEWLY FORMED PHOSPHOLIPID AND PROTEIN IN DEGENERATING AND REGENERATING MOUSE SCIATIC NERVE.
R.M. Gould, R.S. Sinatra, R.E. Madrid*, W.D. Spivack*, V.S. Weir*, and H.M. Wisniewski*, Inst. for Basic Research, Staten Island, NY 10314.

In normal sciatic nerves, autoradiographic studies have pinpointed sites of choline incorporation into phosphatidylcholine and methionine incorporation into protein in the Schwann cell cytoplasm of myelinated fibers. In contrast, tritiated myo-inositol incorporation into phosphatidylinositol was shown to be more prominent in the axon. In the present report, sites of formation of macromolecules from locally injected precursors were determined in sciatic nerves at various times after crush injury (or transection).

In one series of experiments designed to study changes in phospholipid and protein synthesis in response to injury, male mice were anesthetized, the right sciatic nerve was exposed and crushed with watchmaker's forceps #5 for 5-10 sec. The point of crush was marked with India ink or a loosely placed ligature. The wound was closed and the skin sutured. At 6 and 18 hr and 3, 7 and 14 days after crushing the nerve, tritiated precursor (choline, inositol or methionine) was injected into the nerve at points above and below the crush. The animals were sacrificed two hours later and both longitudinal and transverse sections were prepared for light microscopic autoradiography. At the earlier intervals (6 and 18 hr) axons were found to contain label suggesting either local synthesis of lipid and protein or a transfer of newly formed macromolecule into the axon. At later times (3, 7 and 14 days) only inositol lipid synthesis was present in the newly growing axons.

In a separate set of experiments designed to study changes in phosphatidylcholine metabolism in phagocytic Schwann cells, nerves were transected at the sciatic notch and one and three days later radioactive choline was injected into the distal nerve about 3-9 mm from the transection. The animals were sacrificed 20 min after injection of precursor. The pattern of phospholipid synthesis was examined in teased fiber preparations and in serial light and EM sections of individually teased fibers in order to visualize changes in the Schwann cell metabolism along an individual internode. Movement of sites of biosynthetic activity to regions along the fiber where ovoids were formed could be seen. The possible significance of altered local phospholipid and protein metabolism in Wallerian degeneration and axonal sprouting will be discussed.

59.4 ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL CORRELATES OF THE REINNERVATION OF RAT MUSCLES. IMPLICATIONS ON THE ROLE OF MEMBRANE COMPONENTS SUCH AS GANGLIOSIDES IN THE MOTOR NERVE SPROUTING.
A. Gorio, G. Carmignoto* and M. Finesso*. Department of Cytopharmacology, Fidia Research Laboratories, Abano Terme, Italy.

The Extensor Digitorum Longus (EDL) muscle of the rat was denervated by crushing the sciatic nerve.

Results have led to the conclusion that the restored nerve-muscle contact occurs only at the old end-plate regions and in no instance did we observe more than one end-plate on the same muscle fibre. First sign of reinnervation is the recording of subthreshold e.p.p.'s (1-2 mV) at the site of the nerve entrance, 2 weeks after denervation. M.e.p.p.'s are very rare about 1% of the normal level and stimulation with 30mM K^+ raises the frequency to only a few per minute. However the effectiveness of K^+ stimulation is unchanged because the m.e.p.p. rate is increased 100 fold as in normally innervated muscles. The nerve and K^+ evoked releases suggest a quick recovery of the voltage dependent Ca^{++}-channel. However the m.e.p.p. frequency at rest returns normal in 2 weeks suggesting 2 different mechanisms for releasing quantal Ach. The response to hypertonic solution is further delayed and reaches normal value only 7 weeks after the crush.

Multiple innervation is apparent 3 days after recording the e.p.p. and reaches the maximum value of 70% in 2 weeks. Daily treatment of the animal with gangliosides induces a strong neurite outgrowth and the maximum level of polyinnervation is reached in about 2 days. This degree of polyinnervation is maintained and it declines to normal level parallel to the untreated muscles in spite of the continuous treatment. This suggest that gangliosides enhances a natural occurring phenomenon and when the stimulus to sprout ceases the ganglioside effect does also.

To further study the sprouting a good model is the collateral reinnervation of the Soleus muscle after resection of the L_5 root. The data clearly show that the best reinnervation process occurs when the sprouts are myelinated and so the innervation can be held.

The Soleus muscles of the animals treated with gangliosides showed a much better recovery compared to the untreated ones, showing after 30 days a full recovery which was kept even 50 days after resection of the L_5 root. This data again suggest that sprouting may be regulated by a dual phenomenon -an endogenous stimulus, perhaps coming from the muscle, and a need for membrane structures such as gangliosides.

59.5 INTERACTION OF AGE AND SEX IN SYMPATHETIC AXONAL INGROWTH INTO THE HIPPOCAMPUS. Teresa A. Milner, Joyce F. Nonaka* and Rebekah Loy. Dept. of Neurosciences, Univ. of Calif., San Diego, La Jolla, CA 92093

Although axonal sprouting in response to central nervous system damage has been described in a variety of systems, exactly what mechanisms regulate the stimulation of growth or the final distribution of terminals is unknown. One factor that influences the rate as well as the extent of intrinsic afferent reorganization in the hippocampus is the age of the animal at the time of afferent damage. Another factor that appears to influence axonal sprouting is the hormonal environment. Our findings indicate that damage to the septal afferent axons of the hippocampus elicits a post-lesion ingrowth of vascular sympathetic axons that is greater in female than in male rats. This sexual dimorphism may be due to a direct hormonal regulation of axonal growth and post-lesion terminal reorganization or to a differential organizing influence of hormonal exposure during development. To distinguish between these possibilities, as well as to examine the role of other developmental influences on axonal sprouting in this system, we have studied sympathetic axon ingrowth following damage of the septal afferent system in male and female rats of three ages. After 30 days, the brains of these animals were analyzed using either the cryostat glyoxylic acid method of fluorescence histochemistry or high affinity uptake of noradrenaline.

We find that (1) the sprouting which is reduced in adult and juvenile males relative to females, is equivalent in the two sexes after transections at postnatal day 3 (P3) and (2) the period of maximum ingrowth occurs near P3 in the male and P13 in the female.

This suggests that both the age and sex of the animal interact to determine the final extent of sympathetic sprouting. Since sexual determination probably occurs by P7, this might account for the equivalent sprouting in males and females lesioned at P3. While maximum ingrowth occurs during different developmental stages in males and females, both occur prior to adulthood. This could reflect developmental changes in the stimuli released by the damaged afferent, the growth capacity of remaining afferents, or the synaptic receptivity in the deafferented target tissue.

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59.6 SYMPATHETIC AXON INGROWTH INTO HIPPOCAMPUS IN ABSENCE OF PYRAMIDAL CELLS. Gary M. Peterson and Rebekah Loy. Dept. of Neurosciences, UCSD School of Medicine, La Jolla, CA 92093.

Noradrenergic (NA)-containing axons, originating in the superior cervical ganglion, grow into the parenchyma of the hippocampal formation (HF) following disruption of the septal input (Loy, Milner and Moore, 1980). This anomolous innervation apparently arises as collateral sprouting from the normal sympathetic plexus on the longitudinal hippocampal arteries. The primary sites of innervation within the HF are adjacent to the granule cell and CA3 pyramidal cell layers. To determine the target of this anomolous innervation the pyramidal cells of CA3 were selectively destroyed by intraventricular injections of kainic acid (KA).

KA (0.8 µg in 0.8 µl saline, pH 7.4) was injected into the lateral ventricle over a period of 30 min. This produces selective loss of pyramidal cells in field CA3 (Nadler, Perry and Cotman, 1978). Five days after KA injection the fimbria was transected bilaterally. Four weeks after the transection the brains were removed and prepared for fluorescence histochemistry using the glyoxylic acid method of de la Torre and Surgeon (1976). One set of adjacent sections were stained with cresyl violet for assessment of cell loss and a second set were stained for acetylcholinesterase for the assessment of changes in the cholinergic innervation of the HF.

The plexus of coarse, intensely fluorescent catecholamine fibers which appears after fimbria transection was observed in strata lucidum, pyramidale and oriens of CA3 both in regions where pyramidal cells were intact and in regions where they had been destroyed by KA. In the fluorescent material the damaged pyramidal cells could be identified by the appearance of autofluorescent "ghosts" which were either glia or fragments of the destroyed pyramidal cells. The density of fibers did not appear to be increased or decreased in the damaged portion of the pyramidal layer as compared to adjacent areas where the cells were intact.

We conclude from these data that the sympathetic NA axons which grow into the HF following fimbria transection terminate on a cell population other than the pyramidal neurons. Previous studies in this lab have shown that within the fascia dentata these fibers form both terminal-type associations with the intracerebral arterioles and capillaries, and synaptic contacts with neuronal elements adjacent to the stratum granulosum. Whether the target in CA3 is a population of interneurons or glia cannot yet be determined. However, a model put forth by Mosko, Lynch and Cotman (1973) showing the septal input terminating on inhibitory interneurons suggests tha removal of this input might allow ingrowth of sympathetic fibers to these interneurons.

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59.7 SYMPATHOHIPPOCAMPAL SPROUTING: IS ZINC THE LINK? Keith A. Crutcher and James N. Davis, Dept. of Medicine (Neurology) and Pharmacology, Duke University Medical Center and Veterans Administration Medical Center, Durham, N. C. 27705.

We have previously shown that sympathetic neurons appear in the rat hippocampal formation specifically in response to septo-hippocampal (cholinergic) denervation. Such sprouting occurs in the absence of afferent input to the superior cervical ganglion indicating that some change in the target initiates the sprouting response. The fact that both septohippocampal and sympathohippocampal fibers are distributed with a topography similar to that of granule cell axons (mossy fibers) led us to test the hypothesis that granule cells are required for sympathetic sprouting. We partially eliminated granule cells with neonatal irradiation or adult colchicine injections into the hippocampal formation. Septal lesions placed 2 months following irradiation or 2 - 4 weeks after colchicine injections resulted in sympathohippocampal sprouting only in regions where granule cells remained. The sprouts were distributed in the same areas where remaining mossy fibers could be visualized in adjacent sections stained for heavy metals (Timms method). These results support the hypothesis that granule cells, particularly the mossy fibers, are the targets of sympathohippocampal fibers. Since septohippocampal and sympathetic neurons exhibit specific transport of Nerve Growth Factor (NGF), a zinc-containing protein, and since both septo- and sympathohippocampal fibers are distributed in close association with the zinc-containing axons of granule cells (mossy fibers) it is tempting to speculate that granule cells produce a zinc-containing tropic factor. Such a factor might normally be transported by septal fibers. If so, septal lesions would result in accumulation of the hypothetical factor and subsequent sympathetic sprouting. This hypothesis, although speculative, would account for the distribution of sympathohippocampal fibers and for the specificity of the sprouting which occurs only in response to loss of septohippocampal fibers.

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59.8 THE SYMPATHETIC INNERVATION OF THE HABENULA. Ron Philo* and Zehava Gottesfeld. (SPON: J.A. McConnell). Dept. of Neurobiol. & Anat., Univ. of Texas Med. Sch., Houston, Texas 77025.

The use of histofluorescence demonstrated that noradrenergic innervation of the habenula (Hb) stems from central (Fuxe, 1965) and from peripheral superior cervical ganglia (SCG) (Bjorklund et al., 1972). It has been reported recently that following partial deafferentation of the Hb by lesions in the stria medullaris (SM), sprouting of noradrenergic axon terminals was observed in these nuclei (Gottesfeld, 1980). The source of the sprouting neurons has yet to be determined. This work has been undertaken to study biochemically, first-the regional distribution of norepinephrine (NE) within the Hb, second-to assess the contribution of the SCG to noradrenergic innervation of this region and third-to determine whether or not the SCG neurons have the capacity to sprout in deafferented Hb. The experimental paradigm consisted of the following groups of male Sprague-Dawley rats (200g) :1) animals with bilateral superior cervical ganglionectomy (SCGX) were allowed to survive for 5,14 and 42 days following surgery, 2) rats with SM lesions for 25 days were then ganglionectomized and allowed to survive for an additional 10 days, 3) animals with SM lesions for 35 days and 4) sham-treated control rats for each experimental group. The rats were killed by decapitation and brain sections (300 µM thick) were cut in a cryostat at -8°C. Tissue punches were removed from the Hb region in 7 consecutive frontal sections. NE was assayed by the method of Coyle & Henry (1973). The results demonstrate regional differences in NE distribution in the Hb, with the highest NE levels in the core regions. After SCGX, NE was significantly decreased at 5, 14 and 42 days ($P<0.008$, $P<0.03$, $P<0.02$, respectively) in only one site, i.e. A4230, according to Konig and Klippel, 1963. After SM lesion for 35 days, NE levels were increased in all Hb regions, and this increase was not affected by SCGX. It is concluded that noradrenergic sprouts in the Hb observed after SM lesion, are of central origin, not from the peripheral sympathetic system.

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59.9 GANGLIOSIDE MEDIATION OF IN VITRO NEURONAL MATURATION. F. Roisen and H. Bartfeld*, Dept. of Anatomy, CMDNJ-Rutgers Medical School, Piscataway, NJ 08854, and the Amyotrophic Lateral Sclerosis Research Center of St. Vincent's Hospital and Medical Center, NY, NY 10011.

Gangliosides have been suggested to be membraneous components which may play a significant role in the regulation of a variety of neuronal events including: differentiation, defense, growth, regeneration, trophic interaction and transformation. To examine the potential role of these glycosphingolipids on in vitro neuronal maturation, bovine brain gangliosides were incorporated into culture media (in the presence or absence of heat-inactivated fetal calf serum) and applied to established neuronal lines (Neuro 2a and S_{20} neuroblastoma) and primary explant cultures of chick embryonic sensory ganglia (DRG). The effects, of coded samples, of the test media on these different culture systems were evaluated morphologically and biochemically. The mean number and length of cell processes in a given unit area were repeatedly determined with high resolution light microscopy and provided a semiquantitative index of neuronal maturation. A biochemical measure of growth was obtained by determining the activity levels of ornithine decarboxylase (ODC), the rate limiting enzyme in the polyamine biosynthetic pathway.

Media containing the ganglioside mixture enhanced significantly the degree and rate of fiber formation as well as the ODC activity of Neuro 2a neuroblastomal cultures. In contrast, no significant differences were found between S_{20} neuroblastomal cells treated with either control or ganglioside supplemented media (GSM). Primary cultures of DRG exposed to GSM exhibited a significantly greater mean number of neuritic processes than that found in control cultures. The mean neuritic lengths of the control and GSM groups were not statistically different. The GSM also enhanced significantly the ODC activity of the DRG cultures in the presence or absence of serum.

These results are consistent with the notion that gangliosides can be incorporated into the axolemma where they can then function as "acceptor" molecules for growth promoting substances. The possibility of a synergistic relationship between gangliosides and nerve growth factor will be discussed.
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59.10 PUTRESCINE FACILITATES BEHAVIORAL RECOVERY FROM LESIONS OF THE ENTORHINAL CORTEX IN RATS. J. Ramirez*, J. Venditto* and D. Stein (SPON: B. Fass). Brain Res. Lab., Clark University & U. Mass. Med. Center, Worcester, MA 01610

Studies have confirmed that partial deafferentation of the dentate gyrus (DG) caused by entorhinal cortex (EC) damage results in proliferation of remaining septal and hippocampal afferents (Lynch & Cotman, 1975). Additional evidence has suggested that sprouting may play a role in behavioral recovery after EC lesions (Loesche & Steward, 1977). To date, however, little is known about the specific substrates that mediate behavioral recovery and synaptic proliferation. Trophic substances such as NGF have been implicated but polyamines such as putrescine, spermidine and spermine may also be involved. Polyamines are involved in cellular growth and differentiation. They facilitate both translation and transcription by enhancing the activity of DNA replicase, DNA dependent RNA-replicase, DNA dependent RNA-polymerase and t-RNA-methyltransferase (Shaw,1979). Increased turnover of ornithine decarboxylase, the rate limiting enzyme in polyamine synthesis, has been detected in brain during development and regeneration after injury.

The present study examined whether putrescine application to a system undergoing collateral sprouting might facilitate behavioral recovery. Accordingly, rats sustained bilateral lesions of EC followed by either injection of saline or putrescine (0.2 M) bilaterally into the DG of the dorsal hippocampus. Two days after surgery the rats were tested on a Y-maze for retention of a preoperatively learned alternation task.

The results show that animals given putrescine required significantly fewer days (X=22.3) to relearn the alternation task after surgery than rats treated with saline (X=32.2). Previous research suggests that sprouting in the hippocampus may be related to behavioral recovery. Putrescine may have contributed to more rapid behavioral recovery by enhancing the biochemical processes associated with sprouting. Further studies examining the morphological consequences of polyamine treatments are presently being conducted. (Supported by NIA grant 2 RO 1 AG00295-04.)

59.11 LACK OF PLASTICITY IN SOMATOSTATIN SYSTEMS IN CAT LUMBAR SPINAL CORD. A. Tessler, M. E. Goldberger, M. Murray, B. T. Himes* and R. Artymyshyn* (SPON: I. Zimmerman). Dept. of Anatomy, The Med. Coll. of Penn., Phila., PA 19129.

Substance P (SP) and Somatostatin (SOM)-like immunoreactivities have been demonstrated in the spinal cord, transported there, presumably by one or more populations of dorsal root afferent fibers. Using the unlabeled antibody (PAP) technique we have found that the distribution of SOM reaction product in the lumbar spinal cord of the cat resembles that previously described in several species: SOM reaction product is densest in lamina II, is present in lateral lamina VII and sparse in lamina I, V and ventral horn. Thus, its distribution only partly overlaps that of SP. Unilateral complete lumbosacral dorsal rhizotomy diminishes only slightly the amount of SOM reaction product in lamina II; maximal decrease in intensity of staining and in width of the band of staining present in lamina II is apparent by about 10d. Combining deafferentation with additional surgical lesions, including ventral rhizotomy, contralateral complete lumbosacral dorsal rhizotomy, high and low lumbar transections, and hemisections above and below L6 together with commissurotomy, does not abolish SOM staining. These observations suggest that the SOM-containing cell bodies, apparent in various laminae normally or after colchicine application, are responsible for the residual but reduced SOM staining. The reduction in SOM after rhizotomy appears to be permanent. The pattern of change in SOM reaction product following dorsal rhizotomy therefore contrasts with that which we have previously described for SP. Although a decrease in dorsal horn SP reaction product is also apparent 10d after dorsal root section, SP reaction product subsequently demonstrates a partial return. This partial return of SP immunoreactivity mimics the normal distribution of SP staining, but differs in its appearance, being more granular and less globular. Interneurons are at least in part responsible for residual SOM and SP staining and for the subsequent increase in SP.
Supported in part by the Medical Research Service of the Veterans Administration and by NIH Grants NS14477 and NS13768.
Anti SOM antiserum was supplied by Dr. Seymour Reichlin, Endocrine Division, New England and Medical Center Hospital, prepared with support of USPHS Grant #RO1 AM16684.

59.12 ENDOCRINE INFLUENCES ON ADRENERGIC AXON SPROUTING IN THE RAT DENTATE GYRUS. Stephen W. Scheff, Georgia Sasvary* and Carl W. Cotman. Dept. Anat., Med. Center, Univ. Kentucky, Lexington, KY and Dept. Psychobiology, Univ. California, Irvine, CA 92717.

We previously demonstrated that chronic changes in the physiological levels of glucocorticoids can alter axon sprouting in the rat dentate gyrus of the hippocampal formation (Exp. Neurol.,68: 195-201, 1980). In the present study we investigated whether or not similar chronic glucocorticoid changes can modify the sprouting reaction of adrenergic neurons. Sprouting of adrenergic neurons innervating the rat septal and hippocampal areas was studied following a unilateral transection of the fimbria using a modified glyoxylic acid histofluorescence method for cellular localization of monoamines.

Young adult animals were adrenalectomized six to ten days prior to a unilateral transection of the fimbria which partially denervates the septal and hippocampal areas. At the time of surgery the animals were subjected to a subcutaneous implantation of a pellet containing either pure corticosterone or cholesterol depending upon group designation. Control animals were given a sham adrenalectomy followed by a fimbria transection and cholesterol implant. Animals administered pure corticosterone were maintained at circulating levels approximately twice those present in naive animals. Forty-five days after the transection the animals were killed, blood samples collected for RIA analysis of corticosterone levels and the brains processed for fluorescence microscopy. Both control animals and adrenalectomized animals receiving cholesterol, demonstrated an increase in noradrenergic innervation of the septum which was equivalent for the two groups in agreement with previous results. The response of the sympathetic catecholaminergic fibers in the hippocampus appeared extremely robust. Adrenalectomized animals maintained at high circulating levels of corticosterone showed significantly less noradrenergic sprouting in the septum and almost no indication of the sympathetic response. The chronic change in the level of glucocorticoids appears to significantly limit the reactive response of adrenergic neurons. These results support our contention that the effect of this hormone is widespread and can affect many different types of neurons.

Adrenergic axon sprouting has been shown to be greatly reduced in the senescent rat (Science 202:775-778, 1978), and it is also known that glucocorticoids are elevated in these animals. The present results support our contention that the diminished sprouting observed in aged animals may be partially due to the elevated glucocorticoid levels. (Supported by research grant AG 00538)

60.1 PURIFICATION OF THE SAXITOXIN RECEPTOR OF THE VOLTAGE SENSITIVE SODIUM CHANNEL FROM MAMMALIAN BRAIN. R. P. Hartshorne* and W. A. Catterall* (SPON: N. Nathanson). Dept. of Pharmacology, Univ. of Washington School of Med., Seattle, WA 98105.

Saxitoxin binds to a single class of binding sites on the sodium channel and blocks action potential propagation. The saxitoxin receptor solubilized from rat brain membranes by the non-ionic detergent Triton X-100 retains high affinity (K_D = .16nM) and specificity for saxitoxin. The soluble receptor is very labile ($t_{1/2}$ = 5 min at 36°) and requires phosphatidyl choline, Ca^{++}, protease inhibitors and low temperature for stability during purification. Receptor concentration is determined by measuring the specific binding of tritiated saxitoxin using a rapid gel filration technique. Gel permeation chromatography of the crude soluble receptor on Sepharose 6b indicates that the Stokes radius of the receptor -detergent-phospholipid complex is 79 Å. Centrifugation of the crude soluble receptor through sucrose gradients in H_2O and D_2O gives $S_{20,w}$=12.3, $\bar{v}$=0.82, and M_r=630,000 for the detergent-phospholipid-protein complex. Correcting for bound lipid and detergent using the partial specific volume ($\bar{v}$) measurement gives a molecular weight for the receptor protein of approximately 340,000.

The soluble saxitoxin receptor was purified by conventional methods. The first purification step was ion exchange chromatography on DEAE Sephadex at pH 6.0. The peak fractions were pooled, neutralized, applied to a wheat germ lectin-sepharose 4b column and eluted with an N-acetylglucosamine gradient. The peak fractions were pooled and centrifuged through 3-12% sucrose gradients. Analysis of the peak fractions of the sucrose gradient by SDS polyacrylamide gel electrophoresis revealed single major protein species of M_r 250,000. The concentration of this protein correlates well with saxitoxin binding activity in different fractions from the gradient. A protein of the same molecular weight is specifically labeled by a photoactivable derivative of scorpion toxin which binds at a different site on sodium channels in rat brain membranes (Beneski and Catterall, PNAS, 77, 639, 1980). Our results show that an M_r= 250,000 protein is the saxitoxin binding component of the sodium channel from rat brain and this polypeptide is located at or near the scorpion toxin receptor site.

60.2 VALPROATE AFFECTS THE MEMBRANE PROPERTIES AND CYCLIC NUCLEOTIDE LEVELS OF THE CRAYFISH GIANT AXON: THE INFLUENCE OF DILANTIN. Thomas M. Nosek. Dept. of Physiology, Medical College of Georgia Augusta, GA 30912.

This series of experiments was undertaken to compare the effects of valproate (VPA), a new and very effective antiepileptic, with those of dilantin (DPH) on the excitability properties of the crayfish medial giant axon. Electrophysiologic measurements were made on axons that were space and current clamped. Conducted action potentials were generated for the measurement of conduction velocity (CV). All axons were bathed in a control Van Herreveld's solution at 22°C for 1 hour before drugs were added directly to the bathing medium (pH was maintained at 7.4). VPA had a dose dependent (1 X 10^{-4} to 4 X 10^{-3}M) effect on membrane properties that was reversible. Within 30 minutes of application, VPA produced a depolarization of the membrane that was associated with an increase in specific membrane resistance. VPA also decreased the magnitude of the action potential (AP), $+dV/dt_{max}$, CV, and $-dV/dt_{max}$ and increased the duration of the action potential (AP-D) suggesting that it affects both the electrogenic Na^+ and K^+ channels of the axon membrane. 8 X 10^{-3}M VPA proved toxic, it rapidly and irreversibly depolarized the membrane, abolished the action potential, and decreased the resting membrane resistance. These effects are in contrast to those of a maximally effective dose of DPH (0.11 mM) which did not affect the magnitude of the resting or action potential or of the membrane resistance. It s significant effects were limited to a decrease in $+dV/dt_{max}$, CV, and threshold current and an increase in AP-D. The synergistic effects of DPH and VPA were evaluated by pretreating axons with 0.11 mM DPH for 30 minutes before VPA was added to the bathing medium. Under these conditions, a 30 minute exposure to VPA had a significant effect on only one membrane parameter; it decreased $-dV/dt_{max}$. Because elevated cellular levels of cAMP and cGMP have been correlated with the increased membrane activity associated with epilepsy, the effects of VPA and DPH, alone and in combination, on the cellular levels of these nucleotides (measured by radioimmunoassay) were elevated. 4 mM VPA significantly decreased the cellular levels of both cAMP and cGMP while 0.11 mM DPH affected neither nucleotide. Pretreatment of the axons with DPH abolished the effects of VPA on these nucleotides. These experiments demonstrate that: 1) VPA and DPH both depress axon excitability but in different ways; 2) the electrophysiologic effects of VPA are associated with a decrease in the cellular levels of cAMP and cGMP; 3) DPH has an antagonistic effect on the response of the axon to VPA. (Supported by NIH Grant #1-NS-6-2340 to the Georgia Comprehensive Epilepsy Program).

60.3 ACTIONS OF SAXITOXIN ANALOGS ON SQUID AXON MEMBRANES. C.H. Wu, T. Narahashi and Y. Shimizu*.(Spon: J.C. Houk). Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611 and College of Pharmacy, Univ. of Rhode Island, Kingston, RI 92881.

Several dinoflagellate toxins which are chemically related to saxitoxin (STX) have recently become available in pure forms. Neosaxitoxin and gonyautoxin-I, -II, and -IV have been isolated from the dinoflagellate Gonyaulax tamarensis and their chemical structures recently elucidated. Although animal poisoning due to G. tamarensis resembles that of saxitoxin, the mechanism of action has not been established. We now report the pharmacological actions of these new toxins on squid axon membranes under voltage clamp conditions. In addition, comparison of their actions on sodium channels may offer us some insight into the structure-activity relationship of saxitoxin action at the molecular level.

All four toxins selectively blocked sodium currents without affecting potassium currents. Like STX, they were effective when applied externally to the axon. The blocking action was reversible upon washing with the toxin-free saline solution. The kinetics of activation and inactivation of sodium conductance did not seem to be affected. However, the effective concentration of the toxins in suppressing 50% of the maximum sodium conductance varies among the toxins. Neosaxitoxin, with an additional OH group on the 1-nitrogen atom of STX, blocked sodium channels with a K_D of 12 nM. Gonyautoxin-II, with an OSO_3^- substitution on the 11-carbon atom of STX, showed a similar potency (K_D = 14.5 nM). These results are very close to the reported value for STX binding to squid sodium channels at comparable temperature. In contrast, simulaneous substitutions of the OH group on the 1-nitrogen atom and OSO_3^- group on the 11-carbon atom seem to hinder the binding of the toxin to receptors. Gonyautoxin-I and -IV with both substitutions showed a much weaker potency, their apparent K_D being 88 nM and 58 nM, respectively. Thus, we conclude that the binding to the receptors requires an optimal steric size of the toxin molecule. The present results are also compatible with the notion that the guanidinium group around the 8-carbon atom is essential for the blocking action. (Supported by NIH grant NS 14144 to T.N. and PHS grant FD-00619 to Y.S.).

60.4 pH DEPENDENCE OF BATRACHOTOXININ-A-BENZOATE BINDING TO VOLTAGE-SENSITIVE SODIUM CHANNELS. George B. Brown. Neurosciences Program, University of Alabama in Birmingham, B'ham, AL 35294.

Batrachotoxin (BTX) and the potent analog batrachotoxinin-A-benzoate (BTX-B) act specifically at voltage-sensitive sodium channels to stabilize the open conformation of the channel, causing membrane depolarization. Bartels-Bernal et al. (Proc. Natl. Acad. Sci. USA, 74:951, 1977) have previously demonstrated a pH dependence for the action of BTX on eel electroplax. BTX was inactive at pH 6.1 and active at pH 8.5. Since the tertiary amino group of BTX may have a pKa in this range these results could be explained by a lower membrane solubility or lower binding affinity of the charged BTX species, but similar results were obtained with quaternized BTX, suggesting that a titratable residue(s) with a pKa in this range at the BTX binding site might be responsible for the observed effect. I have examined these possibilities by direct binding measurements of labeled ^{3}H-BTX-B to mouse cortex homogenates as a function of pH. A major constituent of non-specific binding in this system is due to intercalation of unprotonated BTX-B into the lipid bilayer and should therefore be a function of pH. Both non-specific and specific ^{3}H-BTX-B binding were measured between pH 6 and 9. As expected, non-specific binding is a linear function of pH in this range. Specific binding however, is a maximum at pH 8.5, decreasing to zero at pH 6.0. These data indicate that specific binding measured in this pH range may be a function of titration of a histidine residue at or near the binding site. Studies have been initiated to test this possibility. Results from selective modification of histidine in situ using dye-sensitized photo-oxidation are consistent with a role for this amino acid at the BTX-B binding site. Supported by NIH grant NS15617.

60.5 DIFFERENTIAL EFFECTS OF 4-AMINOPYRIDINE ON THE EXCITABILITY OF THE PARALLEL FIBERS OF THE CEREBELLAR CORTEX AND THE DORSAL COLUMN FIBERS OF THE SPINAL CORD. J. D. Kocsis*, R. C. Malenka and S. G. Waxman* (SPON: K. CHOW). Dept. of Neurology, Stanford Sch. of Med., and Veterans Administration Medical Center, Palo Alto, CA 94304.

We have compared the effects of 4-aminopyridine (4-AP) on two central fiber systems in the rat -- (1) the fine caliber non-myelinated parallel fibers (Pf) of the cerebellum, and (2) the large myelinated axons of the dorsal columns (d.c.) of the spinal cord. Voltage-dependent potassium conductances (g_K) have been shown in several axon systems to be blocked by external application of 4-AP. Following anesthesia with urethane, field potentials were elicited through local stimulation (glass-coated tungsten microelectrodes), and recorded with glass microelectrodes (3.0 M NaCl). Extracellular DC slow potentials (SP) were also monitored. Agar pools were built around the exposed tissue to allow continuous superfusion of the surface with warmed oxygenated normal Ringer solution (NS) into which 4-AP or other agents could be added. Since the Pfs are embedded in a dense neuropil containing postsynaptic elements, the cerebellar surface was superfused with NS containing Mn^{2+} or Mg^{2+} to block synaptic transmission. A triphasic Pf volley remained after these ions were added to the NS.

Double stimulation experiments indicate that for Pfs superfused in NS or with NS plus Mn^{2+} or Mg^{2+}, conduction velocity is reduced during the relative refractory period (RRP) and is increased during the supernormal period (SNP). 4-AP (0.1 mM) added to the NS-Mg^{2+} solution (1) increased the duration of the Pf volley, (2) increased the duration of the absolute and RRPs, and (3) reduced the SNP. No changes in excitability for the myelinated d.c. fibers after 4-AP application were observed even with higher 4-AP (2.0 mM) concentrations. Superfusion of d.c. fibers with 20 mM K^+ reversibly blocked d.c. fiber conduction indicating the efficacy of the superfusion pool. Following repetitive stimulation, a negative SP was present for the Pfs in NS-Mg^{2+}, but was eliminated by 4-AP. No SP could be recorded from the dorsal columns following repetitive stimulation, but a prominent SP was present when the microelectrode was positioned deeper into synaptic zones of the spinal cord.

These results suggest that (1) g_K may be an important contributor to the recovery characteristics of central nonmyelinated axons, and that (2) central myelinated axons may lack g_K as has been reported (Chiu et al, J. Physiol. 292, 1979) for peripheral myelinated axons. (Supported in part by the NIH (R-5353 and NS-15320), the Veterans Administration, and the National Multiple Sclerosis Society (RG-1231).)

60.7 STRATEGY AND METHODS FOR "BREAKING NEURAL CODES." W. R. Klemm and C. J. Sherry. Brain Research Lab., Dept. Biology, Texas A&M Univ., College Station, TX 77843.

Summarized herein is the evidence that supports the hypothesis that neuronal action potentials (spike trains) are coded not only in terms of simple discharge rate but also are coded by certain patterns of spike intervals. Based on the nonparametric relative interval description method of Sherry (Int. J. Neurosci. 3:259, 1972), the various analyses of single-unit activity from cerebellar cortex neurons of rats seem to disclose 3 principal categories of discoveries: 1) Serial dependence of intervals does exist. This has been demonstrated with a variety of conventional statistical tests. These serial dependencies have also been shown to be independent of the (nonsequential) interval distribution variability. 2) Information theory is appropriate for evaluating spike trains. We have developed and tested methods for computing entropy for a given number of adjacent intervals, for assessing the relative entropy of any one interval in a set of intervals, for computing for a group of neurons the mean and standard deviation of entropy for specified clusters of intervals, and for transforming entropy values so that interval clusters of differing number can all be compared on the same numerical entropy scale (percent maximum entropy). In addition to the descriptive and quantitative value of such entropy measures, we have also demonstrated their utility in testing hypotheses and in making empirical correlations. 3) The nervous system seems to process spike train intervals in "bytes," not "bits," of adjacent, serially ordered intervals. Among the several lines of evidence for this conclusion is the demonstration that drug-induced (ethanol) changes in entropy of specific interval clusters seemed to involve a "linked" combination of certain interval clusters, some which increase and others which decrease in incidence. Also, by using n-dimensional Chi Square methodology, we have demonstrated that the relationships of adjacent intervals represent a Markovian process, at least of the first order, and for many neurons, of the second order (i.e. the duration of a given interval is partially determined by the duration of the immediately preceding two intervals). Finally, we showed that the relative entropy (percent maximum entropy) of interval clusters of different number does not have a Gaussian distribution but rather is distributed in rather surprising ways by the specific number and relative durations of adjacent intervals.

60.6 INHIBITION OF CALCIUM ACCUMULATION IN SYNAPTOSOMES BY PHENYTOIN. S.M. Daniels-McQueen* and J.A. Ferrendelli (SPON: J. Trotter). Div. of Clin. Neuropharm. and Depts. of Neurol. and Pharm., Washington Univ. Med. Sch., St. Louis, MO. 63110.

Although phenytoin (PHT) is one of the major anticonvulsant drugs used for the treatment of several types of epilepsy, its mechanism of action remains poorly understood. Previous studies have indicated that PHT alters conductance of both Na^+ and Ca^{2+} across cellular membranes. To better define its action on Ca^{2+} conductance, we have compared the effect of PHT with that of Mn^{2+} and tetrodotoxin (TTX) on Ca^{2+} accumulation in isolated nerve terminals (synaptosomes).

Synaptosomes were prepared with conventional techniques and Ca^{2+} accumulation was measured by radioisotope methods. In control buffer containing 132 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 10 mM glucose and 20 mM Tris, pH 7.4, synaptosomes accumulated Ca^{2+} at a rate of 2-8 μmoles/g prot/min. Increasing the $[K^+]$ in the media to 64 mM (with an equivalent reduction of $[Na^+]$) or addition of 5 μM veratridine increased Ca^{2+} accumulation 2- to 4-fold. TTX, a selective inhibitor of Na^+ conductance, blocked the response to veratridine (ID_{50}=10 nM) but had no effect on that of K^+. In contrast, Mn^{2+}, a selective inhibitor of Ca^{2+} conductance, blocked both K^+- and veratridine-induced Ca^{2+} accumulation, equivalently (ID_{50}=1 mM). PHT also inhibited K^+- and veratridine-induced Ca^{2+} accumulation. Only 35 μM PHT was needed to produce 50% inhibition of the veratridine effect; however, an equivalent inhibition of the $[K^+]$ effect required 300 μM.

The observation that PHT produces more inhibition of veratridine-induced accumulation of Ca^{2+}, an effect that is closer to that of TTX than to that of Mn^{2+}, suggests that PHT has a greater action on Na^+ conductance than on Ca^{2+} conductance in excitable tissues. Since the therapeutic blood levels of phenytoin are usually between 40 and 80 μM, the present results may also indicate that inhibition of Na^+ conductance has a greater importance than inhibition of Ca^{2+} conductance in the antiepileptic action of PHT.

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61.1 DIFFERENCES IN THE EFFECTS OF LEAD AND CADMIUM ON SYNAPTIC TRANSMISSION. G.P. Cooper, T. E. Kober* and R. S. Manalis. Depts. of Environmental Health and Physiology, Univ. of Cincinnati Coll. of Med., Cincinnati, OH 45267.

In electrophysiological experiments we have found Pb^{+2} and Cd^{+2} to be powerful synaptic blocking agents. Both Pb^{+2} and Cd^{+2} appear to block transmission principally through an interference with the Ca-mediated release of transmitter agents from presynaptic nerve terminals. In experiments using in vitro sympathetic ganglia we have shown that lead prevents the uptake of ^{45}Ca by presynaptic nerve terminals, thus reinforcing the conclusion reached previously in electrophysiological studies. However, ^{45}Ca uptake by presynaptic nerve terminals is not appreciably affected by cadmium even at concentrations great enough to completely block synaptic transmission.

These differences may be accounted for if we assume that a two-step process is involved in Ca-mediated transmitter release, step one being the passage of Ca^{+2} through the presynaptic membrane and step two the binding of Ca^{+2} to some intracellular site. Thus Pb^{+2} would be assumed to interfere at step one and Cd^{+2} at step two.

We have also shown that Pb^{+2} increases the spontaneous release of quanta of ACh in the frog neuromuscular junction. We postulated that Pb^{+2} must cause this effect by the displacement of Ca^{+2} from intracellular sites. If, now, the two-step process of transmitter release is valid and if Cd^{+2} does indeed interfere with Ca-mediated release of transmitter intracellularly, then it may be predicted that Cd^{+2} would be a more potent blocking agent than Pb^{+2} at the neuromuscular junction but would not increase MEPP frequency.

This deduction was confirmed in preliminary experiments performed in vitro on the isolated sciatic nerve-sartorius muscle preparation of the frog (Rana Pipiens). Standard microelectrode techniques were used to monitor intracellular responses from individual end-plates. Responses were recorded while the preparation was bathed in a control Ringer solution, or in Ringer's solution containing either 100 µM $PbCl_2$ or 100 µM Cd Cl_2. Pb^{+2} produced the expected dramatic increase in MEPP frequency while the MEPP frequency in the presence of Cd^{+2} remained approximately at control level. Whether Cd^{+2} may actually decrease the MEPP frequency remains to be determined.

These experiments indicate that, though both Pb^{+2} and Cd^{+2} interfere with Ca-mediated transmitter release, their subcellualr sites and mechanisms of action may be quite different (Supported by NIEHS Grants ES01494 and ES00159).

61.2 EFFECTS OF MEMBRANE FATTY ACID MODIFICATION ON NOREPINEPHRINE RELEASE FROM PHEOCHROMOCYTOMA CLONE PC12. Thomas P. Williams*, and Richard McGee, Jr. Department of Pharmacology, Georgetown University, Schools of Med. & Dent., Washington, D.C. 20007.

The pheochromocytoma clonal cell line, PC12, exhibits many properties consistent with the functional characteristics of neuronal cells, including the ability to release norepinephrine (NE) in response to a variety of stimuli. In order to assess the effects of alterations in membrane lipid composition on neuronal cell function, we have modified the phospholipid fatty acyl composition of the cell by exposing them to fatty acids (FA) as complexes with bovine serum albumin (BSA) (FA/BSA molar ratio <4, [FA] = 25 - 125 µM). We then examined the effects of these alterations on the release of newly accumulated [^{3}H]-NE in response to exposure of the cells to carbachol (2 mM) or K^+ (50 mM).

Addition of linoleate (18:2) or arachidonate (20:4) to the culture medium for 4-9 days resulted in a concentration related increase of these FA in the phospholipids (PL) of the cells. Exposure of the cells to 18:2 (125 µM) increased the PL content of 18:2 from 8.7% to 22.8% with a concomitant decrease in oleate (18:1) from 24.8% to 12.7%. At this same concentration of 20:4, the proportion of 20:4 in the PL component was increased from 14:3% to 29.0%, again with a concomitant decrease in 18:1. Exposure of the cells to 125 M 18:1 increased the 18:1 content of the PL from 24.8% to 37.8%. Similar alterations were observed in the FA composition of the neutral lipids of the PC12 cells.

The FA modified PC12 cells were examined for their ability to release newly accumulated [^{3}H]-NE. The FA modifications caused little, if any, change in the amount of [^{3}H]-NE released in response to the general depolarizing effects of 50 mM K^+. However, carbachol-stimulated release was decreased in PC12 cells modified by supplementation with 18:2 or 20:4. Addition of 18:2 resulted in a 40% decrease from control of [^{3}H]-NE released in response to 4 minutes of stimulation with carbachol. Addition of 20:4 resulted in a decrease to less than 50% of control values. In contrast, 18:1 had little effect on carbachol-stimulated release. These results suggest that the membrane FA alterations have little effect on generalized depolarization-dependent NE release, but may have substantial effects on receptor mediated release.
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61.3 LIPOSOMES CONTAINING CALCIUM CAUSE RELEASE OF ACETYLCHOLINE FROM SYNAPTOSOMES. Richard D. Crosland*, Joseph V. Martin*, and W.O. McClure (SPON: B.C. Abbott). Section of Cellular Biology, Department of Biology, University of Southern Calif., Los Angeles, CA 90007.

Iontophoretic injection of Ca^{2+} into large invertebrate neurons has demonstrated that an increase in intracellular Ca^{2+} causes the release of neurotransmitter. Iontophoretic injection of Ca^{2+} into presynaptic terminals of the mammalian neuromuscular junction is not possible due to their small size. Recent investigations (PNAS, 75: 5214, 1978) have shown, however, that liposomes containing Ca^{2+} can increase the quantal content of evoked release of acetylcholine (ACh) at the mammalian neuromuscular junction, presumably by introducing Ca^{2+} directly into the presynaptic terminals. We have extended these studies to the central nervous system by investigating the effect of Ca^{2+}-containing liposomes on the release of ACh from rat brain synaptosomes.

Small, unilaminar vesicles composed of 3% egg phosphatidylcholine were prepared in the presence of either 113 mM $CaCl_2$ or 113 mM $MgCl_2$. The liposomes were exchanged into a Ca^{2+} free, balanced saline solution containing 1 mM EGTA. Rat brain synaptosomes previously loaded with [^{3}H] choline were incubated for increasing lengths of time at 37 degrees with either Ca^{2+}- or Mg^{2+}- containing liposomes. The suspensions were pelleted, and the supernatants assayed for [^{3}H] choline, [^{3}H] ACh, and lactate dehydrogenase (LDH).

Synaptosomes incubated with Ca^{2+}- containing liposomes released increasing amounts of radioactivity with time, reaching a maximum of 40% above control at 15 min of incubation. Liposomes containing Mg^{2+}, however, caused no increased efflux of radioactivity. After 30 min of incubation there was lysis of the synaptosomes as measured by LDH activity. Tritiated ACh accounted for more than 95% of the Ca^{2+}- induced release of radioactivity, suggesting that the Ca^{2+}-induced release reflects normal physiological processes. We have demonstrated that Ca^{2+} containing liposomes can release ACh from rat brain synaptosomes, presumably by direct introduction of Ca^{2+} into the synaptosomes. These data suggest that, as in invertebrate neurons and mammalian peripheral neurons, release of neurotransmitters in the central nervous system is dependent on the intracellular Ca^{2+} concentration and not on the influx of Ca^{2+} through Ca^{2+} channels in the presynaptic membrane. In addition, liposomes may prove to be useful vehicles for delivery of other ions and membrane impermeable drugs into the synaptoplasm.

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61.4 CALMODULIN IN SYNAPTOPLASM: POSSIBLE INVOLVEMENT IN NEUROTRANSMITTER RELEASE. Hall, M.E., Babitch, J.A., & Cantrell, S.*. Chemistry Department, Texas Christian University, Fort Worth, Texas 76129.

It has been hypothesized that actomyosin, previously isolated from brain, may be involved in the mechanism of neurotransmitter release. In support of this hypothesis, it has been shown that actin and myosin can be identified in synaptic fractions from brain, and that phalloidin, which inhibits the depolymerization of filamentous actin, inhibits neurotransmitter release from synaptosomes. If transmitter release is mediated by an actomyosin mechanism, then based on actomyosin mechanism in muscle, the Ca^{++} sensitivity of transmitter release is probably confered by either Calmodulin (CaM) as in smooth muscle, or by troponin-C (TN-C) as in skeletal muscle.

We have investigated the protein constituents of presynaptic cytoplasm (synaptoplasm) using one- and two-dimensional gel electrophoresis. We have identified CaM, on the basis of comigration with purified CaM and changes in rate of migration with and without Ca^{++}. CaM is one of several synaptoplasmic proteins that binds $^{45}Ca^{++}$. To date, we have not found a protein corresponding to skeletal TN-C. Data will be presented concerning the presence or absence of other forms of TN-C, perhaps specific to brain. (Supported by Research Grant NS-12485 from the NIH.)

61.5 THE Na^+,K^+-ATPASE: A PLAUSIBLE TRIGGER FOR VOLTAGE INDEPENDENT RELEASE OF CYTOPLASMIC NEUROTRANSMITTERS. J.V. O'Fallon[1,2],* R.W. Brosemer[2]*and J.W. Harding[1]. Veterinary and Comparative Anatomy, Pharmacology and Physiology[1], and Biochemistry and Biophysics[2], Washington State University, Pullman, WA 99164.

Neurotransmitters are released from presynaptic nerve terminals in response to depolarizations resulting from the arrival of action potentials. When an action potential reaches a nerve terminal, it initiates the momentary opening of voltage-dependent Ca^{++} channels. The influx of Ca^{++} classically induces the exocytosis of transmitters from synaptic vesicles.

Elevation of K^+ concentrations in the external environment is routinely used in vitro to mimic the depolarizing effect of an action potential. Much evidence indicates, however, that K^+-induced release is only partially Ca^{++} dependent. Partial Ca^{++} dependency can imply the existence of two pools of neurotransmitters; e.g., a vesicular pool whose release would involve exocytosis and be Ca^{++} dependent and a cytoplasmic pool whose release could involve membrane transport processes and be Ca^{++} independent.

Recently, it has been proposed that inhibition of the Na^+,K^+-ATPase triggers neurotransmitter release (Vizi, E.S., Neurosci., 3:367-384, 1978). In order to establish the universality of the Na^+,K^+-ATPase as a possible trigger, the release of eight transmitters from eight regions of mouse brain was simultaneously examined. The release induced by 20 μM ouabain, a specific inhibitor of the Na^+,K^+-ATPase, was compared to that induced by 60 mM K^+. With few exceptions, all transmitters were released by either or both agents from each brain region. Potassium was superior in releasing the biogenic amines and acetylcholine while the putative amino acid transmitters were generally releasable by both agents. Calcium dependency was proportional to the superiority of K^+ over ouabain for initiating release. Measurements of tissue depolarization indicated that 60 mM K^+ is capable of depolarizing brain tissue above the threshold necessary for initiating an action potential while 20 μM ouabain is not. The pattern of release by ouabain, coupled with its failure to depolarize brain tissue at 20 μM, strongly suggests that inhibition of the Na^+,K^+-ATPase is capable of releasing cytoplasmic neurotransmitters in a voltage-independent manner.

61.6 CHOLINERGIC AGONIST-STIMULATED PHOSPHORYLATION OF TWO SPECIFIC PROTEINS IN BOVINE CHROMAFFIN CELLS: CORRELATION WITH CATECHOLAMINE SECRETION. R. W. Holz, C. E. Rothwell* & T. Ueda. Departments of Pharmacology and Psychiatry, and the Mental Health Research Institute, University of Michigan Medical School, Ann Arbor, Michigan, 48109.

The secretion of neurotransmitters and prepackaged hormones from nerve terminals and cells is usually triggered by the influx of Ca^{++}. By unknown mechanisms the rise in intracellular Ca^{++} causes storage vesicles to fuse with the plasma membrane and release their intravesicular contents to the cell exterior (exocytosis). We have investigated the relationship between protein phosphorylation and catecholamine secretion in monolayers of dissociated bovine chromaffin cells maintained in tissue culture. Cells were preincubated for 30 minutes in [^{32}P]phosphate to allow [^{32}P]phosphate to enter cells and become incorporated into ATP and other high energy phosphate compounds which can serve as phosphate donors in protein phosphorylation reactions. Although approximately 20 proteins significantly incorporated [^{32}P]phosphate during the 30 minute preincubation, during the subsequent 2.5 minute incubation carbachol significantly and consistantly stimulated [^{32}P] phosphate incorporation into only two specific protein bands, one of 56,000 daltons and the other of 99,000 daltons. The carbachol-induced phosphorylation of the 56,000 dalton protein was strongly Ca^{++} - dependent and occurred simultaneously with or just preceeded catecholamine secretion. The phosphorylation of the 99,000 dalton protein was not as well correlated with secretion. Carbachol- and Ca^{++} - dependent phosphorylation of the 56,000 dalton protein may participate in the biochemical mechanisms underlying exocytosis.

61.7 RELEASE OF CATECHOLAMINES AND PEPTIDES FROM RAT HYPOTHALAMI: SENSITIVITY DIFFERENCES TO HIGH POTASSIUM. N. B. Thoa*, N. D. Vu*, T. Moody, T. L. O'Donohue and D. M. Jacobowitz. (SPON: R. C. Colburn). NIMH, Bethesda, Md. 20205 and Howard Univ., Washington, D.C. 20059.

Individual hypothalami were dissected from male rat brains and chopped into 225 μm slices, using a Sorvall TC-2 sectioner. The samples were placed into polypropylene centrifuge tubes containing 0.6 ml of a modified Krebs-bicarbonate buffer aerated with O_2 (95%) and CO_2 (5%). Release was observed at 37°C and 2 minute fractions were collected. Epinephrine (E), norepinephrine (NE) and dopamine (DA) were assayed using a sensitive radioenzymatic method. The peptides bombesin, α-MSH and substance P were measured by radioimmunoassay. Fractions collected prior to KCl contained the catecholamines (CA) in the following proportions: NE = 70%, DA = 25% and E = 4%. KCl (50 mM) increased NE release 5 fold, E release 10 fold and DA release 12 fold and the proportions of the CA following KCl were: 46% NE, 47% DA and 6% E. All peptides slightly increased release of CA following KCl (50 mM). At 75 mM, KCl induced about a 3 fold increase in the release of all 3 peptides. Both CA and peptides released by KCl were calcium dependent.

The preferential release of DA by KCl suggests that, in the rat hypothalamus, DA is not just a precursor of NE. The lower sensitivity of the peptide release in response to KCl suggests that peptide and CA-containing vesicles are different with respect to storage and mobilization properties in response to depolarizing stimuli.

61.8 ANALYSIS OF TRANSMITTER RELEASE: NEW EQUATIONS FOR QUANTAL RELEASE PARAMETERS. M.D. Miyamoto. Dept. of Pharmacol., E. Tenn. St. Univ., Col. of Med., Johnson City, TN 37601.

The last decade has seen a proliferation in the use of binomial release parameters. The advantage is that they may allow investigation into the subcellular release mechanism, if, e.g., p represents a Ca-dependent probability of release and n reflects the no. of releasable synaptic vesicles.

However, Brown et al. (PNAS 73: 2913, 1976) and Barton & Cohen (Nature 268: 267, 1977) have indicated that large variations in p (var p) or n (var n) may bias the estimates, with the calculations becoming meaningless. With computer stimulation, they show that the calculated p (w/o compensation for var p) is always larger than the 'true' (programmed) $\bar{p}$, and vice versa for $\bar{n}$. McLachlan (Int. Rev. Physiol. 17: 49, 1978), by contrast, indicates that the example used is an extreme one, with 1/5 of all p = 1 and 4/5 = 0, which raises the question of whether n with small p contributes much to the estimate and whether the smaller estimates for n may not, in fact, be more appropriate.

Nevertheless, the arguments by Brown et al. have drawn enough attention that use of binomial statistics has become widely criticized. The only means of resolving this impasse is to derive equations which describe the exact contribution of $\bar{p}$, var p, $\bar{n}$ and var n. Equations for $\bar{p}$ and var p (eqns. (1) & (2)) are presented here.

Previous equations used the mean and variance of m (quantal content). These are simply the 1st two moments of m (Courtney, J. Theor. Biol. 73: 285, 1978) and are estimated by μ_1 and μ_2, where $\mu_i = E(m - \mu_1')^i$, i = 1,2,3... and $\mu_1' = E(m)$. From the moment generating function, the 3rd moment for the binomial is

$$\mu_3 = \Sigma p(1-p)\ (1-2p) \text{ or } \Sigma p - 3\Sigma p^2 + 2\Sigma p^3$$

By adding and subtracting the term 3/n (Σp) and substituting the term "var p" for its definition (Kendall & Stuart, The Adv. Theory of Statistics, Hafner, N.Y., 1969), one obtains,

$$\text{var } p = \bar{p}/3 - \bar{p}^{\,2} + 2/3\ \bar{p}^{\,3} - (\mu_3\bar{p}/3\mu_1) \quad \text{eqn (1)}$$

From Kendall & Stuart,

$$\bar{p} + \text{var } p/\bar{p} = 1 - \mu_2/\mu_1$$

By substituting eqn (1) into the above formula, one gets,

$$\bar{p} = \{1 - 3/2\ (\mu_2/\mu_1) + \mu_3/2\mu_1\}^{1/3} \quad \text{eqn (2)}$$

The "cost" of the additional information for these calculations is in the use of μ_3, such that one needs a larger sample size, perhaps as many as 300 independent measurements of m. Supported in part by NS 15089 and BRDG 1-S08-RR09171

62.1 FREEZE-FRACTURE OF SYNAPSES IN AN INSECT CENTRAL NERVOUS SYSTEM. Leslie P. Tolbert and John G. Hildebrand. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Synapses in the neuropil of the olfactory lobes of the brain of the moth Manduca sexta have been examined in an effort to determine the freeze-fracture appearance of these synapses. The neuropil consists of a central loose meshwork of coarse fibers surrounded by an array of dense, compact glomeruli, where all synaptic interactions between antennal olfactory axons and intrinsic neurons of the olfactory lobe occur. Olfactory-lobe neurons include both local interneurons and projection neurons, and inhibitory as well as excitatory synaptic interactions are common (Matsumoto, Neurosci. Abstr. 5: 170, 1979).

Thin sections reveal that a typical synaptic contact involves multiple postsynaptic profiles apposed in pairs to an individual presynaptic element. The presynaptic element contains a bar-shaped membrane-associated density which is flanked by synaptic vesicles and which follows a shallow groove in the membrane. Postsynaptic elements are lined by membrane-associated densities in the region opposite to the synaptic bar and vesicles.

Freeze-fracture replicas of the synaptic neuropil are heavily laden with membrane specializations that are thought to represent synapses. The most readily identifiable presynaptic elements have a striking similarity to excitatory terminals in the vertebrate CNS (e.g. Landis and Reese, J. Comp. Neurol. 155:93, 1974): they exhibit numerous plasmalemmal deformations, presumably representing sites of exo- or endocytosis, and a concentration of large (9-13 nm) P-face particles. The clusters of particles are sometimes found without associated plasmalemmal deformations, and rows of plasmalemmal pits are sometimes found without particle specializations; it is not known whether these represent synapses. Postsynaptically, the analogy to vertebrate excitatory synapses persists: large intramembranous particles aggregate in the otherwise bare E face immediately opposite to the presynaptic cluster of vesicles. The pre- and postsynaptic specializations are only rarely of the elongated shape that would have been expected for a bar-type synapse. This raises the possibility that the identified specializations in freeze-fracture replicas represent a type of synapse different from that known to be predominant from examination of thin sections. This possibility, however, seems unlikely from a purely quantitative point of view. Instead, transmitter release may occur at focal points along the bar-shaped density observed in thin sections, and the intramembranous particle specializations may represent the "active zones". (Supported by NIH grant AI 16150-01 and NIH postdoctoral fellowship F32 NS05891-02.)

62.2 QUICK-FREEZING REVEALS DIFFERENCES IN SYNAPTIC VESICLE ARRANGEMENTS IN THE TWO ELECTRIC ORGANS OF NARCINE BRASILIENSIS. Thomas E. Phillips & Alan F. Boyne. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611 & Dept. Pharm. & Exptl. Therap., Univ. MD., Sch. Med., Baltimore, MD 21201.

Narcine brasiliensis has a rapidly fatiguing main electric organ and a rapidly facilitating accessory organ (Bennett and Grundfest, J. Gen. Physiol., 44:805, 1961). Chemical fixation reveals no striking differences in vesicle arrangements that can explain these differing physiological behaviors.

We have used quick-freezing and freeze-substitution to prepare the two organs for transmission E.M. In the main organ, 24% of the presynaptic membrane has vesicles attached to it. This vesicle subpopulation represents 15% of the total terminal vesicle pool (n=16 terminals). In the rapidly facilitating accessory organ, only 7% of the presynaptic membrane has vesicles attached; this represents 5.5% of the total vesicle pool (n=12 terminals).

These results leave open the possibility that the difference in initial stores of vesicles attached to the pre-synaptic membrane can account for the difference in physiological behavior of the two organs. Further experiments on stimulated tissue are being done to investigate this possibility.

Sanes et al. (J. Cell Biol., 78:176, 1978) have shown that the basal lamina plays a role in active zone localization within nerve terminals at the frog nmj. Although there are no apparent thickening in pre- or post-synaptic membrane in the electric organ, we have recognized a dependence of vesicle attachment on the underlying basal lamina. This observation is facilitated by the general high frequency of vesicle attachments and the occurence of distinct separations between the basal lamina and the presynaptic membrane at post-junctional folds into which the basal lamina dips: no vesicles are seen directly over the fold (n=36 cases). On those occasions when the basal lamina is seen to bridge the post-junctional fold, vesicles are found with the same probability as in other regions of the nerve terminal.

Our results are consistent with the suggestion of Sanes et al., that components associated with the basal lamina play a role in organizing the differentiation of the nerve terminal.

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62.3 BASEMENT MEMBRANE (BM) COLLAGEN (TYPE IV) METABOLISM AND NEUROMUSCULAR JUNCTIONS. K. Romstedt* and B.W. Festoff. (SPON: J.L. Voogt). Dept. of Neurology, Univ. of Kansas Med. Ctr. and Neurobiology Res. Lab., VA Med. Ctr., Kansas City, MO 64128.

Recently, several genetic types of collagen (I, III and V) have been identified by immunofluorescence in cultured and developing muscle. The involvement of the extracellular matrix (ECM) in muscle development as well as in nerve-muscle interaction is receiving increasing interest. We have been concerned with the regulation of several synaptic macromolecules at the neuromuscular junction (NMJ), specifically, fibronectin, 16S acetylcholinesterase (AChE), and, recently, Type IV collagen.

The rat anterior gracilis (RAG) muscle allows easy separation of end-plate (EP+) and non-end-plate (EP-) regions as well as for denervation studies. EP+/EP- regions were dissected, homogenized in cold 0.5M acetic acid (HAc) overnight and digested with pepsin for 6 hours. The supernatant was dialyzed and then run on dodecyl sulfate gels (SDS-PAGE) in absence or presence of reductant. A large concentration of Type I collagen was found in both EP+ and EP- regions, which could be removed if extraction in cold HAc preceded pepsin digestion. In EP+ regions, 2-3 bands were detected between $\alpha_1\alpha_2$ and $\beta_{11}\beta_{12}$ bands of Type I on non-reduced gels. These did not disappear with reduction and were present but less obvious in EP- regions. Using a sensitive hydroxyproline (HyP) assay, 2-3 times the EP- content of HyP was found in EP+ regions.

To confirm these observations, ^{3}H-proline (> 100mCi/mmol; 50μ Ci per animal) was injected I.P. into 80-100gm male Sprague-Dawley rats. After 24 hours, rats were killed by cervical dislocation, RAG dissected and EP+ and EP- regions separated. Following extraction in cold HAc labelled residues were digested with pepsin. These pepsin digests were then subjected to SDS-PAGE in 0.1M Na phosphate at neutral pH. Cut dried gels were then analyzed by fluorography and autoradiography. Using this technique quantitative increase in Type IV-like bands, which did not disappear with reducing agent, was found in EP+ regions. These data indicate that (a) Type IV collagen is found in muscle, (b) it is concentrated in EP+ regions and (c) it may be a substrate for proteolytic and/or collagenolytic activity suggesting its role in plasticity and denervation changes in muscle.

62.4 IMMUNOELECTRONMICROSCOPY OF SUBSTANCE P CONTAINING SYNAPSES IN RAT SPINAL CORD. G.Nilaver*,E.A.Zimmerman,J.G.Linner*, L.Chu* and G.P.Kozlowski. Dept. of Neurology, College of P&S, Columbia Univ. New York, NY 10032, and Dept. of Neurobiology and Anatomy, The Univ. of Texas Medical School at Houston, Houston, TX 77025.

The undecapeptide substance P (SP) is found in high concentrations in areas of brain and spinal cord involved in pain perception. SP has been implicated in pain processes and has been reported to interact with the opiate peptides met- and leu-enkephalin. Previous studies (Chan-Palay and Palay,PNAS,74,1977) have reported SP labeling in synapses of rat gelatinosa to be associated with both large and small diameter synaptic vesicles. Other studies (Pickel et al, Brain Res.,122.1977) have demonstrated selective labeling only of the large dense vesicles. The present ultrastructural study of rat substantia gelatinosa immunoreacted for SP was undertaken to compare the SP containing synaptic profiles in this region with these reports, and also with those reported in other brain areas. Rat spinal cords were perfusion fixed with 2% gluteraldehyde and 3% paraformaldehyde in phosphate buffer. 50 μm thick sections through the substantia gelatinosa were cut in the longitudinal and transverse planes using a Vibratome and immunocytochemically labeled for SP using the pre-embedding method of staining. Sections were incubated in specific antiserum generated in rabbit against synthetic SP conjugated to thyroglobulin (#NSP1-4) used at a dilution of 1:1000 for 2 hours. Ultrathin sections were studied both before and after counterstaining with lead citrate and uranyl acetate. SP immunoreactivity was concentrated in lamina I and II of the substantia gelatinosa. The reaction product was localized in unmyelinated axons and axon terminals forming asymmetrical synapses with dendrites. Several of these dendrites also had other non-reactive axon terminals with similar but unlabeled synaptic structures. The majority of synapses showed immunoreactivity in the lumen of large (120 nm) dense vesicles and on the periphery of small (20-40 nm) clear vesicles. Some synapses however, showed labeling only of the large dense vesicles. Within the labeled synapses, reaction product was also noted on mitochondrial membranes. In longitudinally sectioned axons several synaptic contacts could be demonstrated along the length of the axon, with reaction product in the axoplasmic matrix and on microtubules. No positive axo-somatic contacts could be demonstrated in the substantia gelatinosa. The present ultrastructural study of SP in the rat substantia gelatinosa shows the existance of both types of synapses previously reported in this region, and is in agreement with reports of SP-ergic synapses in other brain areas.

(Supported by NIH grants AM-20337 and HD-12781).

62.5 IN VIVO STIMULATION OF THE EDINGER WESTPHAL NUCLEUS PRODUCES MORPHOLOGICAL CHANGES IN THE PRESYNAPTIC TERMINAL OF THE CHICK CILIARY GANGLION. E. Philippe and J. P. Tremblay.* (SPON. Y. Le Beux). Lab. de neurobiologie, Dépt. d'Anatomie, U. Laval, G1K 7P4.

The Edinger Westphal nucleus of one day old chicks was stimulated during one hour at 100 Hz with a monopolar electrode placed stereostaxically. The effectiveness of the stimulation was monitored by the contraction of iris. The ciliary ganglion was fixed by injecting 2% formaldehyde and 1% gluteraldehyde in the ocular cavity while the stimulation was still continued for 2 more minutes. The ganglion was then dissected out and placed O.N. in the same fixatif. Eighty microns thick longitudinal slices of the ciliary ganglion were then obtained with a Smith Falquar tissue chopper. The tissue was then postfixed in osmium, dehydrated in alcool, stained in bloc with uranyl acetate and imbedded in epon. The ultrathin sections were collected on a 150 mesh copper grid. The occulomotor nerve ending almost completely surrounds the ciliary cells in young chicks and for this reason it has been called a calyx. Eight (4 controls, 4 stimulated) longitudinal calyx sections were completely reconstructed using about 40 overlapping pictures (at 53,000X) for each. The ciliary cells whose calyx was reconstructed had to be located in square of the copper grid completely filled with epon to prevent any density modification due to shrinking of the section. The nucleus and nucleolus of the cell had to be present to insure that the section was from the central portion of the ciliary cell. This in vivo stimulation reduces significantly the density of not only the clear vesicles (control: 100.4 ± 10.0/u^2, stimulated: 31.0 ± 1.7/u^2 p=.001) but also of the dense cores (c:1.08 ± 0.53/u^2, st: 0.15 ± 0.05/u^2, p=.025). Stimulation also increases the density of the coated vesicules (c:1.06 ± 0.30/u^2, st:1.94 ± 0.12/u^2, p=.005) and the amount of vacuoles membrane (c:0.47 ± 0.09/u^2, st:0.87 ± 0.27/u^2, p=0.05). An histogram distribution of the clear vesicles also indicated an increase of the size of the clear vesicles following stimulation. These results are similar to those reported at the neuromuscular junction by Heuser (J.Cell Biol.57,315,1973) except for the lowering of dense core vesicles which has not been reported before. The amount of membrane of the different organelles (clear, coated, dense vesicles and vacuoles) was calculated following stereological corrections for the density and the size of the organelles (total organelle membrane in u^2 per calyx section = c:35.48 ± 1.38, st:14.93 ± 1.00, p=.001). We have found that the net loss of organelle membrane following stimulation is not compensated for by an equivalent increase of the plasma membrane (plasma membrane in u^2 per section = c:10.64 ± 1.14, st:12.65 ± 3.35, p=.5). These results differ therefore from those of Heuser who found that the total amount of membrane remains constant at the neuromuscular junction following in vitro stimulation. More experiments are in progress to establish if this net membrane loss is also observed after in vitro stimulation of the ciliary ganglion.

62.6 A LIGHT AND ELECTRON MICROSCOPIC STUDY OF SYMPATHETIC NEURONS INTRACELLULARLY LABELLED WITH HRP. H. Kondo*, N.J. Dun and G.D. Pappas (SPON: R.S. Schmidt). Dept. of Anat. Col. of Med. Univ. Ill. Chicago, Ill. 60612 and Dept. of Pharmacol. Loyola Univ. Med. Ctr. Maywood, Ill. 60153.

Horseradish peroxidase (HRP) was injected intracellularly by iontophoresis into single sympathetic neurons of isolated rat and rabbit superior cervical ganglia and bullfrog paravertebral ganglia. The ganglia were then processed for light and electron microscopy study. Under light microscope, the perikarya of the labelled superior cervical ganglion cells of the rat and rabbit appeared round to oval, and several long processes could be seen emanating from the soma. The majority of processes which branched extensively among the adjacent neurons were identified as dendrites. In about 30% of the neurons labelled with HRP, several varicosities, 20-70 µm in length, could be observed at the distal portion of the dendrites; some of the varicose dendrites appeared to envelop partially the soma of the adjacent neurons. The axons, on the other hand, appeared solitary and varicosities were not observed in any of the axons. Labelled bullfrog sympathetic ganglion cells were unipolar and exhibited no branching; varicosities were not observed in any of the HRP-stained axons. The ultrastructures of cytoplasmic components could be clearly observed in several rat and rabbit sympathetic neurons lightly labelled with HRP. The dendrites which measured about 1 µm in diameter contained abundant small, clear spherical vesicles 50 nm in diameter, and a few large granular vesicles of 100 nm in diameter. The dendrites made contact with perikarya and dendrites of adjacent non-labelled neurons. In these junctions, a cluster of vesicles could be seen at the contact site of the labelled neurons, and the membrane of the non-labelled neuron immediately underneath the cluster of vesicles showed a light band of electron density; the apposed plasma membranes were separated by a space of about 20 nm. Our results using HRP-labelled sympathetic neurons of the rat and rabbit show that dendrites rather than axons give rise to varicose fibers, and that dendro-dendritic and dendro-somatic synapses appear to be present in these ganglia. (Supported in part by NSF Grant BNS 77-28493 and NINCDS Grant NS 15848).

62.7 ULTRASTRUCTURE OF NEUROMUSCULAR JUNCTIONS ON A TONIC AND A PHASIC CRAYFISH MUSCLE. T. Mendelson, R.R. Hoy and M.M. Salpeter. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14850.

In crayfish, synapses on the tonic opener muscle of the walking leg have small facilitating EPSP's which produce slow, prolonged contraction, whereas synapses on the phasic fast flexor muscle of the abdomen have large, poorly facilitating EPSP's producing large graded spikes and fast, brief contractions which fatigue rapidly. Although the morphology of the neuromuscular junctions on the opener muscle has been described (Jahromi, S.S. and Atwood, H.L., J. Cell Biol. 63:559, 1974; Smith, D.O., J. Comp. Neur., 182:839, 1978) that of the fast flexor has not. This study compares the ultrastructure of the neuromuscular junctions on the fast flexor muscles with those on opener muscles.

The axon terminals on opener muscle fibers were many times larger than those on fast flexor fibers; however, the proportion of synaptically-specialized axonal membrane was three times less in opener neuromuscular junctions than in fast flexor junctions. Opener muscle terminals had many more small synapses, a profusion of large synaptic vesicles (440 A mean diameter) which often filled the entire cross section of terminal, and many small mitochondria. They were found surrounded by a thick layer of muscle sarcoplasm containing many large mitochondria.

In contrast, fast flexor terminals were much smaller, with sparsely distributed small synaptic vesicles (377 A mean diameter) and no mitochondria. They were located close to the contractile myofilaments, surrounded by a narrow band of muscle sarcoplasm with few or no mitochondria. Terminals with finestructural features associated with excitatory and inhibitory function were found on both types of muscle but presynaptically inhibitory terminals were seen only in the opener muscle.

The fact that tonic and phasic muscles have different finestructure provides a basis for correlating morphology with physiology.

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62.8 DISTRIBUTION OF COLLAGENASE IN SKELETAL MUSCLE: EVIDENCE FOR END-PLATE LOCALIZATION. B.W. Festoff, K. Romstedt* and Sharon T. Piper*. Dept. of Neurology, Univ. of Kansas Med. Ctr. and Neurobiology Res. Lab., VA Med. Ctr., Kansas City, MO 64128.

This laboratory is concerned with the neural regulation of synaptic macromolecules at the neuromuscular junction (NMJ). One such molecule has been the end-plate (EP) specific form of acetylcholinesterase (AChE) which has a 16S sedimentation co-efficient in mammalian NMJ. We have confirmed its specificity in NMJ regions of muscle, identified it in motor portions of nerve and spinal cord, calculated its transport rate in sciatic nerve and found it to be neurotrophically-regulated in rat anterior gracilis (RAG) muscle. Another molecule of interest found, but not concentrated at the NMJ, is fibronectin which we found to decrease in sarcolemmal membranes after denervation. Of particular interest is that these 2 molecules may interact at the NMJ because (a) 16S AChE appears to contain a collagenous "tail" which may "anchor" it to the basal lamina at the NMJ and (b) fibronectin has a special affinity for collagen. Types I, III, IV and V collagen have been identified in muscle using specific immunofluorescence. Type IV appears concentrated at the NMJ, but quantitative data is lacking. Since collagenase has been implicated in remodeling processes such as wound healing, in embryogenesis and in smooth muscle of the resorbing uterus, we analyzed collagenase in EP+ and EP- regions of RAG using several techniques. Collagenase is felt to be closely attached to the substrate collagen so we first assessed endogenous activity by measuring release of hydroxyproline (HyP) in 6000XG pellets or homogenates previously activated by small amounts of trypsin. Using this method endogenous activity was present in muscle with twice the activity in the EP+ region. Next we extracted collagenase using the method of Woessner and Ryan (1) and assayed activity with 2 different substrates: "native" 14C-proline-labelled guinea pig skin collagen (Dr. John Jeffrey) and ^{3}H-borohydride-reduced calf skin collagen, cross-linked with formaldehyde (as per Dr. Zena Werb). Using these techniques, (a) collagenase activity was present in muscle; (b) ratios of EP+/EP- activity was 1.06 ± 0.09 (N=7); (c) using "native" substrate, fragments of collagen, typical for the action of mammalian collagenase were found on SDS gels; (d) collagenase activity at NMJ must be tightly bound to substrate since 2X the endogenous activity was found in EP+ whereas after extraction, we found equivalent amounts throughout the muscle. Experiments to determine effects of denervation and other perturbations on activity and distribution of collagenase in muscle are in progress.

(1) Woessner & Ryan. BBA 309:397-405, 1973.

63.1 EFFECTS OF TEMPERATURE ON SPONTANEOUS ACTIVITY IN GOLDFISH CEREBELLUM MAINTAINED IN VITRO. E.J. Green* and C.L. Prosser. Department of Physiology and Biophysics, and Program in Neural and Behavioral Biology, Univ. of Illinois, Urbana, IL 61801.

Central nervous system activity in poikilotherms has been shown to be sensitive to temperature and subject to modification by thermal acclimation. Synaptic function is particularly susceptible, but the mechanisms underlying temperature effects are not known. In the present study endogenous unit activity was examined at various temperatures in slices of goldfish cerebellum.

Goldfish were rendered hypothermic by placing them in 4-5°C water until hyporeflexia and loss of equilibrium occurred. The brains were rapidly removed, cooled to 7°C, and parasagittal slices (400 microns thick) were cut with a tissue chopper (Duffy and Teyler, Physiol. Behav. 14:525, 1975). Slices were placed in a recording chamber where they were continuously perfused with oxygenated ringer solution. The slices were allowed to equilibrate in the chamber for one hour prior to data collection.

Extracellular recordings were made of single unit activity in the Purkinje cell body layer. Individual firing rates, as well as the pattern of activity of individual neurons were remarkably similar to those seen in vivo (Friedlander et al., JCP 112:19, 1976). Most units showed clear increases in firing rate (FR) with increases in temperature, and several different classes of responses were noted. For some units, increases were fairly linear, for others, FR varied exponentially with temperature. For 15°C acclimated fish, Q_{10} values (15-25°C) varied widely between neurons (0-4); however there seemed to be no correlation between initial FR (at 15°C) and Q_{10} values for individual neurons.

Temperature-induced changes in the pattern of activity were evident also. Computer generated histograms revealed that slight warming caused many units to fire in progressively longer bursts, with shorter interburst and interspike intervals. With further warming, some units became nonbursters, and assumed a more random pattern of activity. Data from fish acclimated to different temperatures is presented.

Our work with this preparation demonstrates that neuronal activity due to intracerebellar circuitry is temperature sensitive and that individual neurons respond differently to changes in temperature. Temperature modulation, in conjunction with in vitro techniques, may prove useful in the investigation of information processing and synaptic function.

63.2 EFFECTS OF LOW ETHANOL CONCENTRATIONS ON HIPPOCAMPAL CA1 FIELD EXCITABILITY IN VITRO. G. A. McLean and R. J. Person. Dept. of Physiology and Biophysics, Univ. of Okla. Health Sciences Center Oklahoma City, OK 73190.

Rat hippocampal slices were prepared and maintained in vitro with exposure to carbogen atmosphere according to standard methods (Alger and Teyler, Brain Res: 110, 463). After a preliminary equilibration period, stimulating and recording electrodes were placed in the slice and standard and somal responses were observed; thereafter a further 1 hr equilibration period was allowed. Constant current stimuli of 20-150 μA (500 μsec duration) were delivered to CA3 Schaffer collaterals. Responses were recorded in the CA1 stratum pyramidale using glass microelectrodes (2 M NaCl, 1-5 Mohm) with standard extracellular recording techniques. Ethanol was introduced into sidepools of the experimental chamber in amounts calculated to give final bath concentrations of 100-400 mg ETOH/dL bath. Final ethanol levels were verified at the conclusion of experiments by enzymatic colorimetric assay of the bath.

In 10 of 13 experiments both the population (somal) spike and population EPSP responses were significantly reduced in amplitude within 20 min of the addition of ETOH; the extent of reduction was dose-dependent. In these experiments, response amplitudes recovered substantially, returning to control amplitude with ETOH concentrations below 200 mg/dL. After 1-2 additional hours there was an irreversible decline in response amplitudes at higher ETOH concentrations. In the remaining three experiments, no early, transient reversal of the ETOH-induced response depression was observed. Rather, a slow monotonic decline in amplitude was observed reaching a plateau after 1 hour which continued for another 2-3 hours. Thereafter, responses declined again as in the biphasic experiments.

It is evident that physiological concentrations of ETOH can induce reliable changes in CNS excitability. Effects seen below 200 mg/dL were transient, suggesting that both synaptic transmission and somal spike generating machinery may recover function despite the continued presence of ETOH. These results indicate at least a limited ability of the CNS to adapt to sudden challenges of ETOH at low concentrations. At higher concentrations excitability may stabilize at a lower level than normal for a limited time, then decrease precipitously. Given low to moderate levels of ETOH, these experiments tend to support in vivo results suggesting that both the behavioral and physiological effects of ETOH are proportional to the time derivative of the blood alcohol curve.

63.3 EMBRYONIC CHICK SYMPATHETIC NEURONS GROWN IN CULTURE HAVE ACTION POTENTIAL MECHANISMS FOR Na^+, Ca^{++} AND K^+ AND SEVERAL OTHER K^+ CONDUCTANCE MECHANISMS. Barbara Pope*, Alan D. Grinnell and Paul H. O'Lague (SPON: P. M. Narins.) Dept. Biology and Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA. 90024.

The electrophysiological properties of sympathetic neurons dissociated from the superior cervical ganglia of embryonic chick and maintained in culture for several months have been investigated with intracellular microelectrodes. All cells tested (n=75) had overshooting action potentials ranging from 70-90mV in amplitude and from 1-2ms in duration measured at half amplitude. These potentials were shown to be dependent on the external concentrations of both Na^+ and Ca^{++} by use of the sodium channel blocker tetrodotoxin and conventional Ca^{++} channel blockers. In addition, preliminary evidence was obtained in these cells for the presence of several K^+-dependent conductances (gK^+). These include: 1) a gK^+ that is associated with the falling phase of the action potential (i.e. delayed rectification), 2) a gK^+ that is activated by Ca^{++} and gives rise to a long lasting (100-350ms) hyperpolarization following the action potential, 3) a gK^+ that resembles anomolous rectification seen in other neurons and finally 4) a gK^+ that has been reported in several invertebrate preparations which appears inactivated at the membrane resting potential but which can be activated by a conditioning hyperpolarization of -20 to -30mV below rest followed by a depolarization. Detailed pharmacological evidence for the distinct nature of these conductances will be presented. Supported by USPHS Grant NS12901 (PHO) and NS06232 (ADG), and Muscular Dystrophy Association Grants to PHO and ADG, and postdoctoral fellowships to BP from the MDA and USPHS.

63.4 THE CONTRIBUTION OF NEURONAL ACTIVITY TO 2-DEOXYGLUCOSE UPTAKE IN SPINAL CORD CELL CULTURES. Neville Brookes and David R. Burt. Dept. Pharmacol. & Exptl. Therap., Univ. of Maryland Sch. of Med. , Baltimore, MD 21201.

Primary cell cultures derived from the spinal cords (SC) and dorsal root ganglia (DRG) of fetal mice (Peacock et al., Dev. Biol. 30:137, 1973) contain mixed cell populations. We have applied the Sokoloff method to obtain estimates of the proportion of glucose utilization in these cultures that can be attributed to SC neurons and the extent to which this is influenced by neuronal activity.

[^{3}H]2-deoxyglucose ([^{3}H]2-DG) tracer was added to 3-week-old cultures in nutrient medium containing 25 mM glucose. Uptake was measured by liquid scintillation counting of distilled water lysates of the previously washed cultures. The rate of uptake was constant for the first 1.5 hr, but began to decline by 3 hr. The contribution of SC neurons to uptake was determined by utilizing the specific neurotoxic action of monosodium glutamate (MSG) and also by using tetrodotoxin (TTX) or Mg^{++} to suppress neuronal activity. DRG neurons are resistant to MSG neurotoxicity and are not spontaneously active.

In several experiments [^{3}H]2-DG uptake was decreased by 40-60% in cultures pretreated with 1 mM MSG to destroy SC neurons. Addition of 2 μM TTX or 10 mM Mg^{++} to control cultures eliminated 60-80% of the MSG-sensitive uptake, suggesting that most of the neuronal uptake is a consequence of electrical activity. Uptake in MSG-pretreated cultures was not significantly influenced by TTX or Mg^{++}.

Strychnine, which blocks spinal inhibition, was used to increase the electrical activity of SC neurons. Addition of 10 μM strychnine to control cultures increased uptake, but the effect was variable in the range 10-90%. Uptake of [^{3}H]2-DG in the presence of strychnine was as much as 3.7-fold greater than uptake in replicate MSG-pretreated cultures. We conclude that MSG-sensitive uptake of [^{3}H]2-DG provides a useful quantitative measure of integrated neuronal activity in SC cell cultures. (Supported by USPS grants MH29011 and MH29671.)

63.5 HYPOTHALAMIC THERMOSENSITIVE NEURONS IN CULTURE. F. Baldino, Jr. and H.M.Geller. Dept. of Pharmacology, CMDNJ-Rutgers Med. Sch., Piscataway, NJ 08854

The preoptic/anterior hypothalamus (POAH) has been shown to contain thermosensitive neurons which are assumed to play an integral role in the regulation of body temperature. It was the purpose of this study to utilize explant tissue cultures to define the intrinsic thermosensitive characteristics of POAH neurons. Additionally, tuberal hypothalamus was examined as a control to insure that thermosensitivity is not a universal characteristic of all cultured neurons.

POAH and tuberal hypothalamic explants from newborn rats were maintained on coverslips at 37°C for 3-4 weeks prior to experimentation. The temperature of the recording chamber was maintained with a temperature regulator between 30° and 42°C. Recording of single cell discharges was accomplished using a single barrel glass micropipette (5-10 MΩ impedance) and standard laboratory electrophysiological techniques. Firing rate (FR) was assessed on line with a computer at each temperature randomly chosen between 30°-42°C.

This in vitro analysis of 30 cells yielded at least 3 distinct types of spontaneously active POAH neurons. The majority of cells (62%) tested were relatively unaffected by varying the temperature of the bath between 30° and 42°C ($Q_{10} \leq 2$) and were classified as temperature-insensitive neurons. Others (30%) responded with an increasing FR to increasing hypothalamic temperature and were classified as warm-sensitive cells ($Q_{10} > 2$). Cold-sensitive cells (8%) or those whose FR was inversely proportional to bath temperature ($Q_{10} < 0.5$) were also present in POAH cultures. When synaptic activity was abolished by perfusing a high Mg^{++}, low Ca^{++} medium, the thermosensitivity of these neurons persisted. No warm- or cold-sensitive neurons were found in explants of tuberal hypothalamus.

The demonstration that thermosensitive-neurons exist in preoptic but not tuberal hypothalamic tissue culture suggests that the neuronal sensitivity to temperature may be a characteristic of the neuronal network within the POAH and not solely dependent on peripheral or cutaneous thermal information. Furthermore, the maintenance of thermosensitivity by these neurons when synaptic connectivity is suppressed indicates that the ability to perceive temperature may be an intrinsic property of these POAH neurons. Supported by NSF BNS 79-14003.

63.6 PROPERTIES OF GABA-ERGIC DENDRO-DENDRITIC SYNAPSES IN THE IN VITRO TURTLE OLFACTORY BULB. M.C. Nowycky, K. Mori,* G.M. Shepherd, Sec. of Neuroanatomy, Yale Univ., School of Med., New Haven, Ct. 06510.

Dendro-dendritic reciprocal synapses have been demonstrated anatomically in the olfactory bulbs of many species. Mitral cell dendrites have a presumably excitatory action on granule cell dendrites, which then inhibit the same and neighboring mitral cell dendrites. A variety of evidence suggests that the granule to mitral cell neurotransmitter is GABA. We have investigated the physiological and pharmacological properties of this synapses in the in vitro turtle olfactory bulb preparation.

Turtles were decapitated, the brains removed and the olfactory bulbs were placed in a modified Ringer solution. Intracellular recordings were obtained from mitral cells, some of which were identified with HRP injection. Lateral olfactory tract (LOT) stimulation produced in most mitral cells an antidromic spike, followed by a complex series of at least three inhibitory potentials: two early potentials I_1 and I_2, and a late slow potential, I_s (lasting for one to several seconds). Addition of the GABA antagonist bicuculline to the Ringer solution caused elimination of I_1 and I_2 within 25 minutes and a delayed elimination of I_s at 30 or more minutes. The conductance increase and current sensitivity which normally accompany I_1 and I_2 (and to a lesser extent I_s) were all eliminated by bicuculline. Under control conditions paired LOT volleys resulted in suppression of the test antidromic spike from intervals of 10 ms up to several seconds. Bicuculline eliminated the inhibition of the test spike.

Recordings with KCl electrodes were obtained in the absence and presence of bicuculline. Within minutes of penetration with a KCl electrode the initial hyperpolarizing potentials of I_1 and I_2 became depolarizing. I_s was frequently diminished or eliminated but never reversed to a depolarizing potential. Bicuculline greatly diminished the depolarizing potentials I_1 and I_2.

We conclude that I_1 and I_2 are GABA-ergic and chloride dependent, whereas the I_s component is not. Current experiments are directed toward examining substances which modulate the synaptic components of mitral cell responses, and analysing further the mechanism of the I_s response.

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63.7 MULTIPLE ACTIONS OF FLURAZEPAM IN THE PICOMOLAR RANGE ON CULTURED NEURONAL EXCITABILITY. J.F. MacDonald and J.L. Barker. Playfair Neuroscience Unit, T.W.H., Toronto, Ontario, M5T 2S8 and Laboratory of Neurophysiology, NIH - NINCDS, Bethesda, MD., 20205.

Spinal neurons dissociated from foetal mouse cords were grown in tissue culture for 4 weeks or more. Conventional intracellular recordings were made from single neurons and flurazepam was applied extracellularly using pressure microperfusion. Picrotoxin (50 µM) when appropriate was included in the bathing medium (Hank's plus 25 mM HEPES). In order to supress ongoing synaptic activity magnesium levels were elevated to 10 mM ($MgCl_2$).

Three depressant effects of flurazepam on the excitability of neurons were observed:

1) a dose-dependent increase in membrane conductance likely a consequence of a direct activation of membrane chloride permeability. This response was detectable with concentrations as low as 1 pM. But sustained applications of higher concentrations (> 10 nM) led to a complete desensitization. Picrotoxin failed to block this response.

2) a depression of the cell's spike-generating capacities. Low concentrations (100 pM - 10 nM) elevated thresholds for generating spikes and/or prevented repetitive firing. Higher concentrations (> 100 nM) were ineffective on cells that were sensitive to low concentrations (100 pM).

3) a depression of picrotoxin-induced convulsive activity. The presence of this drug evoked paroxysmal depolarizing events (PDEs) on a majority of cells studied. Relatively low concentrations (10-100 pM) of flurazepam attenuated the amplitude and duration of these events. This attenuation could be correlated to the depressant effects described in 1) and 2). However, higher concentrations (>100 nM) were much less effective.

These results suggest that flurazepam has multiple, concentration dependent actions on membrane excitability. However, not all neurons were sensitive nor did each neuron necessarily demonstrate each of these actions. The "bell-shaped" feature of the dose-response relation may help to explain tolerance.

63.8 EFFECTS OF NOREPINEPHRINE IN RAT HIPPOCAMPAL SLICES. M. SEGAL* (SPON: S.R. Sampson). Isotope Department, The Weizmann Institute of Science, Rehovot, Israel.

The effects of topical application of norepinephrine (NE) on membrane properties of about 150 neurons were studied in the rat hippocampal slice preparation. NE at a concentration of 10-100 µM applied in a droplet, produced a 3-5 mV hyperpolarization associated with minimal changes in input resistance, firing threshold, spike size or EPSP's. Higher concentrations of NE (1-10 mM) produced 15-20% reductions in input resistance. Application of NE on the soma produced its maximal effects, indicating that the observed lack of marked conductance changes is not due to activation of remote receptors. The effects of NE were blocked by sotalol, a beta antagonist. The NE induced hyperpolarizations were insensitive to changes in extracellular Ca^{+2} or Cl^- concentration. In the same preparation, 5HT produced hyperpolarizations associated with 25-35% reduction in input resistance and GABA produced a slight depolarization accompanied by 80-90% reductions in input resistance. These data indicate that unlike NE, 5HT and GABA activate K^+ and Cl^- conductances, respectively. The hypothesis that NE activates a Na-K pump was supported by the observations that NE effects were markedly attenuated by ouabain and low temperature.

The possible mediation of NE effects by cyclic AMP was tested in several experiments; it was found that cyclic AMP mimicks effects of NE. Also, the action of cyclic AMP was blocked by ouabain. Furthermore, IBMX potentiated the responses to NE.

It is suggested that NE activates, via generation of cyclic AMP, a Na-K pump which in turn hyperpolarizes the recorded neurone in the hippocampus.

64.1 EFFECTS OF BUSPIRONE ON SPINAL REFLEXES IN THE CAT. G. Keith Matheson and L. A. Riblet. Indiana University, School of Medicine, Evansville, IN 47732 and Department of Biologic Research, Mead Johnson Pharmaceutical Division, Evansville, IN 47721.

Buspirone HCl is a nonbenzodiazepine antianxiety agent which has been shown to be equipotent to diazepam in a double-blind clinical study in psychoneurotic outpatients with a primary diagnosis of anxiety neurosis (Am. J. Psychiatry 136: 1184, 1979). Binding studies indicate that buspirone neither inhibits nor stimulates ^{3}H-benzodiazepine binding to neuronal tissue (Trans. Am. Soc. Neurochem. 11: 193, 1980). Also unlike the benzodiazepines, buspirone has no anticonvulsant activity, interacts minimally with CNS depressants, does not cause muscle relaxation and lacks abuse potential. Thus, buspirone has a markedly different psychopharmacologic profile from other known antianxiety agents and is characterized as being anxioselective (Taylor, D.P., et al., this volume). The present study was undertaken in order to determine the acute effects of buspirone on spinal reflex mechanisms. Random source cats of either sex were anesthetized, intubated and cannulated for intravenous drug administration and blood pressure monitoring. If blood pressure fell below 75 mm Hg experiments were terminated. A paraffin boat was formed around the lumbar region of the exposed spinal cord. The temperature within the paraffin boat and the animal's core temperature was maintained at 37°C. Bipolar Pt stimulating electrodes were attached to the dorsal roots between L_6 and S_1. Recording Pt electrodes were attached to rootlets of the ventral root at L_7 or S_1 and to the dorsal rootlets at L_7. The animal was then paralyzed with gallamine triethiodide and artifically ventilated. Fluid balance was maintained with Lactated Ringer's Solution. Buspirone increased, by 100 percent or more, the amplitude of the dorsal root reflex and the monosynaptic reflex which persisted for over three hours. It was also noted from the monosynaptic reflex elicited after a conditioning stimulus pulse that buspirone increased five-fold the effect of post-synaptic inhibition. Presynaptic inhibitory mechanisms acting on the monosynaptic reflex were not significantly changed during the first two hours following drug administration but were reduced for the remainder of the experiment. This is in contrast to the effect of buspirone on the dorsal root reflex. These findings suggest that buspirone has differential effects on the presynaptic inhibitory mechanisms which generate the dorsal root reflex and modulate the monosynaptic reflex.

64.2 PHARMACOLOGICAL EVIDENCE THAT FIRING OF HIPPOCAMPAL PYRAMIDS IS RELEASED BY DISINHIBITION. N. Ropert*, K. Krnjević and R.J. Reiffenstein* (SPON: R. Capek). Anaesthesia Research and Physiology Depts., McGill University, Montreal Canada. H3G 1Y6.

In a recent study of IPSPs recorded in hippocampal neurons, a striking loss of inhibitory efficacy was observed during repetitive stimulation at frequencies > 2/s (Ben-Ari et al., Can. J. Physiol. Pharmacol. 57, 1462,1979). It was therefore proposed that a frequency-related disinhibition could account for the increase in firing observed during tetanic stimulation. In the present experiments, we have compared the effects of repetitive stimulation with those produced by several convulsants that are known to interfere with GABA-mediated inhibition. In rats under urethane, extracellular field responses were studied with multibarrelled microelectrodes, particularly at depths of about 2.0 mm from the cortical surface, where fimbrial stimulation evokes a maximal positive wave. At a very low frequency (≤ 1/s) this stimulation usually evokes nothing but a positive wave, which corresponds to the IPSP that is readily observed with intracellular recording. If the stimulating intensity is at least 3 x threshold (for the positive wave), increasing the frequency to 2 or more Hz causes a diminution of the positive wave and particularly the appearance of a population spike (initial latency about 6 ms). With increasing duration and/or frequency of stimulation, the spike increases in amplitude, and second and even third spikes appear (at intervals of 4-5 ms), so that a striking burst is generated, evidently reflecting the synchronized repetitive discharge of many pyramidal cells. (Presumably, weaker fimbrial stimulation is ineffective in generating spikes because it does not activate sufficient orthodromic excitation by commissural fibres.) An identical pattern of diminished positive field and population spikes (single or multiple) was readily generated by the following convulsants, *during low frequency stimulation*: picrotoxin (4 mg/kg i.v.), bicuculline methochloride, Na benzyl penicillin and d-tubocurarine (applied by microiontophoresis, in doses ≤ 7 nA). The intensity of firing was graded according to the amount of drug released, and the effects were usually only slowly reversible (over 1-2 min). Since all these agents have previously been shown to antagonize GABA-mediated inhibitions, and there is good reason to believe that GABA is the inhibitory transmitter that mediates fimbrial inhibition of hippocampal pyramids, these results support the idea that the release of firing is caused by reduced efficacy of inhibitory action, which is at least partly due to a diminished effectiveness of GABA (Ben-Ari et al., 1979). It is of interest that at least two naturally-occurring agents (ACh and met-enkephalin) have a comparable disinhibitory action.

Supported by the Canadian Medical Research Council.

64.3 THE EFFECT OF CAFFEINE AND MORPHINE ON ADENOSINE AND ACETYLCHOLINE RELEASE FROM RAT CEREBRAL CORTEX: INVOLVEMENT OF A PURINERGIC STEP IN THE ACTION OF MORPHINE. Z.G. Jiang*, J.W. Phillis, and B.J. Chelack*. (Spon: P.V. Sulakhe). Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Sask., Canada S7N OWO.

Methylxanthines (caffeine and theophylline) have been reported to antagonize several actions of morphine e.g. the analgesic effects of morphine in mice, and the depressant action of morphine on rat striatal neurons. Methylxanthines antagonize the depressant effects of morphine on acetylcholine (ACh) release from the guinea-pig longitudinal muscle myenteric plexus (JPET. 197,379, 1976) and intact rat cerebral cortex (Eur. J. Pharmac. 49,309, 1978). Adenosine also inhibits ACh release from the field stimulated guinea-pig longitudinal muscle preparation and cerebral cortex by a methylxanthine sensitive mechanism (JPET. 197,379, 1976), raising the possibility that morphine may reduce ACh release by a purinergic step. Groups of male Wistar rats were anaesthetized with nitrous-oxide-methoxyflurane. The dura was removed and a cup was placed on each hemisphere. The cortical surface within each cup was then incubated for 40 min with 100µl of ^{3}H-adenosine (0.1mM; specific activity of 0.1 Ci/M ICN) in physiological saline at 37°C and subsequently refilled with 100µl saline. Thereafter the cup contents were withdrawn every 15 min and replaced with fresh saline. Methylxanthines and morphine were administered through a femoral intravenous cannula. The bulk of the labelled material (> 50%) released was in the form of adenine nucleotides, inosine accounted for (20-40%), and adenosine and hypoxanthine together accounted for about 10% of the total activity. Morphine (1 mg/kg) evoked a significant increase (44%; $0.05 > p > 0.01$) in purine efflux rates during the initial 30 minutes following its administration and smaller increases during the next 30 min. Larger doses of morphine (5mg/kg) had a more pronounced effect. Prior injection of naloxone prevented the morphine-evoked increases in purine release. Caffeine (40 mg/kg) did not alter purine efflux rates. Morphine (1 mg/kg), given 30 min following prior treatment with caffeine (20 mg/kg or 40 mg/kg), evoked an increase in purine efflux similar to that of morphine alone. These findings are consistent with the suggestion that morphine suppression of ACh release may be mediated by a purinergic step. Morphine enhances adenosine release by a naloxone-sensitive but methylxanthine- insensitive mechanism. Morphine suppresses ACh release by a naloxone- and methylxanthine- sensitive mechanism. This lack of effect of caffeine on morphine elicited purine release suggests that caffeine is not a morphine antagonist *per se*, but rather that morphine suppression of ACh release involves a purinergic step.

64.4 BENZODIAZEPINE POTENTIATION OF INHIBITION IN HIPPOCAMPAL SLICE PREPARATIONS Herbert J. Doller and Forrest F. Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, 12501 Washington Avenue, Rockville, MD 20852.

Benzodiazepines have been shown to potentiate inhibitory responses in various regions of the central nervous system. The potentiation is thought to be specific for GABA mediated inhibition. We investigated the effect of bath applied benzodiazepines on the inhibition of granule cells in the hippocampal slice preparation.

The hippocampus from male Hartley guinea pigs was sliced in transverse sections with a thickness of approximately 450 um The slices were placed in a trough-like chamber, superfused at a rate of 5 ml/min with a Krebs solution saturated with 95% O_2 and 5% CO_2, and maintained at 37° C. The Krebs solution contained 124 mM NaCl, 5 mM KCl, 2.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.24 mM NaH_2CO_2, 10 mM glucose, and 26 mM NaH_2CO_2. Field potentials from the granule cell body layer were recorded in response to stimulation (0.1 Hz) of the perforant pathway. A second stimulating electrode was placed in the granule cell body layer near the CA_3 pyramidal cells. Stimulation of this region of the granule cells preceded by 10 to 100 msec every second stimulus to the perforant pathway. Such pre-stimulation resulted in the reduction in the amplitude of the perforant pathway-evoked granule cell population spike. The addition of 20 uM diazepam to the superfusing Krebs solution resulted in a potentiation of the inhibition evoked by conditioning stimulation of the granule cell body layer. The results indicate that benzodiazepines potentiate the inhibition of granule cell field potentials in the hippocampal slice preparation.

64.5 β-LEPTINOTARSIN-d: DOES IT ACTIVATE CALCIUM CHANNELS IN SYNAPTOSOMES? Jun E. Yoshino*, Ting H. Hsiao*, and William O. McClure. Section of Cellular Biology, Univ. of Southern Calif., Los Angeles, CA, 90007 and Department of Biology, Utah State University, Logan, UT, 84322.

Leptinotarsin-d (LPTd) is a protein, present in the hemolymph of the Colorado potato beetle, L. decemlineata, which stimulates the release of [^{3}H]acetylcholine from rat brain synaptosomes (Yoshino et al., J. Neurochem. 34, 635, 1980). The toxin has been purified in excess of 200 fold (Yoshino et al., Fed. Proc. 39, 281, 1980), but is not yet homogeneous. In contrast to the results reported by Hsiao and Fraenkel (Toxicon 7, 119, 1969), the most purified preparation of LPTd was not lethal to flies. In order to differentiate the activities in the hemolymph, the protein which is lethal to flies will be designated α-LPTd, and that which stimulates only the release of neurotransmitters will be referred to as β-LPTd.

The neurochemical properties of β-LPTd were examined using synaptosomes incubated with [^{3}H]choline and immobilized on Millipore filters. The relationship between the concentration of β-LPTd and the release of radioactive material produced a sigmoid curve with a Hill coefficient of 2. When the external Ca^{2+} was lowered by the removal of $CaCl_2$ and the addition of 50 μM EGTA, the curve remained sigmoid, but the amount of radioactivity released was depressed by 60%. Since the ability of β-LPTd to stimulate release appeared to be dependent upon the presence of Ca^{2+} in the external media, the uptake of Ca^{2+} into synaptosomes stimulated by β-LPTd was examined. β-LPTd stimulated a dose dependent uptake of $^{45}Ca^{2+}$ into synaptosomes. High concentrations of Mg^{2+} inhibit K^+-induced release of neurotransmitters from synaptosomes by interfering with Ca^{2+} influx through the voltage dependent Ca^{2+} channel. Both K^+-induced release and the activity of β-LPTd were depressed to the same extent when the external concentration of Mg^{2+} was increased. Verapamil (10 μM), a putative blocker of Ca^{2+} channels, inhibited the ability of β-LPTd to stimulate release by 25%. Since the ability of β-LPTd to stimulate release was not impaired by either the presence of tetrodotoxin or lowering the external concentration of Na^+, the toxin does not appear to be utilizing a Na^+-dependent mechanism to open the voltage sensitive Ca^{2+} channels. Our results suggest 2 molecules of β-LPTd are required to stimulate release from synaptosomes, and that these 2 molecules of toxin maybe activating the Ca^{2+} channel and promoting the influx of Ca^{2+}.

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64.6 EFFECTS OF PHENYTOIN ON PYRAMIDAL CELLS IN THE IN VITRO RAT HIPPOCAMPUS. N. Hershkowitz* and G. F. Ayala, Dept. Neurol., Baylor Col. Med., Houston, Tx 77030.

The effect of 35 μg/ml of phenytoin (P) on a number of membrane and synaptic properties of CA1 and CA3 pyramidal cells in the in vitro hippocampus was examined utilizing intracellular recording techniques. Each cell was used as its own control. Effects noted below would occur 15 to 30 min after the introduction of P; reversal of effect on rinse out was usually slower. No significant effect was observed on either the resting membrane potential (RMP) or input resistance (Rn) (cf table) even up to 90 min after exposure to P. However, the amplitude (Amp), rate of rise, and rate of decay of action potentials (AP), evoked by either antidromic activation or cathodal current injection, was consistently reduced (cf table). The following table summarizes combined CA1 and CA3 results in terms of means ± standard error (*indicates $p<0.05$ difference from control by paired t test):

	Rn (MOhms)	RMP (mv)	AP Amp (mv)	AP Rise (v/sec)	AP Decay (v/sec)
n	15	15	10	10	10
Control	22.2±1.4	-56.8±2.5	84.6±1.5	422.0±20.8	112.7±8.0
15-30 min in P	21.1±1.4	-56.9±1.9	77.4±2.2*	362.4±23.5*	98.8±6.2*

The effect of P was studied on IPSPs evoked in CA1 cells by either Schaffer collateral (n=8) or recurrent pathway (n=4) stimulation and in CA3 cells by mossy fiber pathway (n=5) stimulation. P did not change the duration of these events. The conductance change and reversal potential of the early IPSP, measured at its peak response, also appeared unaffected.

The effect of P on hyperexcitability in CA1 cells (n=5) induced by tetanic orthodromic Schaffer collateral stimulation (50 Hz, 10 sec) was examined. This hyperexcitability, which may last up to 1 min following tetanus, is characterized by the ability of a single orthodromic stimulation to elicit a high frequency burst of action potentials arising from an underlying depolarization. P markedly reduced the number of action potentials in the burst as well as the underlying depolarization. This effect was observed when the neuron did not exhibit a decreased ability to generate an action potential for each stimulus during the tetanus. In a few neurons, however, continued exposure to P would cause a reduction in the ability to follow tetanus. (Supported by NIH Grant NS 11535)

64.7 NEUROPHARMACOLOGY OF AFFERENT PROJECTIONS FROM THE SUBSTANTIA NIGRA AND LATERAL HABENULA TO THE ANTERIOR RAPHE IN THE RAT. W.C. Stern, A. Johnson*, J.D. Bronzino* and P.J. Morgane. D. Dix Hosp., Raleigh, NC 27611, Trinity College, Hartford, CT 06106 and Worcester Fndn. Exptl. Bio., Shrewsbury, MA 01545

The present experiments characterized the neurotransmitter(s) likely to be involved in the suppression of raphe unit activity produced by electrical stimulation of the afferent projections from the lateral habenula (LHb) and substantia nigra (SN). Wang and Aghajanian (Science 1977, 197:89-91) reported that the LHb-raphe projection was probably mediated by GABA since the GABA antagonist, picrotoxin, blocked the effects of LHb stimulation. In the first experiment, this effect was confirmed in anesthetized rats using extracellular glass microelectrodes and i.p. doses of 2-10 mg/kg of picrotoxin. Picrotoxin produced a 50% reduction in the percent of raphe cells inhibited by LHb stimulation. In other experiments recording from over 500 raphe units, we attempted to characterize the neurotransmitter mediating the inhibitory effects of the SN on the raphe (dorsalis and medianus). Dopamine and GABA, both of which are present in high concentrations in nigral neurons, do not seem to be involved since moderate-high doses (i.p.) of blockers of these transmitters (picrotoxin, haloperidol, or chlorpromazine) failed to reduce the suppressing effects of nigral stimulation on raphe activity. Similar negative findings occurred with drugs that block acetylcholine (atropine, scopolamine), opiates (naloxone), glycine (strychnine), histamine (diphenhydramine, cyproheptadine) and norepinephrine (propranolol, phenoxybenzamine).

The effects of all the above drugs on the spontaneous discharge rates of populations of raphe dorsalis and raphe medianus neurons are also being evaluated. For raphe dorsalis units, only picrotoxin produced a significant overall change in mean firing rate (38% increase), an effect consistent with the blockade of LHb-produced suppression by picrotoxin. This suggests that the spontaneous firing rate of raphe units is influenced by tonic gabergic inhibition originating in the LHb. In the raphe medianus, the drugs which significantly altered (increased) activity were cyproheptadine (5-20 mg/kg) and yohimbine (5-10 mg/kg). Since the antihistamine, diphenhydramine (10-30 mg/kg), did not increase raphe medianus discharge rates, it is more likely that cyproheptadine's effects result from its anti-serotonin activity. These results also show that the effects of various drugs on spontaneous activity are not necessarily the same in the dorsalis and medianus subdivisions of the midbrain raphe. These results support prior findings of differential inputs, outputs, and circuitry for these two raphe nuclei. (Supported by funds from Burroughs Wellcome and NSF grant BNS 77-16512.)

64.8 PENTOBARBITAL-INDUCED PROLONGATION OF SPONTANEOUS AND EVOKED IPSPs IN CA3 HIPPOCAMPAL NEURONS. F. J. Lebeda, T.H. Brown and D. Johnston. Dept. of Neurology, Baylor Coll. of Med., Houston, TX 77030 and Div. of Neurosciences, City of Hope Research Inst., Duarte, CA 91010.

A variety of convulsant and anticonvulsant agents have been proposed to interact with inhibitory synapses in the mammalian central nervous system. The in vitro hippocampal slice preparation offers accessible inhibitory pathways for investigating these postulated pharmacological effects at the cellular level. For example, the convulsant picrotoxin has been demonstrated to antagonize spontaneously occurring miniature inhibitory postsynaptic potentials (IPSPs) in the CA3 pyramidal neurons of guinea pig hippocampal slices (Brown and Johnston, SFN Abst. 6,1980). In the present study, initial experiments were performed to determine an effective concentration range of sodium pentobarbital which counteracted the 10 μM picrotoxin-induced spontaneous epileptiform discharges observed in CA3 field potential recordings. Since pentobarbital (100-500 μM) was able to block reversibly these picrotoxin-induced discharges, the spontaneous and evoked IPSPs were therefore examined at these concentrations.

With bath-applied pentobarbital (150 μM), mossy fiber stimulation produced IPSPs in which the half-decay times were prolonged from about 50 to 150 msec in CA3 neurons. Qualitatively similar results have been obtained with pentobarbital in CA1 hippocampal neurons in vivo and in vitro (Nicoll et al., Nature 258:625,1975; Alger and Nicoll, Nature 281:315,1979).

Spontaneous IPSPs were examined by utilizing a single electrode clamp circuit and the recently introduced technique of employing Cs-filled microelectrodes (2M Cs_2SO_4; Johnston and Brown, Fed. Proc. 39:2071,1980). After injecting Cs intracellularly (causing an increase in input resistance), the spontaneous IPSPs were augmented during maintained depolarizations to membrane potentials between -40 and +20 mV. After exposure to 125 μM pentobarbital, median values of spontaneous IPSP amplitudes at 0 mV were only about 30% larger, while times-to-peak and half-decay times were approximately 3-fold longer than control (increased from about 8 to 30 msec and from 30 to 90 msec respectively). These alterations are consistent with the hypothesis that part of the mechanism of action of pentobarbital involves the enhancement of inhibitory synaptic transmission. (Supported by a grant from the Epilepsy Foundation of America and by NIH Grants NS15772 and NS11535).

64.9 HISTAMINE MODULATES SYNAPTIC TRANSMISSION IN RAT SUPERIOR CERVICAL GANGLION AND AT FROG NEUROMUSCULAR JUNCTION VIA TWO PHARMACOLOGICALLY DISTINCT RECEPTORS (H_1 & H_2). R. W. Snow, C. Stein* and D. Weinreich. Dept. Pharmacol. & Exp. Therap., Univ. MD., Sch. Med., Baltimore, MD 21201.

Previous work has suggested that histamine (HA) depresses synaptic transmission at frog neuromuscular junction (NMJ) by both pre- and postjunctional mechanisms (Scuka, M., Neuropharm., 12:441, 1973), but the type of receptors (H_1 or H_2) involved was not investigated. In addition, other work has suggested that HA can either depress transmission via H_2 receptors or facilitate transmission via H_1 receptors in mammalian superior cervical ganglion (SCG) (Brimble, M.J. & Wallis, D.I., Nature, 246:156, 1973). We sought to more clearly establish the receptor types and the mechanisms mediating this synaptic modulation by HA.

In the rat SCG, the compound action potential (CAP) recorded extracellularly following sub-maximal preganglionic stimulation was facilitated by low doses of HA (10^{-7} M) and depressed by higher doses (10^{-5} M). The H_1 agonist 2-thiazolylethylamine (2-TH, 10^{-7} M to 10^{-5} M) also facilitated transmission while the H_2 agonist impromidine (IMP, 10^{-6} M to 10^{-5} M) depressed the CAP.

Using intracellular recording techniques, HA (10^{-5} M) had no effect on membrane potential, input resistance, spike threshold, the amplitude of the spike afterpotential or acetylcholine (ACh) sensitivity. However, HA or IMP (10^{-5} M) decreased the epsp amplitude and quantal content by 15-25%. In addition, 2-TH (5×10^{-7} M) increased the epsp amplitude and quantal content by 20-25%. These H_1 and H_2 agonist effects were blocked by H_1 and H_2 antagonists. We conclude that in the rat SCG, HA mediates facilitation and depression of transmission by presynaptic H_1 and H_2 receptors.

At the frog NMJ, HA (10^{-5} M) had no effect on postjunctional ACh sensitivity. Scuka (1973) has shown that higher doses of HA interact with the postjunctional ACh receptor-ionophore complex, similar to the action of atropine. We believe these postjunctional effects of HA are not mediated by HA receptors.

HA had variable effects on miniature endplate potential frequency (m-Hz), either increasing or decreasing m-Hz in different preparations. IMP (10^{-5} M) consistently decreased m-Hz, while 2-TH (10^{-6} M) consistently increased m-Hz. These results imply the presence of both H_1 and H_2 receptors in the motor nerve terminals.

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64.10 GANGLIONIC DISCHARGES INDUCED BY TYRAMINE. Daryl Christ* (SPON: D.L. Avery). Dept. of Pharmacology, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

d-Amphetamine produces discharges in the stellate ganglion. If this effect is due to a sympathomimetic action of d-amphetamine, other sympathomimetics should also induce ganglionic discharges. The effects of tyramine were explored in the isolated stellate ganglion of the hamster using extracellular recording methods. Tyramine HCl, at concentrations between 10^{-3} and 10^{-2}M, reduced the amplitude of the postganglionic action potential from preganglionic nerve stimulation at 0.2 Hz. The ganglionic blocking action of tyramine was much weaker than the ganglionic blocking action of d-amphetamine or norepinephrine. 10^{-4}M tyramine induced asynchronous discharges in the postganglionic nerve. These discharges were considerably larger at 10^{-3}M tyramine. The discharges were not maintained during continuous exposure to tyramine but tended to subside after approximately 5 minutes exposure to tyramine. The discharges from tyramine were potentiated by 10^{-5}M pargyline, a monoamine oxidase inhibitor. Although tyramine produced discharges, it did not increase the ganglionic discharges from DMPP (10^{-4}M).

Tyramine produces discharges in the stellate ganglion which are very similar to the discharges from d-amphetamine. This indicates that the discharges from these drugs are due to sympathomimetic actions of the drugs. The potentiating action of pargyline supports this conclusion. On the other hand, the large difference in potency of tyramine and d-amphetamine for ganglionic blockade, and the difference in abilities to potentiate the actions of DMPP indicate that these effects are not due to sympathomimetic actions of the drugs. (Supported by NIH Grant NS-15528)

64.11 EFFECTS OF VANADIUM ON RAT BRAIN SYNAPTOSOMAL ATPases AND BINDING OF ^{3}H-OUABAIN AND ^{45}CALCIUM. D. Desaiah, S. K. Mishra, and R. L. Osborn*. Neurology and Research Services, Veterans Administration Medical Center, and Department of Neurology, University of Mississippi Medical Center, Jackson, MS. 39216.

Vanadium has been shown to inhibit Na^+-K^+ ATPase in heart and brain tissue homogenates. However, a comparative study on the effects of vanadium on different ATPases in one tissue preparation is lacking. We have determined the sensitivity of Na^+-K^+, Ca^{++} and Mg^{++} ATPases of purified rat brain synaptosomes to vanadium. The effects of vanadium on binding of ^{3}H-ouabain and ^{45}Calcium to rat brain synaptosomes were also studied. Preincubation of synaptosomes with varying concentrations of vanadium chloride resulted in a significant and concentration-dependent inhibition of Na^+-K^+ and Ca^{++} ATPase activities. However, Na^+-K^+ ATPase was more sensitive to vanadium than Ca^{++} ATPase. The Mg^{++} ATPase activity was unaffected by vanadium at any concentration tested. A 50% inhibition of Na^+-K^+ and Ca^{++} ATPases were obtained at 10^{-6}M and 10^{-4}M vanadium chloride respectively. Preincubation of synaptosomes with vanadium chloride resulted in a decreased binding of ^{3}H-ouabain. The inhibition of ouabain binding was significant and dose-dependent reaching a maximum inhibition of 90% at 10^{-3}M vanadium chloride. However vanadium chloride had no appreciable effect on ^{45}Calcium binding to synaptosomes. These results and other kinetic data indicate that vanadium may be a more effective inhibitor of Na^+-K^+ ATPase than other ATPases. Also the present data suggest that vanadium may not be a specific inhibitor of Na^+-K^+ ATPase like ouabain. (Supported by Medical Research Service of Veterans Administration).

65.1 PROPERTIES OF ELECTRICAL CONNECTIONS FORMED BY ISOLATED LEECH NEURONS IN CULTURE. P. A. Fuchs and D. F. Ready* Neurobiology Dept., Stanford Univ. Med. School, Stanford, California. 94305.

Single, identified neurons isolated from the leech CNS will establish electrical connections with one another in culture. We have investigated the extent to which these de novo contacts resemble the normal connections of cells within the ganglion. In ganglia from adult leeches Retzius cells are electrically coupled to their contralateral homologues, but not to pressure (P) sensory cells. P cells, as well as nociceptive (N) sensory cells, normally make electrical connections with the longitudinal (L) motoneuron, and L motoneurons are electrically coupled to their contralateral homologues.

These connections are reproduced by isolated cells in culture. Retzius cells become electrically coupled to other Retzius cells, but not to P sensory neurons. P and N sensory cells make electrical connections with L motoneurons, and L cells become electrically coupled. Thus, neurons maintained in culture interact with a variety of targets to display the pattern of connectivity they show in the animal.

The properties of the electrical junctions formed by cells in culture are similar to those of the corresponding synapses in the ganglion. The electrical connections formed by pairs of Retzius cells or pairs of L motoneurons is non-rectifying, while P and N sensory cells make rectifying connections with L motoneurons. For the most part, the sites and structures underlying these various forms of electrical coupling between leech neurons are unknown. Using isolated pairs of nerve cells in culture it is possible to examine such interactions in detail and to begin to define the mechanisms involved in different forms of electrical connectivity between neurons.

65.2 DEVELOPMENT OF RAT NEUROMUSCULAR JUNCTIONS IN ORGAN CULTURE. L. Ziskind-Conhaim and M.J. Dennis*. Dept. of Physiology, University of California Medical School, San Francisco, CA 94143

Explants of d15-d18 embryonic rat intercostal muscles together with their thoracic spinal cord segments were maintained in organ culture for up to 6 days. Muscle fibers continued to grow and differentiate after explantation although not as extensively as during the same interval in utero. The small tufts of nerve sprouts which were observed at d15 began to branch and extend along the center of the muscles after 1-2 days in culture.

Acetylcholine receptors, as detected by autoradiography, were uniformly distributed along the fibers at d15 and began to cluster in the mid-region of the fibers one day after explantation. Furthermore, receptor clusters were associated with acetylcholinesterase, indicating that the cholinergic receptors accumulated at the endplates of the muscle fibers. The time course of receptor aggregation was identical to that seen in vivo and the formation of the clusters was dependent on the presence of nerve terminals.

The resting membrane potential of the explanted muscle fibers varied between -70 mV and -85 mV and the properties of spontaneous and evoked synaptic potentials were comparable to those recorded in embryonic fibers in vivo (Dennis, Ziskind-Conhaim and Harris. Develop. Biol., in press). The number of functional synaptic contacts on individual muscle fibers increased during the culturing period at a rate similar to that observed in vivo. Our observations establish the developing rat intercostal muscles in organ culture as a favorable preparation in addressing questions which could not be easily studied in the embryos.

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65.3 DEGREE OF SPECIFICITY MAINTAINED BY SPROUTING AND REGENERATING MOTOR NERVES TO CAT TIBIALIS ANTERIOR MUSCLE. J. Ungar-Sargon* and M. E. Goldberger. Dept. Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Cats were allowed to survive up to 2 years following a unilateral spared (L7) nerve preparation (lumbosacral spinal nerves cut sparing L7) in order to permit collateral sprouting by L7 and then regeneration by L5, L6, S1, S2. Reflexes were tested sequentially. Initially the tibialis anterior (T.A.) myotatic reflex was present but extremely weak on the operated side indicating that only some fibers to T.A. were carried in the L7 nerve and others had been cut. Later (1 week) the reflex recovered and became exaggerated suggesting collateral sprouting of L7 fibers into the muscle. Two years later the motor innervation to T.A. was examined by using retrograde transport of HRP injected into the T.A. muscle of both legs. In the normal cat, labeled cells innervating T.A. are found in a narrow range of L6 (caudal) and L7. Thelabeled cells are symmetrical in their rostrocaudal extent on the two sides and are found in a discrete nucleus also symmetrical bilaterally in the lateral-ventral horn consistent with the localization found by Romanes. T-test showed there is no significant difference in size on the two sides. On the operated side in the segment of the spared nerve, the discrete nucleus is still observed; cells of other nuclei are only occasionally labeled. The labeled cells on the operated side are up to twice as large as the labeled cells on the control side in the spared nerve segment. In segments of the cut but regenerated nerves, the cross sectional organization of the motor representation of T.A. appears to be disrupted. Labeled motor neurons are not found in a particular nucleus; moreover, their location is not necessarily similar to the position of the T.A. nucleus. Some appear to lie in the medial motor nucleus. The normal rostro-caudal extent is also changed. Labeled cells are found in L5, L6 and S1 as well as L7. The cells outside of the spared nerve segment are not consistently enlarged. Apparently a normal representation is maintained by cells undergoing collateral sprouting. For the most part, the normal topography suggests that specificity is retained by collateral sprouting and motor neurons which sprout into the partly denervated muscle already maintained connections there (or lie among cells that do). This is not the case for regenerating motor neurons which project to a muscle they do not innervate normally.

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65.4 INNERVATION OF THE CILIARY GANGLION IN ADULT AND NEONATAL RABBITS. David A. Johnson* and Dale Purves. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO. 63110.

An important feature of the postnatal development of synaptic connections in mammalian autonomic ganglia is the elimination of some of the synapses initially formed. Neither of the ganglia studied to date (submandibular ganglion of the rat; hamster superior cervical ganglion) is ideally suited to further exploration of synapse elimination. The rules which govern this phenomenon might be studied to best advantage in a ganglion which is a) anatomically well defined, b) comprised of a small number of neurons, c) comprised of ganglion cells each of which is innervated by only a few preganglionic axons in maturity, d) innervated by a small number of preganglionic neurons whose location is well defined, and e) amenable to physiological modification of ganglion cell activity. The ciliary ganglion of the albino rabbit meets these criteria.

The number of neurons in the ganglion, as well as the preganglionic population, is indeed small. Cell counts show that the ganglion is comprised of about 500 neurons (range 366-593, n=15). Moreover, the average number of cells in ganglia of one day-old animals is not significantly different from the number of cells in adult ganglia. Horseradish peroxidase applied to the proximal cut end of the preganglionic stalk labeled fewer than 300 neurons in the ipsilateral brainstem just ventral to the fourth ventricle at the level of the oculomotor nucleus (n=3). Since we have not ruled out some uptake by damaged oculomotor axons, this figure is a maximum estimate of the number of preganglionic cells.

Intracellular recordings from ganglion cells during graded stimulation of the preganglionic nerve in vitro show that adult neurons (older than 10 weeks) are innervated by an average of 2.2 different axons (range 1-6). In contrast, neonatal neurons (from animals 1-7 days old) are innervated by more than 5 different axons on average; only occasional cells in newborns receive inputs from one or two axons, compared to 64% of neurons in adult ganglia. The average number of inputs to ganglion cells declines gradually during the first few postnatal weeks, reaching the adult level by about 9-10 weeks of age.

A possible reason why some ciliary ganglion cells remain innervated by up to 6 different axons in maturity while others lose their multiple innervation is considered in an accompanying abstract (R.I. Hume and D. Purves, this volume).

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65.5 RELATION OF MULTIPLE INNERVATION TO THE GEOMETRY OF RABBIT CILIARY GANGLION CELLS. Richard I. Hume and Dale Purves. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO. 63110.

Neurons in the rat submandibular ganglion, which generally receive innervation from only one preganglionic axon, have few if any processes; on the other hand, neurons in the guinea-pig superior cervical ganglion, which receive several different inputs have a complex dendritic arborization. This suggests a correlation between multiple innervation of autonomic ganglion cells and the number of dendrites they possess (see Lichtman and Purves, 1980). We have examined this possibility directly by determining both the number of inputs and the geometry of individual cells in the ciliary ganglion of adult albino rabbits.

We impaled 100 ganglion cells (1-3 cells per ganglion) with micropipettes filled with 5% horseradish peroxidase (w/v) in buffered 0.2 M potassium acetate. The number of axons making synapses upon each ganglion cell was determined by counting increments in the postsynaptic response during graded stimulation of the preganglionic nerve. HRP was then pressure-injected into the cells for 2-20 minutes. One to three hours later the ganglia were processed according to a modification of the Hanker-Yates procedure. Cells marked in this way were viewed in whole mount and drawn with the aid of a camera lucida.

There was a strong correlation between the complexity of the geometry of individual neurons and the number of inputs they received. Most cells (20/29) receiving one input lacked dendrites altogether; conversely, all those cells with 5 or 6 different inputs (n=9) had extensive dendritic arbors (5.5 primary dendrites, on average). In general, cells with 2-4 inputs (n=62) had an arborization of intermediate complexity (2.4 primary dendrites).

On average, neonatal ciliary ganglion cells are innervated by a greater number of axons than adult ganglion cells (see D.A. Johnson and D. Purves, this volume). That mature cells receive a number of inputs that varies according to the number of dendrites they possess suggests that the geometry of each neuron may be important in determining the outcome of synapse elimination in early postnatal life.

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Ref: Lichtman, J.W. and Purves, D. J. Physiol. (Lond.) 301: 213 (1980).

65.6 CENTRAL REGULATION OF THE MORPHOLOGICAL AND BIOCHEMICAL MATURATION OF SYMPATHETIC GANGLIA. R.W. Hamill, J.M. Lawrence, P. Cochard, G. Raisman and I.B. Black. Div. Develop. Neurology, Cornell Univ. Med. Coll., New York, N.Y. 10021; Natl. Inst. for Medical Research, London, Eng. NW7 1AA; Institut D'Embryologie Du CNRS 94130 Nogent Sur Marne - France.

The effects of interruption of descending central pathways on the morphological and biochemical ontogeny of peripheral sympathetic ganglia were examined in the sixth lumbar (L-6) sympathetic rat ganglia. Previous studies defined the normal maturation of presynaptic choline acetyltransferase (CAT) activity, postsynaptic tyrosine hydroxylase (T-OH) activity, and total protein in the L-6 ganglion. More recent studies indicated that neonatal spinal cord transection prevented the normal ontogeny of presynaptic CAT, postsynaptic T-OH and ganglion protein but the morphological substrate of these biochemical abnormalities remain to be defined. In the present investigations ganglion synapse numbers and adrenergic neuron numbers were examined in transected and sham-operated littermate controls 6 weeks after surgery at 10 days of age and correlative biochemical studies were performed.

Spinal transection resulted in a reduction in synapse number in ganglion cell bodies (53.5% reduction) and neuronal processes (55.8% reduction). This abnormality in synapse number was associated with a reduction of CAT activity to 56% of control. Although T-OH activity also failed to develop normally and was 25% of control, there was no associated alteration in adrenergic neuron number.

These studies suggest that descending central pathways regulate the maturation of presynaptic cholinergic terminals (and hence synapse number) in sympathetic ganglion and that CAT activity serves as an accurate marker for these terminals. Additionally, the ontogeny of ganglion T-OH activity is altered by central lesions and this effect is not secondary to a decrease in adrenergic neuron number.

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66.1 COLLATERAL INPUT TO THE NEOSTRIATUM FROM DESCENDING AXONS OF THE RAT SOMATIC SENSORY-MOTOR CORTEX. John P. Donoghue and S.T. Kitai, Dept. of Anatomy, Michigan State University, East Lansing, Michigan 48824.

Using the technique of intracellular injection of horseradish peroxidase (HRP) it was demonstrated that single neurons in the rat somatic sensory-motor (SSM) cortex project both to the brainstem and to the neostriatum. Brainstem projecting neurons were identified during intracellular recording by antidromic activation from the cerebral peduncle or by tracing HRP labeled axons into the internal capsule after histological processing. A total of 17 HRP-injected cells, all in layer V, were identified in this manner. Five of these neurons gave a collateral branch to the striatum.

Branched cells are typical layer V pyramidal neurons with spiny dendrites. Their cell bodies, measuring 22-26μm, are among the largest in rat SSM cortex. Similar to other layer V cells, the main axon issues several fine intracortical axon collaterals that terminate mainly in layers VI and V, but also ramify near the apical dendrite in the upper cortical layers. The main axon, measuring 2.0-2.5μm in diameter, does not branch in the white matter. About 300μm after entering the striatum a single axon collateral, approximately 0.5μm in diameter arises from the main axon of these cells. Although the HRP reaction product faded in most cases after a few hundred μm, one collateral was followed 1.3mm within the striatum. Typical of other striatal collaterals this axon projects laterally and anteriorly with relatively little arborization. This branched striatal and brainstem projecting cell was antidromically activated from the peduncle at a latency of 0.6 msec, which was the shortest latency found for any of the layer V neurons recorded. All branched cells were excited by peripheral somatic sensory (electrical) stimulation.

Thus, the results demonstrate that some large, rapidly conducting layer V pyramidal cells of rat SSM cortex that project into the brainstem give a fine axon collateral to the neostriatum Further, these cells respond to somatic sensory inputs and would relay this information to lower centers and also to the striatum. The large size of these cells suggests that they may project to the lower brainstem or even to the spinal cord. Supported by NIH Grants NS 14866 and NS 06249.

66.2 INVOLVEMENT OF A CORTICO-STRIATAL AXON REFLEX IN THE RESPONSES OF STRIATAL NEURONS TO SUBSTANTIA NIGRA STIMULATION IN THE RAT. C. J. Wilson*, H. T. Chang & S. T. Kitai, Dept. of Anatomy, Michigan State University, E. Lansing, MI 48824.

Stimulation of substantia nigra has been previously shown to elicit an extracellular field potential in rat striatum and a complex pattern of excitation and inhibition in rat striatal neurons. The early response appears in intracellular recordings as a depolarization beginning as early as 2 msec after nigral stimulation and often containing more than one component. The earliest component of the excitatory response has been interpreted as a monosynaptic nigral induced EPSP. The present evidence, however, suggests that this EPSP and the associated field response arise from inadvertent stimulation of cortical efferents projecting both to brainstem and striatum.

Intracellular recordings were obtained from rat striatal neurons using bevelled glass microelectrodes containing 4% horseradish peroxidase (HRP) in Tris buffer (pH 7.6) and .5M potassium chloride or potassium acetate. Neurons were identified by microscopic examination after iontophoretic application of HRP through the recording electrode and subsequent histochemical processing. Most identified spiny neurons responded to stimulation of substantia nigra with short latency (2-6 msec) monosynaptic EPSP. In many cases, this depolarizing response exhibited more than one distinct component. Stimulation of the rostro-ventral pons more than 2 mm caudal to substantia nigra, however, resulted in a nearly identical response in these same neurons. When both sites were stimulated simultaneously the two responses failed to summate, suggesting that they may be mediated by the same population of axons. Since both of these areas contain a high density of cortical efferent fibers, the experiments were repeated in rats subjected 3-4 days earlier to large ipsilateral aspirations of frontal cortex. After this treatment, the short latency EPSP and the field response to both nigral and pontine stimulation were severely attenuated or absent. In the decorticate rats, nigral stimulation elicited long latency (8 to 16 msec) low amplitude depolarizations which could not be elicited from the pontine stimulation site. Despite its long latency, this depolarization appeared monosynaptic by virtue of its constant latency upon variation of stimulus intensity or frequency. This response to nigral stimulation may represent the activation of slowly conducting dopaminergic nigro-striatal axons. (Supported by USPHS grant NS 14866).

66.3 EXCITATORY POSTSYNAPTIC POTENTIALS RECORDED IN NEURONS OF THE RAT CAUDATE-PUTAMEN FROM STIMULATION OF THE DORSAL RAPHE NUCLEUS ARE SEROTONERGIC. M.R. Park, J.A. Gonzales-Vegas*, J.W. Lighthall and S.T. Kitai, Dept. of Anatomy, Michigan State University, East Lansing, Michigan 48824.

Stimulation of dorsal raphe nucleus (DR) has previously been shown (VanderMaelen, et al., Brain Res., 175:356, 1979) to produce monosynaptic EPSPs in neurons of the rat caudate-putamen (CP). It has not been determined, however, whether these excitatory responses are due to the known serotonergic DR-CP projection. In order to answer this question, intracellular recording of the responses to DR stimulation was performed in male hooded rats (275-350 g) in which the levels of serotonin (5-HT) were first depleted then restored (as: Wang & Aghajanian, Neuropharm. 17: 819, 1978). In the period 48 to 12 hours before the beginning of the recording session, the animals were injected with 2 or 3 divided doses of p-chlorophenylalanine methyl ester (PCPA), total dose 375 mg/kg, in order to deplete 5-HT. Later, during the acute recording session, the immediate precursor of 5-HT, 5-hydroxytryptophan (5-HTP) was injected (7.5-15 mg/kg) in order to raise the level of 5-HT available as neurotransmitter. In all, recordings from 83 neurons in the 5-HT depleted state can be compared with 77 post-5-HTP recordings. In addition, 5 untreated animals (55 neurons) provide a comparison to normal responsiveness. Internal control was possible in those cases where a neuron could be recorded from both before and as the 5-HTP became effective. In these cases the EPSP evoked from DR stimulation was observed to increase in amplitude (e.g. from 0.8 to 19 mV in one case and 0.9 to 9 mV in another). The effect of systemic injection of 5-HTP was not immediate but required up to 30 minutes to develop. Responses to DR stimulation were not entirely absent in the PCPA treated animals prior to the injection of 5-HTP. Low amplitude (ca. 1mV) long latency responses were observed, conceivably due to residual 5-HT in the DR terminals. In addition, EPSPs of short latency (3-5 msec), relatively large amplitude (>5mV) and fast rise time were observed in 20% of the neurons encountered in PCPA treated pre-5-HTP injected animals. These fast responses are distinct from the bulk of DR evoked responses, as recorded either after 5-HTP injection or in non-PCPA treated animals, in that they are not reduced in amplitude (shunted) by a preceeding DR, substantia nigra, or cortex stimulation (interstimulus interval 20 msec) and are resistant to shunting by recurrent inhibition. The observed recovery of EPSP amplitude following 5-HTP injection strongly suggests that 5-HT is an excitatory neurotransmitter in CP. There also exists a short latency non-serotonergic excitation following DR stimulation which is not necessarily due to a mid-brain projection to CP. (Supported by NIH Grant BRSG 05772-04 and USPHS Grant NS 14866).

66.4 EVIDENCE FOR INHIBITION IN RAT NEOSTRIATAL BRAIN SLICES. J.W. Lighthall, M.R. Park, S.T. Kitai. Dept. of Anatomy, Mich. State Univ., East Lansing, Michigan 48824.

On the basis of both extracellular field responses and intracellular recording, inhibition could be observed in brain slices of rat caudate-putamen (CP). Slices, cut parasagittally, 350-400μm thick, where taken from a rectangular core of brain measuring 2 by 5 mm and containing both the right and left CP. The slices were maintained in artifical media at 37°C. Glass micropipettes were used for recording. Intrastriatal bipolar stimulation (0.05-0.30 msec, 10-90 V, delivered at 1 Hz) elicits a field potential lasting 1-8 msec. With low stimulation intensity, the field potential, which consists of two negative components N-1 and N-2, exhibited characteristics similar to those previously described in CP brain slice (Misgeld, U., et al., Brain Res. 34: 575, 1979). The amplitudes of both N-1 (latency 0.5-2.0 msec) and N-2 (latency 1.5-5.0 msec) vary within the range 0.5-1.0 mV. Stimulus intensity and/or duration could be adjusted to evoke a maximum N-2 response measuring 2.0-5.0 mV in amplitude. The amplitude of the N-2 response also increased as the recording pipette was advanced toward the cell and was maximum prior to pentration. Double shock at low stimulus intensity, given at a 5 msec interstimulus interval (ISI), and delivered at 0.75 Hz, either produced a slight potentiation of, or did not change the amplitude of, the N-2 portion of the test response. Increasing the frequency of stimulation from 0.75 Hz to 5 Hz resulted in a reduction of the N-2 portion of the second response. This phenomenon was observed at ISIs up to 50 msec. When stimulation levels were adjusted to evoke a maximum N-2 response, double shock resulted in total reduction of the test N-2 component at a 5 msec ISI. This reduction occured at stimulus frequencies of 1 Hz or less. The N-2 response returned to control levels at ISIs of 20 to 30 msec.

Intracellular records indicate the N-2 response to be a mass response of synaptically induced action potentials. Immediately before penetration, the N-2 component is superimposed on a positive potential. The positive potential gradually increases in amplitude (0.5-1.0 mV) and duration (1.0-6.0 msec) as the microelectrode is advanced toward the cell. Upon penetration, EPSPs were observed, often triggering APs whose latencies correspond with those of the N-2 component. Double shock stimulation delivered at 1Hz resulted in failure of the test spike to be triggered from the EPSP. Intracellular injection of HRP (Sigma type VI, 2M in tris buffer, pH 7.6, and 0.5M K-acetate) identified the recorded cells as CP spiny neurons.

(This work was supported by USPHS Grant 14866).

66.5 EFFECT OF PERIPHERAL NERVE STIMULATION ON THE ACTIVITY OF DOPAMINERGIC NEURONS: INVOLVEMENT OF NON-DOPAMINERGIC NIGRAL NEURONS AND STRIATONIGRAL PATHWAYS. D. W. Hommer* and B. S. Bunney (SPON: M. Bowers). Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510

Biochemical (Nieoullon et al., Nature 269: 340-342, 1977) and electrophysiological (Chiodo et al., Brain Res., in press, 1980) studies have demonstrated an effect of sensory stimulation (light flashes, hind paw stimulation, tail pressure and cervical probing) on dopaminergic (DA) cell function in the substantia nigra. In unpublished experiments we have observed that noxious stimuli (toe pinch) also can induce changes in DA activity. To investigate these effects further we have used extracellular single unit recording techniques to study the effects of sciatic nerve stimulation on the activity of nigral DA and non-DA neurons in chloral hydrate anesthetized male albino rats. The sciatic nerve was stimulated with single 50 u sec duration, 40-80 uA square wave pulses. Unit responses to stimulation were analyzed using post stimulus time histograms. Two populations of DA neurons could be distinguished. A minority of DA cells responded to stimulation with a single period of inhibition. Sixty percent of the DA neurons tested responded to stimulation with an initial inhibitory period followed by one or more oscillations between excitation and inhibition. These oscillations were blocked by i.v. administered haloperidol (0.1 mg/kg) or by cutting the striato-nigral pathways (without damaging DA axons). The initial inhibitory period was decreased by haloperidol but was increased by the lesion. A population of identified non-DA zona reticulata neuron, which has been shown to exert an inhibitory influence on DA neurons (Grace and Bunney, Eur. J. Pharmacol. 59: 211-218, 1979) was found to respond to stimulation with an initial excitation. The latency for this stimulus-induced excitation was similar to the latency of the initial inhibition of DA neurons.

These results suggest that sensory stimuli may induce an initial inhibition of DA neurons, at least in part, through excitation of an inhibitory non-DA zona reticulata neuron. The ability of haloperidol to decrease the initial inhibition suggests that dopamine release, perhaps at dendrodendritic synapses, may also play a role in the DA neuron's response to peripheral stimuli. The subsequent oscillations in DA neuron activity appear to be mediated through reciprocal striato-nigral, nigro-striatal pathways, since lesioning these pathways abolishes the oscillations. The fact that not all DA neurons demonstrated oscillations in their activity after stimulation suggests that not all zona compacta DA neurons receive innervation from striato-nigral feedback pathways. (Supported by NIMH grants MH-28849, MH-25642, MH-14276 and the State of Connecticut.)

66.6 LIGHT MICROSCOPY OF ISOLATED CAUDATE. L. A. Marco and R. B. Chronister. Departments of Psychiatry and Pharmacology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107 and VA Medical Center, Coatesville, PA 19320; Department of Anatomy, College of Medicine, University of South Alabama, Mobile, AL 36688.

Recent electrophysiological recordings indicate that the surgically isolated caudate nucleus of rats and cats in situ has retained an appreciable amount of internal circuitry and that most of its neurons are highly responsive to iontophorized acetylcholine (Marco et al., Soc. Neurosci. Abstr. 5:75, 1979). To determine 1) the extent of the isolation, and 2) the status of caudate neurons, the brains examined physiologically were fixed in either 10% formalin or 10% formalin/80% alcohol and embedded in either paraffin (10-20 μ sections) or celloidin (40 μ sections). In every brain so far examined 14-60 days post-isolation, the isolation was complete in both rats and cats. Numerous areas which project to the caudate had degenerating neurons; paramount among these were the substantia nigra and the dorsal raphe. This degeneration was brought about by section of the medial forebrain bundle. Two parasagittal sections, one separating the caudate from the septal area and accumbens and the other immediately lateral to the caudate, isolated the nucleus further. Isolation was completed by removal of the overlying cortex and a coronal transection through the rostral pole of the caudate. Examination of the caudate revealed that indeed the intrinsic neuronal circuitry of the caudate was to a large extent intact. The medium sized neurons and the large neurons did not show any consistent patterns of chromatolysis. The 40 μ sections showed a number of degenerated neurons. These had a uniform distribution and seemed to be exclusively the medium sized neurons and not the large ones. On the other hand, phosphotungstic-acid hematoxylin stains revealed that a glial reaction was in process. Furthermore, silver impregnations of the paraffin sections revealed that degenerating fibers with associated terminals were present. These observations suggest that although most of the somata of the intrinsic neurons are still fairly normal, the input systems are degenerating with a concomitant glial cell and glitter cell clean-up. Critical staining (toluidine blue) of the 10 μ paraffin sections revealed that dendritic alterations were present. Main dendrites were no longer linear with a smooth appearance but twisted and irregular in contour. This type of dendrite is suggestive of major de-afferentation. Golgi impregnations are underway to determine the extent of this de-afferentation and concomitant post-synaptic spine/dendrite changes. (Supported by NINCDS Grant #NS14712-02 and VA Merit Review funds.)

66.7 INCREASED NEUROANATOMICAL RESOLUTION USING PEROXIDASE-LABELLED LECTIN TRANSPORT: DEMONSTRATION OF A PALLIDOSTRIATAL PATHWAY Wm.A. Staines*, H. Kimura*, H.C. Fibiger and E.G. McGeer
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The characteristics of a conjugate of horseradish peroxidase with wheat germ agglutinin (WGA-HRP) as a neuroanatomical tracer were determined in the CNS. The main technical finding was the high degree of control over the injection site allowed by this technique.

Adsorptive tracers, such as WGA, have a high affinity for the sugar moieties of membrane glycoproteins and/or glycolipids. It has been suggested that this allows for uptake by a process of adsorptive endocytosis, rather than fluid phase endocytosis as is the case for HRP. The usefulness of WGAas a retrograde/anterograde tracer has been shown using a number of different methods for marking the lectin[1]. The most convenient marking technique so far developed is the direct chemical conjugation of the lectin to HRP (WGA-HRP)[2]. This conjugate has been shown to be transported from the periphery with a sensitivity much higher than that of free HRP. It was reasoned that the lectin character of the conjugate should not only enhance sensitivity but also limit diffusion from the injection site and thus confer an important improvement over free HRP for studies in the CNS.

Apparent injection site diameters could be controlled by changes in either the volume or concentration of the WGA-HRP solution. The concentration dependence of the injection site size indicates saturable binding of the conjugate to the striatal neuropil. Upon injection of dilute solutions of WGA-HRP into the striatum of the rat, injection site diameters on the order of 500 μm could be easily and reproducibly obtained. The retrograde labelling of cell bodies in the substantia nigra was correspondingly very restricted. Using both the diaminobenzidine and the tetramethylbenzidine protocols for the visualization of peroxidase activity, anterograde transport was in evidence, but could be abolished if the WGA-HRP was co-injected with kainic acid.

After discrete striatal injections of WGA-HRP, in addition to a highly localized labelling of known striatal afferents, a pallidostriatal pathway was demonstrated at both light and electron microscopic levels.

1.Schwab, M.E., Javoy-Agid, F. & Agid, Y. (1978) Brain Res 152: 145-150
2.Gonatas, N.K., Harper, C., Mizutani, T. & Gonatas, J.O. (1979) J Histochem Cytochem 27:728-734

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66.8 PRECISE LOCATION OF NIGROTECTAL NEURONS IN THE MONKEY, CAT AND RAT. R.M. Beckstead, S.B. Edwards and A. Frankfurter. Dept. of Anatomy, University of Virginia Sch. of Med., Charlottesville, Va. 22908.

Although the projection from the substantia nigra's pars reticulata (SNR) to the deeper layers of the superior colliculus has been mapped in the monkey by anterograde methods, the precise location of the nigrotectal cells within SNR has not been documented. Moreover, retrograde cell-labeling studies in the cat and rat indicate some species differences in the location of nigral neurons that send axons to the superior colliculus. In the present study, the intranigral location of nigrotectal neurons was compared in the monkey, cat and rat using the horseradish peroxidase (HRP) retrograde cell-labeling technique. The histochemical reaction employed was that of Mesulam in which the chromogen is tetramethylbenzidine. In each species, it was possible to obtain a number of cases in which the HRP deposit involved essentially the entire extent of the superior colliculus of one side with only minimal involvement of adjacent brainstem structures.

In the monkey, labeled nigrotectal cells were particularly numerous in the extreme rostrolateral portion of SNR. A progressively decreasing number of cells spread medially from this region of high concentration in a ventral stratum of SNR immediately dorsal to the pes pedunculi. No labeled cells were found in the caudal one-half or so of SNR, nor in its extreme medial part. A substantial number of HRP-positive cells were present in the contralateral SNR in a similar distribution. In the cat, labeled cells were less selectively localized in SNR's mediolateral expanse, being distributed more or less randomly in its middle portion with a scattering of cells in both medial and lateral parts of SNR. As in the monkey, all cell-labeling was confined to the rostral half of SNR. Although some cell-labeling occurred in the contralateral SNR, it was less substantial than in the monkey. In the rat, the HRP-positive cells were especially concentrated throughout the mediolateral extent of a ventral stratum of SNR immediately dorsal to the pes pedunculi. Although some labeled cells were located more dorsally in SNR, they were far fewer in number and consistently less heavily labeled with HRP reaction product. As in the cat and monkey, the cell-labeling in SNR did not invade its caudal half. Only one or two HRP-positive cells could be detected in the contralateral SNR of the rat.

Thus, these three common laboratory mammals exhibit some differences in the precise location of the nigrotectal neurons and in the degree of bilaterality of the nigrotectal projection. Common to all three species, however, is the confinement of the nigrotectal cells to the rostral part of SNR. Supported by NIH Grant 5 S07 RR05431-18.

67.1 HETEROGENEITY OF EXCITATORY AMINO ACID RECEPTOR-IONOPHORE COMPLEXES IN THE RAT STRIATUM. A. Luini*, O. Goldberg* and V.I. Teichberg. Depts. of Neurobiology and Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel.

We have developed a new biochemical method allowing quantitative studies of the interactions of excitatory amino acids with their respective receptor-ionophore complexes in brain. After loading rat striatum slices with either $^{22}Na^+$, $^{42}K^+$, $^{86}Rb^+$ or $^{36}Cl^-$, we measure the rate of efflux of the tracer ion into a non radioactive physiological medium. The addition of excitatory amino acids to this medium increases the rate of $^{22}Na^+$ efflux in a dose dependent manner. This effect is not modified by 10^{-7}M TTX, a high Mg^{++}/Ca^{++} ratio, 1 mM theophylline and low temperature and is still observed in a Na^+-free medium indicating that the amino acid induced increase in $^{22}Na^+$ efflux rate is due to the opening of receptor linked ionophores. The order of potency of excitatory amino acids measured in this system is the following: N-methyl-D-aspartate (NMDA) > DL-homocysteate > quisqualate (QA) > kainate (KA) > D-glutamate > L-glutamate(L-Glu) > L-aspartate. The K_m values for L-glutamate and kainate are 1.2mM and 0.15 mM respectively. NMDA, in the striatum is about 50 fold more potent than L-glu. We have also studied the effects of various antagonists on the above-mentioned agonists. 2-amino-5-phosphonovalerate at 0.2 mM was found to block NMDA >> L-Glu but did not block QA or KA. γ-D-glutamylglycine at 0.2mM blocks NMDA >> KA > L-Glu and not QA. DL-aminosuberate at 1 mM blocks only NMDA but not KA, QA or L-Glu. DL-aminoadipate at 3mM blocks NMDA >> KA > Glu > QA. Kainic acid lactone, a derivative of kainate newly synthesized in our laboratory, used at 3mM blocks NMDA > KA but did not affect L-Glu or QA. On the basis of these results one can conclude that there exist at least four types of excitatory amino acid receptors in the striatum. Further evidence for difference between L-Glu and KA receptors is the finding that L-Glu increases the efflux rates of both $^{22}Na^+$ and $^{42}K^+$ where KA, while increasing $^{22}Na^+$ efflux rate, does almost not affect $^{42}K^+$ efflux rate. Our results raise the question of the nature of the excitatory neurotransmitters in the striatum.

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67.2 GABA Analogues: High Correlation Between Channel Duration on Cultured Mouse Spinal Neurons and 1/IC50 Values at Low Affinity GABA Receptor Sites on Frozen Rat Brain Synaptic Membranes. Jeffery L. Barker and David A. Mathers* Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, Md. 20205

Intracellular recordings from voltage-clamped mouse spinal neurons in tissue culture were used to study the effects of γ-aminobutyric acid (GABA) analogues on membrane excitability.Thirteen agonists tested depressed excitability in a dose-dependent, reversible manner through an increase in Cl^- ion conductance like GABA.Fluctuation analysis of membrane current responses provided estimates of the elementary events underlying these responses,all of which were associated with an increase in variance.Estimates of the single channel conductance activated by each analogue did not differ significantly from one another.Power density spectra of the agonist responses could be fit by single Lorentzian equations,from which estimates of agonist-induced single channel durations were calculated.These estimates all differed significantly from that for GABA, their ratios relative to GABA (=1) being 0.1 (taurine, GuAC, BABA, IAA), 0.3 (3APS, DAV, BGP,P4S), 0.5(THIP, isoguvacine, GABOB), 0.8(TAC), and 2.5 (muscimol).The results indicate that these analogues activate channels of the same conductance but of different durations.

We cannot be certain that all of the analogues are agonists at GABA receptors.Investigators using frozen rat brain synaptic membranes as a source of GABA receptor sites have shown that GABA binds to low and high affinity sites.Competitive binding assays have revealed that many structural analogues can displace GABA from the low affinity site.We have found that single channel conductance is not significantly correlated with the reciprocal of the analogue concentration displacing 50% of the bound GABA(IC50). However, single channel duration is correlated with 1/IC50 values for those analogues used in both binding and electrophysiological studies.For three independent binding studies the correlation coefficients were 0.95(n=10 analogues),0.97(n=7),0.97(n=5), all highly significant ($p<0.001$).The close correlations strongly suggest that the biochemical and biophysical assays involve a common parameter important to GABA receptor activation of Cl^- ion channels and that the low affinity site described in the binding studies is related to post-synaptic GABA receptors coupled to Cl^- ion channels. Although the method of assay used in binding studies does not provide a reliable estimate of the affinity of each analogue for the GABA receptor, the data suggest that agonist affinity may be the rate-limiting step in determining channel kinetics at GABA receptors.

67.3 CHARACTERIZATION OF ENDOGENOUS INHIBITORS OF Na^+ INDEPENDENT ^{3}H-GABA BINDING TO SYNAPTIC MEMBRANES (SM). S. Mazzari* and A. Guidotti. Lab. Preclin. Pharmacol., NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

Endogenous inhibitors of ^{3}H-GABA binding different from GABA were extracted from SM, washed twice with Triton X-100. When this extract was applied to a Biogel P-2 column, two major peaks endowed with inhibitory activity of ^{3}H-GABA binding were obtained. The first one was not retained by the column, while the second corresponded to a molecular weight of 400-800 dalton. The first peak, reapplied to a Sephadex G-50 column was eluted in position similar to that of ribonuclease A (a marker for 14,000 dalton). This peak (GABA-modulin) can be characterized as:

1) inhibiting high affinity ^{3}H-GABA binding non competitively
2) preventing GABA-stimulated ^{3}H-diazepam binding
3) inhibiting ^{3}H-diazepam binding competitively
4) being trypsin and pronase sensitive and 5) not dyalizable.

When GABA-modulin was incubated at 4°C for 24 hr and then reapplied to a Biogel P-2 column, a decreased activity of the GABA-modulin peak was observed. This decreased activity was associated with the appearance of a small molecular weight material (400-800 dalton) which binds GABA and inhibits ^{3}H-GABA binding noncompetitively. Furthermore, the 400-800 dalton material inhibits competitively ^{3}H-diazepam binding, prevents GABA-stimulated ^{3}H-diazepam binding and is dyalizable. These two molecular forms of endogenous inhibitors of ^{3}H-GABA binding can be obtained either from fresh or microwaved rat brains after the endogenous GABA is removed from the extracts using fast purification procedure according to Penefsky et al. (J. Biol. Chem. 252:2891, 1977).

67.4 ENHANCEMENT OF [^{3}H]GABA BINDING BY THE NOVEL ANXIOLYTIC ICI 136,753 AND BENZODIAZEPINES. B. A. Meiners* and A. I. Salama* (Spon. J. B. Malick), Biomedical Research Dept., ICI Americas Inc., Wilmington, DE 19897

ICI 136,753, an anxiolytic pyrazolopyridine, enhances specific [^{3}H]GABA binding to frozen and thawed, Triton X100 treated rat brain synaptic membrane fragments. The maximal enhancement in chloride-free Tris-citrate buffer was 20-30% over control and half maximal values are achieved at 10μM ICI 136,753. The enhancement of [^{3}H]GABA binding thus requires higher concentrations than the enhancement of [^{3}H]flunitrazepam binding by ICI 136,753 (EC_{50} 1 μM) (Salama and Meiners, 1980).

Two other pyrazolopyridines, cartazolate and SQ20,009, were also found to enhance GABA binding. When compared to ICI 136,763 cartazolate gives a larger maximal enhancement with about the same EC_{50}.

The benzodiazepines, flunitrazepam, diazepam and chlordiazepoxide, were found to enhance GABA binding in this preparation. The maximal enhancement for the three compounds was about 16%. This enhancement is lower than seen for cartazolate in chloride-free buffer. The rank order of potency for enhancement of GABA binding by the three benzodiazepines was the same as that for their displacement of [^{3}H]flunitrazepam binding. The range was from 10^{-6} to 10^{-9}M.

The binding isotherm for GABA in the presence and absence of ICI 136,753 (20 μM) gave a curvilinear Scatchard plot. In the presence of the drug there was a parallel shift of the points to the right. This is consistent with an increase in the number of both high and low affinity binding sites with little, if any, change in the affinities. In contrast, the changes in the GABA binding isotherm caused by chlordiazepoxide appeared to reflect affinity changes.

The effects of ICI 136,753 and flunitrazepam on [^{3}H] GABA binding were additive even at high concentrations of flunitrazepam (10^{-5}M) where additional flunitrazepam had no additional effect.

It has been suggested that the benzodiazepines exert their anxiolytic effect by altering GABA binding. Given the enhancement by ICI 136,753 of GABA binding, it is reasonable to suggest that the pyrizolopyridine anxiolytics such as ICI 136,753 may also be exerting their effects by altering GABA binding. ICI 136,753 appears to bind to a site distinct from the [^{3}H]GABA binding sites and the high affinity [^{3}H]benzodiazepine binding site. The site to which ICI 136,753 binds is, however, linked to both the [^{3}H]GABA and [^{3}H]benzodiazepine sites.

67.5 Enhancement of Benzodiazepine Binding by the Novel Anxiolytic ICI 136,753

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ICI 136,753 is one of a series of pyrazolo (3,4) pyridines possessing some cyclic AMP phosphodiesterase inhibitory activity. It has also been shown to restore behavior suppressed by punishment in animal conflict procedures. Unlike the benzodiazepines, ICI 136,753 enhances the specific binding of ^{3}H-flunitrazepam in fresh rat brain P_2 fraction suspended in Tris-HCl buffer. The maximal effect was about 40% with an EC_{50} about 1μM. Scatchard plot analysis revealed that a change in the binding affinity is the cause for the enhanced binding. ICI 136,753 at 10^{-5}M changes the K_D of ^{3}H-Diazepam from 8.1 nM to 4.5 nM with no significant change in the number of binding sites. The enhancement of ^{3}H-flunitrazepam binding caused by GABA (1-5 μM) could be blocked by the GABA antagonist bicuculline at a concentration (10 μM) which reduced the binding slightly. Similarly, this same concentration of bicuculline also prevented the enhancing effects of ICI 136,753 (0.5-5 μM). This suggests that the effects of ICI 136,753 are mediated through a GABAergic component. At a concentration of GABA (100 μM) where maximal enhancement is obtained on ^{3}H-flunitrazepam binding, addition of 5 μM ICI 136,753 caused a further enhancement of this binding. The effects of GABA and ICI 136,753 were compared in a preparation which had been frozen and thawed. In this situation, the enhancement due to GABA was greater than in the fresh P_2 fraction, while ICI 136,753 produced a concentration dependent (0.5-50 μM) displacement of ^{3}H-flunitrazepam binding (10-30%).

Thus it is concluded that the anxiolytic effects of ICI 136,753 seem to be manifested by a mechanism different from that of the benzodiazepines. The enhancing effect seen with ICI 136,753 may involve GABAergic mechanisms, at a site which is different and perhaps allosteric to the GABA binding site.

67.6 THE INTERACTIONS OF 5HT AND QUIPAZINE ON NEUROBLASTOMA.

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Cells of the neuroblastoma line NIE-115 respond to 5HT (applied by pressure injection) at $5X10^{-6}$M or higher by a depolarization. Desensitization of the response occurs. We were interested in the effects of quipazine [2-(1-piperazinyl) quinoline maleate] on these cells since the drug's strong stimulatory effect on smooth muscle is thought to be mediated by 5HT receptors (cf. Hong et al. (1969) Eur. J. Pharm 6:274). At 10^{-5}M, quipazine applied for 1-2 sec had no effect but 10^{-4}M produces depolarizations of up to 10 mV (but usually less than 5mV), and 10^{-3}M quipazine causes depolarizations of up to 20 mV which repolarize slowly (several minutes) or not at all. If 5HT and quipazine act as agonists on the same receptor, they should exhibit simple cross-desensitization. However, quipazine (at 10^{-3}M) in the bath does not just reversibly diminish the depolarizing response of 10^{-5}M 5HT, but causes the residual 5HT-induced depolarization to be prolonged like a quipazine depolarization. Furthermore, the response of cells to 10^{-3}M quipazine is virtually unaltered by the presence of 10^{-3}M 5HT in the bath. The actions of 5HT and quipazine on NIE-115 cells may not, therefore, be confined to a single receptor, but further study is required to clarify the mechanisms involved in their interactions.

68.1 INTERSTIMULUS INTERVAL EFFECTS ON CLASSICALLY CONDITIONED FLEXION REFLEX FACILITATION IN SPINAL CAT. R.G. Durkovic, Dept. Physiol., Upstate Med. Ctr., Syracuse, N.Y. 13210.

In the unanesthetized, decerebrate, spinal cat repeated trials of paired stimuli delivered to two hindlimb cutaneous nerves produces an increase in the anterior tibial muscle response to the first (conditioned) stimulus (Durkovic, Physiol. & Behav. 14:297, 1975). Control treatment animals which receive the same stimuli in an unpaired sequence do not exhibit reflex facilitation.

The present study was carried out to investigate the effects of the interval between conditioned stimulus (CS) and unconditioned stimulus (US) onset on the reflex response. Experiments were conducted on ten groups of animals, each with a different interstimulus interval (ISI). CS alone trials were interspersed among 30 conditioning trials to assess response alterations in all groups.

With simultaneous CS-US onset and with CS onset before US onset (forward conditioning) an inverted U shaped function was obtained with maximum flexion reflex facilitation at +0.5 and +1.0 sec. and little or no facilitation in groups with ISIs of 0.0, +0.25, +3.0 and +30.0 secs. With US onset before CS onset (backward conditioning) increases in reflex response to the CS were also produced with maximum facilitation at -0.25 sec and a drop off of facilitation with larger backward ISIs (-0.5, -1.0 and -3.0 secs).

These data suggest the following:

(i) Classically conditioned flexion reflex facilitation is brought about by the application of two stimuli paired closely together in time.

(ii) This classical conditioning effect can be obtained with both "forward" and "backward" conditioning paradigms.

(iii) There appears to be a mechanism at the spinal level which effectively blocks the classically conditioned response at ISIs near zero. This holds even though maximum overlap of CS and US occurs with such ISIs.

The results of the simultaneous and forward conditioning groups are in agreement with the vast majority of classical conditioning studies in whole animals. The finding of backward conditioning is also consistent with a substantial number of studies reporting successful backward conditioning in intact animals including man.

These results reinforce the concept that spinal conditioning can be viewed as an appropriate model for the study of neural mechanisms of classical conditioning.

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68.2 THE FIXATION OF CENTRALLY AND PERIPHERALLY INDUCED REFLEX ALTERATIONS. J. E. Steinmetz*, M. M. Patterson and J. Cervenka*. Dept. of Psych., Ohio Univ., Athens, OH 45701.

Over the past 50 years a body of research has appeared focusing specifically on retention or memory-like processes in the spinal reflex arcs. These studies have found that postural alterations in the limbs caused by various CNS lesions are maintained after spinal section if a temporal interval between lesion and section called spinal fixation time (SFT) is allowed. To replicate these earlier studies, unilateral lesions were placed in the anterior cerebellum of 30 rats to produce flexion-extention asymmetry in the hindlimbs. Fifteen of the rats were allowed a SFT of 20 min while the remaining animals had a SFT of 50 min. At the end of the fixation times, the degree of asymmetry was measured by suspending weights from the flexed leg until the hindlimbs were level. A spinal transection at T-7 was then performed and asymmetry was again measured. No differences in the amount of asymmetry was found between groups prior to the transection. However, the 50 min SFT group showed a persistent although reduced asymmetry after spinal section while the 20 min SFT group failed to show any asymmetry retention. The temporal parameters involved in spinal fixation of a cerebellar lesion induced asymmetry were more closely examined by varying fixation times from 25 to 45 min in five groups of rats. The data indicate that only an SFT of 45 min results in a consistent retention of asymmetry. Another study explored the possibility of demonstrating the fixation phenomenon through a peripherally induced asymmetry. Hindlimb flexion was produced in 10 male rats who received either 20 or 45 min of unilateral DC hindlimb stimulation. The asymmetry was measured by suspending weights from the flexed limb, a spinal section was performed at T-7 and the asymmetry again assessed. No differences in asymmetry were found before spinal section but differences were noted after the section. The 45 min stimulation group demonstrated a significantly greater amount of asymmetry retention than did the 20 min group. To assess the local effects of DC stimulation on the retention of the peripherally induced asymmetry, the dorsal and ventral spinal roots between T-12 and L-6 were cut in 20 rats. Upon completion of the rhizotomies, the animals were stimulated for either 10, 20, 30, 40 or 50 min after which the resulting asymmetry was measured. The stimulation failed to produce a consistent display of asymmetry in any of the animals. This series of studies suggest that a cerebellar lesion-induced postural alteration can be retained after spinal section if a minimum of 45 min is allowed between the lesion and a cord section. Likewise, a peripherally-induced asymmetry can be retained after spinal section and appears not to be a result of local stimulation effects.

68.3 SUCCESSFUL OBJECT DISCRIMINATION LEARNING AFTER COMBINED AMYGDALOID-HIPPOCAMPAL LESIONS IN MONKEYS DESPITE 24-HOUR INTERTRIAL INTERVALS. B. L. Malamut*, R. C. Saunders* and M. Mishkin. Lab. of Neuropsychology, NIMH, Bethesda, MD 20205

Combined ablation of amygdala and hippocampus in monkeys produces severe impairments on tests of both one-trial object recognition memory (Mishkin, Nature 273:297,1978) and one-trial object-reward associative memory (Spiegler & Mishkin, Neurosci. Abstr. 5: 323, 1979). Although the operated animals' rate of forgetting after a single acquisition trial in the associative memory test is unknown, their rate of forgetting in the recognition memory test seems to be extremely rapid, with performance approaching chance after an unfilled interval of only 2 minutes. Despite this rapid forgetting, animals with the combined lesions have no difficulty learning object discriminations,at least in the standard situation where trials are repeated 3-4 times per minute. In an attempt to resolve this discrepancy between rapid forgetting and successful learning,we tested whether object discrimination learning would be prevented in animals with limbic lesions if intertrial intervals exceeded the putative memory span.

Rhesus monkeys were trained preoperatively in a simultaneous visual discrimination paradigm in which a set of 20 pairs of junk objects were presented only once every 24 hours. The positive and negative objects within each pair, as well as the order of the pairs, remained constant across sessions. When the monkeys reached criterion of 90 correct in 100 trials, they were trained on a second set of 20 new object pairs in the same manner as before. The animals learned each of the sets in an average of 10 sessions, i.e. 10 trials. On completion of both tests, half of the animals received bilateral amygdaloid-hippocampal ablations and half remained as unoperated controls. Postoperatively, all animals were retrained with the same object pairs as before, one set at a time, followed by a third set of 20 new object pairs. Whereas the controls showed nearly perfect retention of the 2 sets learned preoperatively, the operated monkeys showed no retention, a result implying retrograde amnesia. However, they did relearn these sets in approximately twice the number of trials as before, and, in addition, they learned the new set in about the same number of trials as the control animals. In view of this surprisingly successful learning, we subsequently tested these animals in one-trial visual recognition and found the same rapid forgetting as in the original study. Thus, although animals with combined limbic lesions have an extremely short memory span on some tests, they can retain and accumulate information gained from single discrimination learning trials separated by 24-hour intervals. This paradoxical success implies the existence of an important memory mechanism outside the limbic structures of the temporal lobe.

68.4 IMPAIRED SERIAL ORDER PERFORMANCE AFTER TRANSECTION OF SUBICULUM-ANTERIOR THALAMIC FIBERS IN THE RAT. C.J. Rogers*, R.E. Davis, M. Karukan*, R. Smith*, C. Ahrens*, and C. VanHartesveldt. (spon: C. VanHartesveldt). Psychology Dept., Univ. of Fla., Gainesville, Florida 32611.

Recently, we demonstrated that animals with selective transections of the subiculum-anterior thalamus component of the postcommissural fornix learn operant response sequences in a manner different from undamaged animals. These transected animals readily adopt rigid response sequences which they maintain under a broad range of stimulus conditions. In this experiment, we investigated the effects of procedures designed to disrupt the formation of rigid response sequences in animals with these transections. These included changes in the intertrial intervals (0,10, 30,60 secs) and interresponse intervals (0,10,20 secs).

Initially, male, Long-Evans hooded rats were given CRF training in an operant chamber with 4 closely placed levers extending from one wall. Once stable performance was achieved, animals were required to press all 4 levers in any order to complete a trial. Ten trials were given each day. Only the first press on each bar was reinforced. The intertrial interval (ITI) was maintained at 10 secs (days 1-14), 30 secs (days 15-21), 60 secs (days 22-28), and 1 sec (days 23-35). Animals with transections of the subiculum-anterior thalamus connections (AT) were similar to controls at all ITI levels except 60 secs. At this level AT animals demonstrated increased perseverative responding relative to normal animals. In addition, AT animals exhibited significantly more lever pressing than did control animals during all ITI's.

To further interfere with the formation of rigid response sequences, we instituted inter-response delays of 4, 10, and 20 secs. During the inter-response delay period, animals were allowed access to the levers but responses were not reinforced. Onset of a house light signaled the inter-response interval (IRI) while a tone and house light compound stimulus signaled onset of the ITI. All animals exhibited similar increases in error rates during performance of the response sequence. Also both groups demonstrated similar numbers of responses during the IRI delay periods. However, AT animals failed to discriminate between IRI and ITI delays in a manner similar to controls. AT animals exhibited similar response rates during ITI and IRI delays while undamaged animals demonstrated lower rates during ITI delays than during IRI delays.

Taken together, these results suggest that when the formation of rigid response sequences are prevented, the performance of AT animals is impaired. AT animals fail to adopt alternative response strategies whereas normal animals exhibit more flexible behavior.

68.5 MEDIAL SEPTAL LESIONS AND THE ENTORHINAL THETA RHYTHM: CORRELATING THETA LOSS WITH RADIAL ARM MAZE LEARNING.
J. N. P. Rawlins*, S. J. Mitchell, D. S. Olton, Dept of Psychology, The Johns Hopkins University, Baltimore Md 21218, and O. Steward, Dept. of Neurosurg., Univ. Va. Sch. Med., Charlottsville Va. 22901. (SPON: E. Blass)

A regular slow wave theta rhythm can be recorded from the hippocampus (HPC) and medial entorhinal cortex (MEC) of freely moving rats. Lesions of the medial septal area (MSA) abolish theta in HPC and deplete acetylcholinesterase (AChE) in hippocampus and entorhinal cortex. In this experiment MSA lesions are shown to have equivalent effects on HPC and MEC theta, and can abolish both. AChE depletion is related to theta loss at these sites.

Adult rats were trained to walk in a treadmill and then had an electrode implanted in MSA and pairs of recording electrodes implanted in the right dorsal HPC and bilaterally in MEC. After recovery from surgery recordings were made between a frontal bone screw and each one of the recording electrodes in turn while the rat walked. Recording sessions took place over a 2-3 week period. A small lesion was then made in MSA using the implanted electrode. Subsequently recordings were made as before, and compared for amplitude and regularity to the activity seen earlier; thus the degree of theta retention was assessed. Recordings were continued over periods of up to 14 weeks, during which the rats were trained to obtain food in an 8-arm radial maze baited with one food pellet at the end of each arm.

In each recording the peak to peak amplitude of 20 consecutive theta waves was measured. The theta retention on the day after the lesion ranged from 0%-66% (median 0%) in HPC and from 0%-89% (median 4%) in MEC; one week later the retention was 0%-74% (median 49%) in HPC and 0%-94% (median 41%) in MEC. No further significant recovery was seen, and the extent of theta retention in HPC was well matched by that in MEC.

The rats were ranked for theta retention, and also for choice accuracy during the first ten sessions of maze learning. A Spearman's rank correlation test yielded a positive, but not significant, coefficient of 0.63 between rankings on these two measures. The rats showed a slight impairment in acquisition of the maze task, but were able to attain a criterion level of performance in spite of their loss of theta activity.

Since the degree of theta retention in HPC and MEC covaried well, MEC theta might depend on pacing by HPC theta activity. A further series of lesion experiments is exploring this possibility.

68.6 ASSOCIATIVE AND RECOGNITION MEMORY IMPAIRMENTS IN MONKEYS AFTER HIPPOCAMPAL RESECTIONS. M. Moss*, H. Mahut and S. Zola-Morgan* (SPON: D.Rosene) Dept. of Psych., Northeastern U. Boston, MA 02115

Deficits of memory have been seen in monkeys with combined bilateral removals of hippocampus and amygdala, but impairments following selective resections of the hippocampus have been inconsistent. Accordingly, we re-examined the effects of selective hippocampal removals on memory in three separate experiments. In the first, behavioral effects of hippocampal resections were compared to those of either fornix, entorhinal or anterior inferotemporal lesions on a visual concurrent discrimination task. This task involves the presentation of eight pairs of objects in an intermingled fashion until animals learn to discriminate every pair, and, thus, it tests the animals' ability to learn associations between reward and given stimulus objects in the presence of proactive and retroactive interpair interference. The second experiment was based on the clinical finding that medial temporal lobe damage in patients produces a modality non-specific impairment of memory. Accordingly, the performance of monkeys with hippocampal resections was compared to that of monkeys with anterior inferotemporal ablations on the concurrent task in the tactual modality. In the third experiment, the performance of monkeys with either hippocampal or fornix lesions was compared on a non-matching-to-sample task (cf. Mishkin, 1978). This task was administered under eight conditions: with delays between presentation of the sample and choice of either 10, 30, 70 or 130 sec, or with the number of object samples shown prior to pairing each one with a new object numbering 1, 3, 5 or 10 (lists). Both versions of this task require an ability to recognize whether or not a given object had been seen before, rather than whether it had been rewarded before.

In the first experiment, monkeys with either hippocampal, entorhinal or inferotemporal ablations, but not those with fornix sections, were significantly impaired. In the second experiment, both types of lesions resulted in an impairment on the concurrent task in the visual modality, however, in the tactual mode, monkeys with inferotemporal ablations were no longer impaired, whereas four of the six monkeys in the hippocampal group were still markedly impaired. In the third experiment, monkeys with hippocampal resections, but not those with fornix sections, were significantly impaired at all delays and with all list lengths.

Taken together, the results reveal that 1) Selective resections of hippocampus are sufficient to produce memory impairments in non-human primates and 2) These impairments are general since they are found both on tasks which tax discrimination learning ability in the presence of interference as well as on those which demand only recognition of familiar objects after varying time intervals.

69.1 THE INTRACELLULAR DYNAMICS OF A VERTEBRATE CONE. G.A. Carpenter Dept. of Math., Northeastern University, Boston, MA 02115 and S. Grossberg, Dept. of Math., Boston Univ., Boston, MA 02215.

A quantitative model of the intracellular dynamics within a vertebrate cone is described and used to explain parametric data from the turtle cone. The model differs qualitatively from the one suggested by Baylor, Hodgkin, and Lamb in its description of the rules whereby the intracellular transmitter acts. These rules can be derived from simple hypotheses about optimal transduction, and are similar to the transmitter rules that operate in nonvisual neural systems. Thus the cone transmitter is suggested to be a special case of a general principle of neural design.

69.2 CHEMICALLY MIMICKING THE EFFECT OF LIGHT ON THE cGMP CONCENTRATION OF THE VERTEBRATE PHOTORECEPTOR. Edward P. Meyertholen, Meegan J. Wilson, and Sanford E. Ostroy. Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

The major decreases in cGMP concentration which are initiated by light in the vertebrate rod photoreceptor exhibit many of the characteristics required of the phototransduction process. We have critically tested the role of this process by using a chemical procedure which mimics the effect of light on the cGMP concentration of the photoreceptor cell in the excised vertebrate retina. This procedure alters the intracellular level of cGMP in the dark, without requiring injection or extrinsic artificial inhibitors. The chemically induced decreases in cGMP concentration were 80-100% of the change normally observed on illumination; under these conditions the light-induced changes were completely eliminated or reduced to 20% of normal. Electrophysiological experiments done under comparable conditions exhibited a compression of the Light Intensity-Signal Amplitude relationship, but unlike continuous light, no significant changes in sensitivity were observed. The data suggest that light-induced changes in cGMP concentration are not responsible for the phototransduction or light adaptation processes of the vertebrate photoreceptor. (Supported in part by USPHS GRANT EY00413 and GM07211).

69.3 EFFECT OF TEMPERATURE ON SENSITIVITY AND KINETICS OF THE ROD PHOTOCURRENT. G. Matthews, K.-W. Yau* and D. A. Baylor*. Dept. of Neurobiol., Stanford Univ. Med. Sch., Stanford, CA 94305

The time-scale of the light response of vertebrate photoreceptors is strongly dependent on temperature (Penn & Hagins, 1972; Baylor et al., 1974). We have further examined this effect by measuring the photocurrent of single toad rod outer segments drawn into a suction electrode connected to a current-to-voltage transducer. Temperature was controlled by circulating heated or cooled water through channels in the walls of the chamber containing small pieces of toad retina. Temperature in the chamber was measured by a calibrated thermistor mounted within 0.5 mm of the tip of the suction electrode. Photocurrents were faster at higher temperatures. To quantify this effect, the reciprocal time-to-peak, $1/t_p$, of the average response to dim flashes was measured as a function of temperature. In 16 experiments of this type, the Q_{10} was 2.57 between 20° and 30°C, corresponding to an activation energy of 16.7 kcal mole^{-1}. In cones, corresponding values were 1.8 and 10 kcal mole^{-1}(Baylor, et al., 1974). The average dim flash response at 20°C was usually fitted by the expression for the impulse response of a series of four low-pass filters with equal time constant (Baylor, et al., 1979). The characteristic time constant of a cell was usually about 500 msec at 20°C. In most cases, the dim flash response at different temperatures was fitted by the same expression as at 20°C, but with different time constants. This implies that the four delay stages were roughly equally sensitive to temperature changes. In a few cases in which high temperature reduced the time constant to about 250 msec or less, the number of delay stages increased reversibly to five. This suggests that when the time constants of the four delays are reduced sufficiently, a fifth, relatively temperature-insensitive delay with a time constant of about 200 msec is revealed.

Sensitivity, defined as the flash sensitivity in darkness or the half-saturating light intensity, declined with increasing temperature, as did the average amplitude of the single photon response. The reduction in sensitivity could be accounted for quantitatively by the observed decrease in single photon response amplitude. This indicates that the quantum efficiency of isomerization of rhodopsin was unaffected by temperature in the range 15°-35°C.

Baylor, D.A., Hodgkin, A.L. & Lamb, T.D. (1974) J. Physiol., 242, 685-727. Baylor, D.A., Lamb, T.D. & Yau, K.-W. (1979) J. Physiol., 288, 589-611. Penn, R.D. & Hagins, W.A. (1972) Biophys. J., 12, 1073-1094.

69.4 $[Ca^{+2}]_i$ MODULATES SODIUM CONDUCTANCE IN ROD OUTER SEGMENTS. B. Oakley II and L. H. Pinto. Dept. of Biological Sciences, Purdue University, W. Lafayette, Indiana 47907.

It has been suggested that light causes calcium ions to be released from the intracellular disks in vertebrate rod outer segments, and that the resulting increase in intracellular free calcium concentration, $[Ca^{+2}]_i$, decreases the sodium conductance, g_{Na}, of the plasma membrane of the outer segment. We tested the hypothesis that $[Ca^{+2}]_i$ modulates g_{Na} in the isolated, superfused retina of the toad, Bufo marinus. While the input conductance of an impaled outer segment was monitored, EGTA was injected into the cell in order to reduce $[Ca^{+2}]_i$.

Rod outer segments were impaled with double-barrel micropipettes; one barrel was used to measure membrane voltage and the other was used both to pass current and to pressure-inject EGTA. Since the injection of EGTA produces a membrane depolarization, the membrane was (point) voltage clamped to its dark resting potential to prevent changes in voltage-sensitive conductances. The input conductance was assessed by measuring the incremental membrane current required to produce a small (10 mV), hyperpolarization of the membrane.

During voltage clamp, the injection of EGTA evoked an inward membrane current of ≈250 pA and an increase in input conductance. In 21 cells, the input conductance averaged 8.7 nS, and increased by 38% during the injection of EGTA. The EGTA-evoked increase in input conductance was not an artifact due to any limitations of the point clamp, since it was present even after voltage-sensitive conductances were abolished by bathing the retina with TEA^+, Cs^+, Co^{+2}, and 4-AP (3 cells). The observed effects were specific to EGTA; in control experiments, injection of K^+ acetate$^-$ did not detectably alter membrane current (5 cells). Superfusion with low Na^+ Ringer's solution (choline$^+$ substituted for Na^+) caused a membrane hyperpolarization. When the cell was clamped to its new resting voltage, the EGTA-evoked changes in membrane current and input conductance were greatly attenuated (11 cells). Thus, the EGTA-evoked changes in input conductance observed in normal perfusate were due primarily to changes in g_{Na}. Injection of a Ca^{+2}/EGTA buffer having equimolar Ca^{+2} and EGTA did not produce a detectable change in membrane current (3 cells). It is unlikely, therefore, that EGTA produced its effects by chelating a trace metal for which it has a high affinity. Since EGTA chelates Ca^{+2} with an affinity ≈6 orders of magnitude greater than it chelates Mg^{+2}, we conclude that a decrease in $[Ca^{+2}]_i$ increases g_{Na} in rod outer segments. (Supported by EY01221 and EY07008).

69.5 EFFECT OF ADAPTATION ON PHOTORECEPTOR RESPONSE DUALITY IN LIMULUS. Lolin T. Wang* and Gerald S. Wasserman. Sensory Coding Lab., Dept. of Psychological Sciences, Purdue University, West Lafayette, IN 47906.

Intracellular recordings were obtained from the photoreceptors (retinular cells) of excised mature Limulus lateral eyes. At low intensities two types of discrete waves are seen in dark adapted (DA) cells: Small slow potential fluctuations (SPF's)-previously described by Fuortes and Yeandle (1964)- and large fast potential fluctuations (LPF's)-previously described by Dowling (1968). At high intensities, the receptor response is more continuous. Light adapted cells (LA) also exhibit continuous responses, even when low intensity stimuli are used.

A second indicator of adaptation-dependent duality was obtained from measurements of the intensity-response function of the later portions of the response. In DA, this function is inflected-the response grows with intensity up to an intermediate intensity, then levels off and ultimately grows again. In LA, this inflection disappears and the intensity-response function has only one segment.

A third indicator of response duality was given by examining the late components of the response (Wulff and Fahy, 1979). At least three late components could be seen in the DA state. The time course of these late components seems relatively stereotyped. The wavelength dependence of the various components (Adolph, 1968) is being investigated.

These data indicate that there are two mechanisms governing the receptor response in Limulus. A high sensitivity mechanism which yields discontinuous responses made up of distinct components in dark adaptation and a low sensitivity mechanism which yields continuous responses in light adaptation. Some of these data are similar to those obtained by Dowling (1968) from juvenile eyes and some are similar to data obtained by Kaplan and Barlow (1975) from intact eyes. But since these data were obtained from adult excised eyes, they indicate that adaptation is the main variable affecting duality, not age or intactness of the eye.

69.6 INCREASED SUSCEPTIBILITY TO CONSTANT LIGHT-INDUCED RETINAL DEGENERATION IN DIABETIC RATS. D. D. Johnson and W. K. O'Steen, Dept. of Anatomy, Bowman Gray Sch. of Med. of Wake Forest Univ., Winston-Salem, NC 27103.

Albino rat retinal photoreceptors degenerate rapidly following exposure to continuous low-intensity fluorescent illumination. Albino rats were rendered diabetic with streptozotocin to determine if the severity of photoreceptor damage was significantly influenced during diabetogenesis. Young adult female rats were injected intravenously with streptozotocin (65 mg/kg) dissolved in buffered saline, while the control animals received only the vehicle solution. Immediately following the injections, rats were exposed to continuous, low-intensity fluorescent lighting (110 ft-c.) for 9 or 19 days. Additional animals injected with streptozotocin or saline were maintained in a cyclic photoperiod. On day 3 after injection blood glucose assays indicated that the average blood glucose value for the streptozotocin-injected rats was 418 + 22 mg%, as compared to 95 + 6 mg% for the saline-injected group. Both streptozotocin and saline-injected animals had a severe destruction and reduction in numbers of photoreceptor cells following the 9 and 19 day exposure periods, as compared to identically-treated animals housed in a cyclic photoperiod. Measurements of the retina (ONL, outer nuclear layer; and RT, entire retina) showed a 64% and 84% reduction in ONL thickness in the streptozotocin-treated rats exposed to 9 and 19 days of constant light, respectively (both $p<0.001$), and a 42% and 82% reduction in the ONL of saline-injected animals exposed to 9 and 19 days of constant light, respectively (both $p<0.001$), as compared to the cyclic light controls. The RT was also significantly reduced in both the constant light exposed streptozotocin and saline groups as compared to the cyclic light controls (all $p<0.001$). The ONL thickness of the saline and streptozotocin-treated animals kept in cyclic light were nearly identical, as were the RT measurements. When streptozotocin-induced diabetic rats and nondiabetic animals were exposed to constant light, the diabetic animals were more susceptible to retinal damage as judged by ONL and RT measurements. The ONL in the 9 and 19 day streptozotocin groups was reduced in thickness 34% ($p<0.001$) and 31% ($p<0.001$) as compared to the control animals. Similarly, the RT in the 9 and 19 day diabetic groups was reduced 16% ($p<0.01$) and 11% ($p<0.02$) as compared to the control animals. These results indicate that streptozotocin-induced diabetic animals are more susceptible to constant-light induced retinal degeneration, a process which occurs by an as yet undetermined mechanism. (Supported by NIH Grant #EY 02359)

69.7 PHOTORECEPTOR DEVELOPMENT IN THE ZEBRAFISH. T.A. Branchek* and G. Streisinger. Inst. Molecular Biol. and Dept. of Biol., U. of Oregon, Eugene, OR 97403.

The zebrafish, Brachydanio rerio, is highly vision dependent and undergoes rapid embryological development. We have studied its retinal differentiation at the light microscopic level. Of specific interest is the timing of the appearance of rods and cones. Reports in the literature indicate that cones appear before rods (at least in some fish species). This study provides a specific characterization of the anatomical substrate for visual function in young zebrafish and is also of interest from a comparative point of view.

Light microscopic analysis of Epon embedded material shows that at two days (d2) post-fertilization, the layering pattern characteristic of the adult retina is already established. By d4, cone-like outer segments (OS) appear, and they are arranged in a mosaic pattern. Higher resolution electron microscopic analysis is in progress to determine if rods are present at this stage. Appearance of photoreceptors at d4 correlates well with the behavioral repertoire of the zebrafish: vision-dependent feeding behavior begins on this day as does the onset of coordinate eye movements.

In d8 specimens, we observe presumptive rods which have short, bent OS scleral to the cone OS. At this same time, we note the beginning of pigment migration. In other species, pigment migration has been associated with the presence of functional rods.

Cone OS reach their adult dimensions by d12. Rod OS growth continues until d24 when approximate adult length is attained. The four cone types distinguishable in the adult retina are readily identified at d24.

Physiological experiments are in progress to determine the state of functional maturation of rods and cones in this retina at selected ages. In combination with the anatomical data, this work will provide a correlated analysis of the structural and functional development of rods and cones in this vertebrate retina.

70.1 ATTENTIONAL UNITY FOLLOWING BRAIN BISECTION IN MAN. Jeffrey D. Holtzman*, John J. Sidtis*, Bruce T. Volpe*, Michael S. Gazzaniga (SPON: Steven A. Hillyard). Division of Cognitive Neuroscience, Department of Neurology, Cornell University Medical College, New York, New York 10021.

Patients who have undergone a complete midline section of the corpus collosum are unable to perform interfield comparisons of lateralized visual information on a variety of perceptual tasks. Accurate performance would require perceptual access to both visual half-fields, and, at least for certain perceptual tasks, such bilateral representation appears to be unavailable to these patients. Despite this, perceptual-motor performance outside the psychology laboratory is remarkably unimpaired. These patients can carry out directed and coordinated motor activity within the environment: walk, run, avoid physical obstacles, even, in one case, operate a motorcycle.

In two such patients we asked whether the selective allocation of visual attention, an important aspect of directed motor behavior, has access to a unified perceptual representation of the visual world. The paradigm we employed was a modification of one originally used by Posner and his colleagues. Essentially, performance at a detection task under conditions in which a target stimulus was preceded in time by a cue to its spatial location was compared with performance under conditions in which no spatial cue or a misleading cue preceded the target. In general, detection latencies were shortest when attention was directed to the cued location via the valid spatial cue.

In our paradigm, a 3X3 grid appeared on each side of a central fixation point. In the Within-Field condition, the valid spatial cue and the target stimulus were presented with a one second delay to the same cell within the same grid; the invalid prime to a diffferent cell within the same grid. In the Between-Field condition, they appeared in homologous cells in different grids or in different cells in different grids, respectively. Performance was facilitated when a valid spatial cue preceded the target stimulus, not only in the Within-Field condition, but also in the Between-Field condition. These data suggest that the mechanisms which control the allocation of visual attention in these patients have access to a unified representation of the visual environment. Since each grid square subtended only 5 deg of visual angle, the specificity of the spatial priming effect is impressive. It remains to be determined whether or not cortical-cortical (anterior commissure) connections or cortical-subcortical (e.g., parietal-pulvinar) connections mediate this effect.(Aided by USPHS Grant NS15053 and the Alfred P. Sloan Foundation.)

70.2 INTERHEMISPHERIC COGNITIVE INTERACTION FOLLOWING PARTIAL COMMISSUROTOMY. John J. Sidtis*, Bruce T. Volpe*, Jeffrey D. Holtzman*, M.S. Gazzaniga Division of Cognitive Neuroscience, Department of Neurology, Cornell University Medical College, NY,NY

Because of the recent modifications in the surgical technique of commissurotomy, the effects of partial as well as complete callosal section can now be examined in the same patient. The present observations are made on a 26 yr old right handed male, case J.W., who underwent mid-line section of the corpus callosum in two stages. The posterior half of the corpus callosum including the splenium was sectioned first, with the remaining anterior portion sectioned in a second operation approximately 10 wks later. Following the initial posterior section, the primary visual, auditory and somatosensory systems of the right hemisphere were disconnected from the expressive language system of the left hemisphere.

Unlike the completely sectioned patient, J.W. did not deny having "seen" anything following left visual field stimulation. When asked to verbally identify what was presented, however, the patient was unable to do so although he still claimed to be able to "see" the stimulus in his mind. After several such instances, the investigators instituted an interactive probe with the patient that took the form of a game of 20 questions. Such questions began with "Is it an object or a living thing?" and would include questions about form, function and class. At the first interoperative testing, J.W.'s left visual field picture naming was slightly but significantly, better than chance ($p < .02$), by virtue of the 20 questions interaction. His naming of left visual field information improved throughout the interoperative period and by the sixth week, J.W. had adopted a self-generated inferential strategy based on his description of a mental image. Following complete callosal section, left visual field naming fell to chance ($p > .10$). The inference strategy was no longer attempted and the patient denied any experience following left field stimulation.

In the interoperative period, rather than having direct access to sensory information from the right hemisphere, the expressive language system appeared to be acting on a somewhat diffuse activation of stimulus related to semantic information that reflected an end stage result of semantic or gnostic processing within the right hemisphere. The availability of this activation was mediated largely, if not solely, by the anterior portion of the corpus callosum.

(Aided by U.S. PHS grant number 2 R01 NS15053-02 and the Alfred P. Sloan Foundation)

70.3 ELECTROPHYSIOLOGICAL INDICES OF CONSONANT INVARIANT CUES FROM SCALP RECORDED POTENTIALS OF PRESCHOOL AGE CHILDREN DURING SPEECH PERCEPTION. D.L.Molfese, R.J.Erwin*, and M.E.Deen*. Dept. of Psychology, Southern Illinois Univ., Carbondale, IL 62901.

Investigators have been unable to identify the acoustic invariant in stop consonants - the cue that enables a listener to identify particular consonant sounds independent of context. Consonant transition frequencies change as a function of the sounds that precede or follow these transitions. Nevertheless, consonant identification remains unchanged (Liberman, A., et al., Psychol. Rev., 74:431, 1967). The initial consonants of the syllables /di/ and /du/ are perceived as /d/ even though the acoustic cues differ for the two consonants. The present study reports for the first time the identification of neuroelectrical responses recorded from the scalps of preschool children which reflect the encoding of consonant invariant as well as consonant context deoendent cues. Auditory evoked responses (AERs) were recorded from the left and right hemisphere temporal (T_3, T_4, T_5, T_6) and parietal (P_3, P_4) regions of 12 right handed 4-year-old children in response to computer synthesized consonant-vowel syllables /bi, bæ, bɔ, gi, gæ, gɔ/.Amplified AERs for each sound were averaged independently at 8 ms intervals over a 600 ms period follwoing stimulus onset (for 75 points). These averages were then formed into a correlation matrix and subsequently submitted to a principal components analysis. Eleven factors (80% total variance) were isolated and then rotated using the normalized varimax criterion to improve factor distinctiveness. Factor scores were computed for each factor for each averaged AER. Independent analyses of variance with repeated measures for Consonant (2) X Vowel (3) X Formant Structure (2) X Hemisphere (2) X Sites (3) were performed on the factor scores to determine if any factors varied systematically as a function of the experimental manipulations. Factor 3 accounted for 10% of total variance and indicated that the right hemisphere differentiated between consonants independent of context, $F(1,11)=19.18$, $p < .005$. Factor 5 (8% total variance) discriminated between the vowels independent of initial consonant sounds, $F(2,22)=8.10$, $p < .005$. Factor 8 (5% total variance) discriminated between consonats as a function of the vowel sounds, $F(4,44)=6.58$, $p < .001$. These findings suggest that speech perception involves multidimensional processes which involve a variety of lateralized and bilateral hemispheric processes, some of which are sensitive to context independent and some of which are sensitive to context dependent speech cues.

70.4 A SEARCH FOR MORPHOLOGICAL ASYMMETRIES IN AREAS 9 AND 39 IN HUMAN MALE CEREBRAL CORTEX. Marian C. Diamond, Ruth E. Johnson and Amir Dehghan, Department of Physiology-Anatomy, University of California, Berkeley, CA 94720.

Recent studies on morphological asymmetries in the male, rat cerebral cortex have provided intriguing results. An earlier publication (1975) showed that the right cortex was thicker than the left in 15 male, Long-Evans rats per age group in 15 separate age groups in a study beginning at 6 days of age and terminating at 650 days of age. In a more recent study (1980) on 21 standard colony, 90-day-old, S_1, male rats, the right hemisphere was again thicker than the left, and Areas 17 and 39 reached differences which were statistically significant. Additional results with 92 S_1 male rats from various environmental conditions also showed Area 39 (along with some other areas) to be significantly thicker on the right than on the left.

With this information as a guideline, a sample of Area 39 was removed from the right and left hemispheres of 15 human male brains, ranging in age from 45 to 67 years. Since Area 9 in the rat showed the right hemisphere to be greater than the left but not significantly so, a sample of Area 9 was removed from both hemispheres in the human brains to compare with the findings in Area 39 from the same brains.

In order to study neuronal and glial populations, 20 micra, frozen sections were cut and stained with Kluver-Barrera stain. Beginning at the crest of the gyrus, two red lines were drawn 2 mm apart, beginning at the pial surface and extending to the underlying white matter. Another line was marked perpendicular to these two at the junction between the grey and white matter. These lines served as guides to count cells, differentiating neurons, astrocytes and oligodendrocytes. A comparison of neuron-glia ratios, cell numbers and number of microscopic fields/hemisphere will be presented.

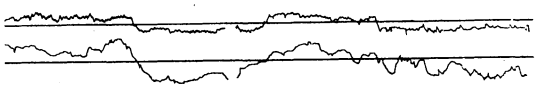
We are interested in area 39 in human brains in particular because the right-left differences shown in the male rat at 90 days of age were not seen in the female at this age and also because Area 39 is a most elaborate sensory integrating area. The question we eventually hope to answer is: Is there really a sex difference between hemispheres in this area and does it exist in human brains?

70.5 CEREBRAL HEMISPHERIC ACTIVITY AND AUTONOMIC NERVOUS FUNCTION
∇¶Deborah A. Werntz*,∇ Reginald Bickford,§ Floyd E. Bloom and ¶§David Shannahoff Singh Khalsa*(Spon. A. Traynor).∇ EEG Lab. UC San Diego Sch. Med. Dept. Neurosience, La Jolla CA 92093. §The Salk Institute, La Jolla CA, ¶ Kundalini Research Institute.

The nasal cycle is an ultradian rhythm with 75-200 min. periodicity in which air flow through the nares predominates unilaterally. This activity is presumed to be regulated via peripheral vasomotor activity with unilateral sympathetic dominance in one nostril coordinated with unilateral parasympathetic dominance in the other. We have compiled experimental evidence which directly correlates the nasal cycle with an alternation of cerebral hemispheric activity exhibited by the integration of EEG amplitudes. The nasal cycle was measured with thermistors to determine the relative air flow through each nostril. This information was recorded simultaneously with the primary EEG, at various montages, and then both were subjected to a continuous integration over time yielding data as seen in Fig.1. This study consisted of 43 males and females, right and left handed, recorded for a minimun time of 85 min. and a maximum of 190 min., with a mean of 2 hours. 22 subjects exhibited a nasal cycle during their recording period and a shift in hemispheric activity was observed to correlate in phase. The remaining subjects did not exhibit nasal cycles. Dominant right nostril air flow is associated ($p<10^{-6}$) with relatively greater left hemisphere amplitude and vice versa Fig.1.

Our data confirm the existence of the nasal cycle as an easily measurable indicator or 'window' of the basic organization of autonomic nervous function. This cycle may be viewed within the context of other ultradian rhythms, to support the concept of a single oscillator system. Dominance of right nostril air flow is correlated with a dominance of sympathetic activity greater on the right side of paired body structures, and left cerebral hemisphere EEG 'activity'. Conversely, left nostril dominance is a relative predominance of sympathetic activity on the left side and relatively greater EEG amplitude in the right hemisphere. Such phenomena could include the basic physiology of other known cyclic phenomena.

Fig.1



Top Tracing: Points above midline indicate a dominance of right nostril air flow; points below midline left nostril air flow. Bottom Tracing: Points above midline indicate greater integrated EEG amplitude (0-30 Hz.) in the left hemisphere; points below greater right hemisphere amplitude. Total time 185 min.

70.6 INTRAHEMISPHERIC AND THALAMIC CONNECTIVITY OF THE POSTERIOR PARIETAL CORTEX IN THE RACCOON. Sharleen T. Sakai Dept. of Psychology and Neuroscience Program, Michigan State University, East Lansing, MI 48824.

The purpose of this study was to investigate the intrahemispheric projections of the posterior parietal cortex with particular regard for the interconnectivity of this region with MI in the raccoon. In addition, the thalamic projections of the posterior parietal area was investigated.

The neural connectivity was determined using the horseradish peroxidase (HRP) tracing technique. Electrophoretic/pressure injections of 30-50% HRP were made into electrophysiologically identified areas of MI or the posterior parietal cortex in chloralose anesthetized raccoons. Following a survival period of 24-72 hours, the animals were transcardially perfused with a buffered aldehyde mixture. The brains were processed for HRP histochemistry using tetramethyl benzidine and diaminobenzidine as the chromogens on adjacent sections.

Following an injection into the MI forepaw area, labelled cell bodies were observed in the posterior parietal cortex in a region caudal to the SI arm area. Evoked potentials were recorded in this area of the posterior parietal cortex following stimulation of the contralateral forepaw. An HRP injection of this region resulted in labelled cells throughout a wide extent of MI forepaw area. The cells of origin were arranged in clusters. Within a cluster, retrogradely labelled neurons were found primarily in layers III and V, although some cells were observed in layer VI. In addition, HRP anterograde dust was observed overlying a cluster of retrogradely labelled neurons. The HRP dust was heaviest in layer I but occasionally was found to extend throughout all cortical layers. Following the posterior parietal cortical injections, labelled cell bodies were also observed rostral to MI in a region corresponding to MII. In addition, labelled neurons were also observed just lateral to SII.

The thalamic afferents of the posterior parietal area were arranged in a dense patch-like configuration. HRP anterograde dust was observed overlying and extending beyond the distribution of labelled thalamic cell bodies. The primary thalamic projection was observed arising from the dorsal cap region of VL throughout much of its rostrocaudal extent. Labelled cells were also observed in VA and dorsal LP. CL also contained HRP positive neurons.

The interrelationship of the posterior parietal cortex and motor structures observed in the present study suggests the posterior parietal cortex maybe involved with the integration of sensory and motor information.

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71.1 ON THE USE OF THE LIPOPHILIC CATION TETRAPHENYLPHOSPHONIUM (TPP^+) AS A MONITOR OF MEMBRANE POTENTIAL IN MITOCHONDRIA CONTAINING PARTICLES. S. Rochel*, A.J. Blume and F.L. Margolis. Department of Physiological Chemistry and Pharmacology, Roche Institute of Molecular Biology, Nutley, NJ 07110.

Several laboratories have used TPP^+ and other lipophilic cations to monitor transmembrane potential in prokaryote and eukaryote systems. Possible problems in calculation of $[TPP^+]$ due to mitochondrial contributions have been noted. However, details of the influence of TPP^+ interaction with mitochondria in eukaryotic systems have generally not been carefully evaluated. We have monitored the membrane potential ($\Delta\Psi$) of rodent olfactory bulb synaptosomes with TPP^+, which distributes across membranes according to the Nernst equation (Lichtshtein, Kaback & Blume, PNAS 76:650-654 [1979] and Ramos et al., PNAS 76:4783-4787 [1979]). Intrasynaptosomal mitochondria also exhibit a negative $\Delta\Psi$ and would therefore be expected to accumulate TPP^+ in a cascade fashion as a function of cytoplasmic $[TPP^+]$. Thus, the effects of TPP^+ on mitochondrial function were studied. We observed that when the effect of external potassium concentration on the synaptosomal potential was monitored in the range from 0 to 90 mM K^+, the calculated $\Delta\Psi$ was 25 mV lower in the presence of oligomycin/Argon (Ar) than in its absence. This indicated that there is accumulation of TPP^+ into mitochondria. It is represented by the difference in accumulation of TPP^+ in the absence and in the presence of the mitochondrial inhibitor oligomycin/Ar which eliminates the mitochondrial potential. This difference was used to calculate an apparent mitochondrial potential of -106 mV and a plasma $\Delta\Psi$ of -65 mV. In addition, the mitochondrial accumulation of TPP^+ decreased as a function of increasing TPP^+ concentration in the medium in the range from 0.1 µM to 200 µM TPP^+, while no such change in accumulation of TPP^+ was observed under oligomycin/Ar, where the only potential maintained is that of the plasma membrane. This suggests that TPP^+ interfered selectively with maintenance of mitochondrial $\Delta\Psi$ under aerobic conditions. Finally, TPP^+ exhibited toxicity in vivo at levels comparable to those used in vitro. As a result of the in vivo experiments, precaution with regard to exposure to the compound is recommended. In conclusion, TPP^+ accumulation is an accurate monitor of the response of synaptosomal $\Delta\Psi$ to the presence of drugs such as veratridine or tetrodotoxin, as well as to alterations of external K^+ concentrations (Rochel & Margolis, Soc. Neurosci. Abstr. 5:745 [1979]) when intrasynaptosomal mitochondrial $\Delta\Psi$ is eliminated by oligomycin/Ar. Our results suggest that TPP^+ at high concentrations (100 µM) may be used for both synaptosomal plasma membrane $\Delta\Psi$ measurements and elimination of mitochondrial $\Delta\Psi$.

71.2 EVIDENCE THAT HIGH FREQUENCY STIMULATION INFLUENCES THE PHOSPHORYLATION OF PYRUVATE DEHYDROGENASE AND THAT THE ACTIVITY OF THIS ENZYME IS LINKED TO MITOCHONDRIAL CALCIUM SEQUESTRATION. Browning, M., Baudry, M.*, Bennett, W.*, Kelly, P., and Lynch,G. Psychobiology Dept., Univ. of California, Irvine, Calif. 92717

Brief bursts of high frequency stimulation of the Schaffer-commissural axons of the rat hippocampal slice induce long-lasting potentiation of the stimulated synapses and a marked change in the endogenous phosphorylation of a 40,000 dalton (40K) brain protein. Since only potentiating stimulation was effective in producing the effect on the 40K protein, we hypothesized that this polypeptide might play some role in producing or triggering LTP. Subsequent experiments (Finn, Browning, and Lynch, Neurosci. Lett., 1980, in press) indicated that trifluoperazine which interferes with the induction of LTP, also blocks the endogenous- and clamodulin-stimulated phosphorylation of the 40K protein. Given the potential importance of this phosphoprotein to synaptic potentiation, we have been interested in establishing its identity and determining the significance of its phosphorylation

The 40K phosphoprotein is enriched in the mitochondrial fraction and since the α-subunit of pyruvate dehydrogenase (α-PDH) is the only mitochondrial phosphoprotein known to be close to that molecular weight, we compared the two proteins. We concluded that the 40K protein was α-PDH on the basis of partial and total proteolytic fingerprint comparisons as well as comparisons of ionic and pharmacological manipulations of the two proteins. We next used Dichloroacetate (DCA) which inhibits PDH kinase. to assess the effects of changes in the phosphorylation of PDH on various mitochondrial functions. (It is known from the work of Linn et al., (1969), Proc. Nat. Acad. Sci., U.S.A., 64, 227-234 that PDH is inhibited by phosphorylation.) DCA stimulated pyruvate-supported calcium accumulation by brain mitochondria and was half-maximally effective at a concentration (0.5mM) which was reasonably close to that at which it inhibited phosphorylation of PDH (0.16mM) and increased pyruvate dehydrogenase activity (0.3mM). DCA had no effect on calcium uptake supported by succinate or ATP, a result which indicates that the drug is acting selectively on the PDH step of mitochondrial metabolism. It appears then that calcium sequestration by mitochondria is tightly linked to the phosphorylation of PDH. Since the available evidence strongly suggests that α-PDH and the 40K material are the same protein, it follows that repetitive stimulation of the type which produces LTP, influences the phosphorylation of PDH and hence perturbs calcium buffering by the mitochondria. A perturbation in intracellular calcium levels could then act on any of several calcium sensitive processes related to the organization of the pre- and post-synaptic elements to produce an enduring change in the efficacy of the transmission process.

71.3 EXTRACELLULAR ALTERNATING CURRENTS CHANGE FIRING RATE IN APLYSIA PACEMAKER NEURONS. A. R. Sheppard*, E. French* and W. R. Adey (SPON: T. J. Willey). VA Hospital, Loma Linda, CA 92357 and Dept. of Physiol., Loma Linda Univ., Loma Linda, CA 92350

Electrical coupling of a neuron to an external current is weak because: 1) most current is shunted around the cell by the highly conductive extracellular medium, and 2) there is no net polarization of a spherical cell. Yet, Terzuolo and Bullock (PNAS, 42:687, 1956) observed modulation of the firing rate (FR) in crayfish stretch receptors by direct currents at extracellular gradients of about 10 mV/cm, and Wachtel (DOE CONF-781016, 1979) reports a frequency dependent change in FR of Aplysia pacemaker neurons at current densities as low as 2 µA/cm .

We report several phenomena affecting the FR of beating pacemaker neurons of the abdominal ganglion of Aplysia californica during exposure to sinusoidal, low frequency currents passed between two Ag-AgCl electrodes immersed in the artificial seawater medium (17C) that surrounds the ganglion.

Electric fields of 0.01 to 0.2 Hz (slow compared to cell FR) at about 20 mV/cm (peak) produce prompt changes in FR, although the correspondence between current maxima and either acceleration or slowing of the FR may differ from cell to cell, and sometimes the correspondence reverses upon a change in frequency for a given cell. The dual phases of the current/rate relation suggest that transduction of the extracellular field occurs at localized sites, and is not due to a net cellular polarization.

Secondly, smaller electric fields (1-4 mV/cm) at frequencies near the endogenous FR (~1 Hz), and at 60 Hz, were applied for 10 or 20 minutes. At all frequencies, the effects, when apparent, occurred after considerable delay. We observed altered FR, increased variability in FR, a field-related hyperpolarizing shift in previously stable neurons, and paroxysmal activity. In particular, the hyperpolarizing potential resembles the one associated with the slow K^+ current in bursters, and in the absence of prompt changes in FR, suggests a coupling of the external field to selected membrane functions.

We estimate that only 5 ppm of the extracellular current crosses the membrane of a 150 µm radius cell (membrane resistance, $9 \cdot 10^4$ Ωcm^2). For a cell in a 4 mV/cm field, bathed in a medium of 19 Ωcm resistivity, the trans-hemisphere current is only $\sim 10^{-12}$A. The localized membrane potential change at a pole of the cell is about 90 µV, while any net potential change is far less, as it depends upon assymetries in cell shape or conductance, and the effects of axonal loading. (Funding: DOE DEAI101-79ET29078, FDA RO1-FD00963, and Southern California Edison Company.)

71.4 VOLTAGE AND CURRENT TRANSFER ALONG DENDRITES OF LOBSTER STRETCH RECEPTOR NEURONS. William H. Calvin and Dennis A. Turner* (SPON: A. A. Ward, Jr.). Department of Neurological Surgery, University of Washington School of Medicine, Seattle WA 98195.

Membrane leakiness in the dendritic tree affects the transfer of voltage and current to the impulse trigger zone at the axon's initial segment. Unlike the losses along an infinite cable, current loss may be minor in a dendritic tree even though voltage is markedly attenuated (see Graubard & Calvin 1979, in The Neurosciences Fourth Study Program). A typical sensory neuron, SN_1 in *Panuliris interruptus*, was investigated by 1) cobalt staining and anatomical reconstruction out to 2 µm diameter distal processes; and 2) intrasomatic measurement of input resistance (yielding 1.09 megohm using the spike height method) in a similar group of neurons. An average SN_1 neuron had an 80 µm soma diameter, a surface area of 7.7×10^4 μm^2, and about eight major dendrites. A simple resistive-only reconstruction of the electrotonic properties was done using the original exact method of Rall (1959; *not* assuming $d^{3/2}$ conservation). Not knowing the anatomy distal to the 2 µm points, one brackets the possible range of R_m by alternately assuming a sealed end or an infinite extension distally. This causes an uncertainity in the computed R_m value (that value which will yield 1.09 megohm intrasomatic input resistance) of 800 to 3600 $ohm \cdot cm^2$. However, voltage and current transfer from a single distal tip input to the soma are little affected by this large range of R_m: voltage transfer averaged 22% for both extremes, while current transfer was 89-83%. The large voltage loss is thus not primarily caused by current loss along the path; it is primarily created by the central loading of the major dendrite by the other dendrites without simultaneous inputs. For inputs simultaneously on all major dendrites, we adapted the method of Rinzel & Rall (1973, which assumed $d^{3/2}$ conservation) and obtained changes from the single-input case qualitatively similar to theirs.

It is clear that R_m alone cannot characterize the electrotonic properties of a dendritic tree; geometry, particularly branching pattern, is usually a far more important variable. One must also ask whether voltage or current is the relevant variable for the neuron's operation. While voltage is relevant when the neuron is silent but approaching the (voltage) threshold for the first impulse, current is considered (Calvin, 1975) to be what elevates firing rate, once rhythmic firing is in progress. The low-loss aspects of SN_1 for current are, however, in contrast to the moderate current losses of spinal motoneurons (Barrett & Crill 1974) and hippocampal pyramidal neurons (Turner & Schwartzkroin 1980). Dendritic trees are often rather good funnels for current; the more branching, however, the greater the loss. [NIH grants].

71.5 REVERSIBLE BLOCKAGE OF ACTION POTENTIALS BY APHANTOXIN. T. Hoshi, W. Watson, J.J. Sasner. (SPON: H. Levitan). Zoology Dept. University of New Hampshire, Durham, NH 03824.

Toxic blue-green algae (Aphanizomenon flos-aquae) bloom in eutrophic, freshwater lakes and ponds in New England. Preliminary studies on frog nerve and muscle preparations indicate that lethal effects are due to the toxin's ability to block the generation of action potentials (Sasner and Ikawa, 1975).

In this investigation, semipurified aphantoxin (M.W.<500 daltons) was applied to partially desheathed nerve cords of crayfish to test its effects on the membrane properties of the medial and lateral giant fibers. At a concentration of 4 µg/ml, aphantoxin reversibly blocked the generation of action potentials in 20 seconds. The time required for this effect was dose-dependent, with a minimum effective concentration of 0.4 µg/ml. Complete recovery following a 4 µg/ml application was obtained after 10 minutes of washing with toxin-free saline solution at a flow rate of 4.5 ml/min. No significant change in resting potential or resting membrane resistance was observed during repeated application of the toxin. The toxin decreased the rate of depolarization much faster than the rate of repolarization.

It is suggested that aphantoxin blocks voltage-sensitive sodium channels much like saxitoxin (STX) and tetrodotoxin (TTX). Aphantoxins' effects are more readily reversible than either STX or TTX and therefore may prove to be a more useful tool for some neurophysiological investigations.

71.6 INACTIVATING FAST POTASSIUM CONDUCTANCE IN VAGAL MOTONEURONS IN GUINEA PIGS: AN IN VITRO STUDY. Y. Yarom*, M. Sugimori* and R. Llinás (SPON: R. A. McCrea). Dept. Physiology & Biophysics, New York Univ. Med. Ctr., 550 First Ave., New York 10016.

A fast voltage-dependent potassium conductance, normally inactive at rest membrane potential level and which may be de-inactivated by hyperpolarizing, is present in mammalian vagal motoneurons. This conductance is observed when the membrane is depolarized from a hyperpolarized level and is in every way similar to that reported in invertebrates by Hagiwara et al. (J. Physiol. 155: 410, 1961) and Connor and Stevens (J. Physiol. 213: 21, 1971). Intracellular recordings from these cells were obtained in brain stem slices from 300 gm. animals. Steady-state measurement of membrane resistance indicates stable properties if taken at rest (-65 mV) and at 10 mV depolarization or hyperpolarization from rest. It was noted, however, that following a hyperpolarizing pulse the potential at the break of the current pulse did not return to the resting level with the expected passive time constant but, rather, remained at a hyperpolarized level having a time course of approximately 30 msec. Measurement of slope conductance following break of the hyperpolarized pulse indicates a large conductance change concomitant with the duration of the after-hyperpolarization. Biphasic current pulse injections demonstrate that this after-hyperpolarization has an equilibrium potential similar to that produced by the after-hyperpolarization following an action potential or the potassium activation which follows calcium electroresponsiveness in this cell. It is, therefore, concluded that the I_A current present in invertebrates is also represented in mammalian CNS neurons. The significance of this current in determining the repetitive firing properties of this neuron will be discussed. (Supported by USPHS grant NS-13742 from NINCDS)

72.1 EFFECTS OF OVARIAN STEROIDS ON SEROTONIN METABOLISM WITHIN DISCRETE BRAIN REGIONS OF OVARIECTOMIZED RATS. Ric. I. Cone, Gary A. Davis* and Robert W. Goy. Wis. Reg. Primate Res. Ctr. and Neurosciences Training Program, Univ. Wisconsin, Madison, WI

Brain serotonin (5-HT) metabolism within the dorsal raphe nucleus (DR) was elevated in ovariectomized (ovx) rats following ovarian steroid replacement compared to metabolism in controls given oil (O) as vehicle. Females received either estradiol (E, 20 µg/kg) and progesterone (P, 2 mg/kg) sc 48 hrs apart or O + O. The animals also received either the monoamine oxidase (MAO) inhibitor, pargyline (75 mg/kg, i.p.) or vehicle (saline) at designated times 10-40 min prior to sacrifice. Decapitation occurred 5 hrs following the second steroid injection and 4-6 hrs into the dark phase of a 14:10 light/dark cycle. Basal 5-HT (5-HT_b) levels and the rate of 5-HT accumulation (5-HT_r) following MAO inhibition were estimated by radioenzymatic assay in select anatomically-defined regions punched from frozen brain tissue. Values within DR listed in the table are based on results from several experiments.

	O + O	E + P
5-HT_b*	37.1 ± 0.7 (30)	42.2 ± 0.7 (29)
5-HT_r†	36.2 ± 1.6 (44)	44.6 ± 2.5 (48)

* ng 5-HT/mg protein ± S.E. (N)

† ng 5-HT/mg protein per half-hour ± S.E. regression (N)

Within the DR, 5-HT_b levels and 5-HT_r rates were 13.8% ($p < .001$) and 23.2% ($p < .025$) higher in E+P treated females compared with O+O treated controls. Differences in 5-HT_b were found within the median raphe nucleus, but these were inconsistent from experiment to experiment. No differences were found in tissue immediately adjacent to these nuclear regions or within other brain regions including the ventral medial nucleus and suprachiasmatic nucleus of the hypothalamus.

It is not clear whether the effects on 5-HT metabolism reported here resulted from the presence of E, P or sequential E+P. In an earlier study, females given either E+O or E+P were found to have significantly higher 5-HT_b levels than controls in grossly dissected tissue from the brainstem raphe area. In addition, plasma levels of corticosterone and progesterone were significantly higher in these animals than in the control group. This suggests that with respect to the effects reported here, adrenal release of P following a low dose of E may have been sufficient to eliminate any distinctions between treatment with E+O versus E+P. Consequently, examining the endocrine specificity of this increase in 5-HT metabolism within DR would require appropriate controls for the effects of E on adrenal activity. Research supported by grants #RR00167 and #MH21312.

72.2 THE EFFECT OF CHRONIC, APERIODIC CORTISOL LEVELS ON THE DIURNAL FLUCTUATION OF SEROTONIN AND DOPAMINE ASSOCIATED BEHAVIORS IN THE GUINEA PIG.
P.CARVEY*, P.NAUSIEDA, W.WEINER, C.GOETZ* AND H.KLAWANS*
Rush-Presbyterian-St.Luke's Medical Center, Chicago, IL 60612

Chronic corticosteroid (CS) therapy, Cushing's syndrome, and, in a significant percentage of cases, long term levodopa therapy, are associated with elevated, non-fluctuating levels of CS. These clinical states are frequently associated with thought disorders, disruption of sleep architecture, fragmentation of the sleep-wake cycle, and myoclonus. Interest in the mechanisms involved in these side effects prompted us to study the effect of chronic, elevated, aperiodic CS levels upon two behavioral models of central monoaminergic activity.

Stereotypic chewing behavior (SB) induced by the direct dopamine (DA) receptor agonist, apomorphine, is a quantitative measure of striatal DA activity. Myoclonic jumping behavior (MJB) induced by the serotonin (5HT) precursor 5-Hydroxytryptophan, is a quantitative measure of brainstem 5HT activity. Prior to chronic CS treatment, the sensitivity to a fixed dose of each test drug was monitored at five time points over a 24 hour period in 110 guinea pigs. The behavioral response to each test drug varied independently, demonstrating statistically distinct circadian variations. We then delivered cortisol succinate (12.5mg/kg/day) to one-half of the animals in such a fashion that daily intake was continuous and aperiodic. Both behaviors were again assessed over 24 hrs in control and experimental animals on days 14 and 28 of treatment. 48 hrs after the final behavioral assessment, animals were sacrificed at their respective time-points and tissue samples were removed and immediately frozen.

Results indicated that elevated, aperiodic CS levels affect both absolute activity within and periodicity of central monoaminergic systems. 14 days of continuous, aperiodic CS treatment abolished rhythmicity and decreased behavioral sensitivity to 5HT precursors, but had no effect on apomorphine behavioral sensitivity or rhythmicity. After 28 days of CS treatment, enhanced sensitivity to 5HT appears to develop and dopaminergic sensitivity and rhymicity are diminished. It is possible that the initial pertubation of sensitivity and periodicity within the 5HT system may have been responsible for subsequent, similar changes in the DA system. These data, when applied to clinical observations of psychomotor toxicity in states of chronic aperiodic hypercortisolemia suggest that manifestation of these side effects may depend upon similar CS mediated alterations of central monoaminergic activity. The mechanism of such putative neuroendocrine interactions is currently being assessed in binding and transmitter level analysis of discrete brain regions.

72.3 BINDING OF ^{3}H-DIHYDROERGOCRYPTINE TO AN α-ADRENERGIC RECEPTOR IN THE BOVINE STALK-MEDIAN EMINENCE. H.T. Chen* and R.I. Weiner. Reproductive Endocrinology Center, Univ. Calif. Med. Sch., San Francisco, CA 94143

Dopaminergic and noradrenergic terminals, implicated in neuroendocrine regulation, densely innervate the external and internal layers of the median eminence, respectively. A high affinity, saturable binding site for ^{3}H-dihydroergocryptine (DHE) was reported in the stalk median eminence (SME) of the sheep (Endocrinology 103:302, 1978). DHE is a potent dopamine agonist and α-adrenergic antagonist and has been used extensively as a radioligand to study both receptors. In the present study, we have further characterized ^{3}H-DHE binding to the SME of the steer. Particulate fractions of the SME were incubated in concentrations of ^{3}H-DHE from 0.1 - 20nM in Tris buffer (pH 7.4) at either 22°C for 90 min. or 37°C for 60 min. Bound ^{3}H-DHE was separated from free by filtration on glass fiber filters. Specific binding defined by 10^{-5}M phentolamine was high affinity (K_d = 1.78 ± 0.22nM) saturable (Bmax = 481 ± 39 fmol/mg protein) and stereoselective. The K_d (2.8 ± 0.1nM) calculated from the ratio of the rate constants K_2/K_1 (K_1 = 1.47 ± 0.15 x $10^7 M^{-1}$ min^{-1} and K_2 = 0.04 ± 0.002 min^{-1}) at 37°C was in good agreement with the K_d determined in equilibrium experiments.

Agonist	Ki(nM)	n	Antagonist	Ki(nM)	n
L-epinephrine	292±67	4	phentolamine	3.0±0.8	3
apomorphine	792±248	2	yohimbine	38±7	3
L-norepinephrine	801±142	4	D-butaclamol	105±19	3
dopamine	6110±1210	3	spiperone	744±34	2
D-norepinephrine	15700±3560	2	prazosin	798±190	3
isoproterenol	71300±19000	4	chlorpromazine	849	1
serotonin	75600	1	propranolol	22300	1
			L-butaclamol	61100	1

The rank order of potency of agonists and antagonists to displace ^{3}H-DHE binding was consistent with an α-adrenergic site. The affinity of dopaminergic agonists to displace DHE was shifted approximately 10 fold to the right relative to their potency at known dopamine receptors while the affinity of dopaminergic antagonists was shifted 100 fold. The rank order of potency of the α-antagonists (phentolamine > yohimbine > prazosin) is consistent with an α-2 receptor. α-adrenergic receptors in the SME may play a role in the neuroendocrine regulation of anterior pituitary hormone secretion. Further studies are needed to determine if these sites are localized on neuronal elements or tanycytes in the SME.
(Supported by NIH Grant HD-08924 and the Rockefeller Foundation.)

72.4 QUANTITATION OF NORADRENERGIC INPUT TO MAGNOCELLULAR PERIKARYA IN THE RAT SUPRAOPTIC NUCLEUS. H. Khachaturian and J.R. Sladek, Jr. Department of Anatomy and Center for Brain Research, Univ. Rochester School of Med. and Dentistry, Rochester, N.Y. 14642.

Noradrenergic mechanisms have been implicated in the central control of neurohypophyseal hormone release. The supraoptic nucleus (SON) receives a dense noradrenergic input originating from monoamine cell groups located in the brain stem. Many fluorescent, norepinephrine-containing varicosities are arranged pericellularly to magnocellular neurons. That these varicosities might represent functional noradrenergic "contacts" has been suggested by quantitative electron microscopy, and direct microiontophoretic applications of norepinephrine onto antidromically identified neurosecretory neurons of the SON.

The present study is aimed at the direct quantitation of the number of fluorescent varicosities juxtaposed to SON neurons, through serial reconstructions of magnocellular neurons. Hypothalami from adult male, Fischer 344 rats of 3 months of age were processed for formaldehyde-induced fluorescence of catecholamines. 4µm paraffin sections were cut on a microtome and the rostral, middle and caudal regions of the SON were photographically reconstructed. Magnocellular neurons were traced through serial sections; the total number of fluorescent varicosities in juxtaposition to the entire perikaryal surface was determined for 120 magnocellular neurons. The results are shown below:

SON region	Zone	Mean(hits/cell)	Range(hits/cell)	N(#cells)
Rostral	Dorsal	3.8	1-9	10
	Ventral	13.3	7-23	13
Middle	Dorsal	4.4	0-9	22
	Ventral	14.5	6-29	53
Caudal	Dorsal	4.7	4-6	4
	Ventral	17.7	10-25	18

Magnocellular neurons in the ventral SON were more heavily "contacted" than those in the dorsal regions of the nucleus. The mean number of varicosities per perikaryon was 15.0 (range 6-29, n=84) for neurons situated ventrally, and 4.3 (range 0-9, n=36) for those located more dorsally. The majority of neurons in the caudal SON belong to the more heavily "contacted" group, while at middle and rostral levels of the nucleus a more uniform dorsoventral separation was seen. It is known that vasopressin neurons predominate in the caudal and ventral parts of the SON. This taken with the present findings suggests that perikarya of vasopressin neurons may receive a richer noradrenergic input than those of oxytocin neurons in the rat SON. Supported by USPHS Grants GM 07230, AG 00847, AG 01456, and NS 15816.

72.5 AGING OF ENDOCRINOLOGICALLY-IMPORTANT CENTRAL NEURONS. John R. Sladek, Jr. and Gloria E. Hoffman, Dept. of Anatomy and Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642.

Several neuroendocrine mechanisms are compromised with age; these include the hypothalamo-pituitary gonadal axis, prolactin secretion, growth hormone release, and others. In order to better define a possible mechanism for these functional alterations a combined analysis of specific peptidergic neurons and functionally interactive dopaminergic neurons was undertaken in the aged rat. Male rats (Fischer 344) of 3, 12, 20, and 30 months of age were examined for formaldehyde-induced histofluorescence of monoamines and PAP immunochemistry of neuropeptides. Antisera generated against LHRH (Nilaver-Silvermann#3) and somatostatin (Stewart#9) were employed. Major declines were noted in the immunochemical staining of LHRH and somatostatin fibers throughout all rostral to caudal levels of the median eminence. In general the density of fibers was decreased although in some instances, especially for somatostatin fibers, exceptionally large profiles were noted which appeared reminiscent of Herring bodies of the neurohypophyseal system. These age-related changes were first noted in the 12 month old animals and were most severe in the 30 month old animals. Dopamine histofluorescence in the external zone of the median eminence also appeared depressed in the aged animals. However, perikarya of origin of this system within the arcuate-periventricular region appeared to contain an increased amount of fluorescence in aged rats. For 30 month old rats this increase was determined microspectrofluorometrically to be about 50-65% greater than 3 month old rats; the dopamine fluorescence of the external zone was 30-40% less than controls.

Two other unusual fluorescence parameters were noted; first what appeared as a hyperinnervation of neurons located in the lateral arcuate nucleus was seen in aged animals and secondly, a thin rim of supraependymal catecholamine fluorescence was seen overlying the region of the arcuate nucleus in the third ventricle.

Whether decreased staining and intensity of dopamine, LHRH, and somatostatin in the median eminence is the result of some change in synthesis and/or release, or alternately some modification of axonal transport of synthesized neurochemicals is unknown. Nevertheless these findings may point to a central locus of altered endocrine function in the senescent rat. Supported by USPHS Grants AG 00847, AG 01456, NS 15816, NS 13725, and RCDA NS 00321.

72.6 CATECHOLAMINE INNERVATION OF THE SUPRAOPTIC NUCLEUS OF THE BRATTLEBORO RAT. J. Schöler* and J.R. Sladek, Jr. (SPON. I. Shoulson) Dept. Anatomy and Center for Brain Res., Univ. Rochester Sch. of Med. and Dentistry, Rochester, N.Y. 14642.

The Brattleboro rat carries a congenital diabetes insipidus due to an inability to synthesize vasopressin as opposed to a neuronal cell loss. Magnocellular hypothalamic nuclei of normal rats receive a dense noradrenergic innervation from the medullary and pontine brain stem which favors the perikaryal innervation of vasopressin neurons. Oxytocin neurons are sparcely innervated. Considering the possibility that the presence of vasopressin may be essential to the maintenance of these innervation patterns, this phenomenon was examined in the Brattleboro rat which is deficient in the target neuron peptide, but not the target neuron. Normal, adult male Long Evans rats and their genetically deficient counterparts were examined for formaldehyde-induced histofluorescence and rat neurophysin immunocytochemistry utilizing a combined demonstration technique. Comparator bridge microscopy was employed to identify peptide-specific target neurons. Alternate section analysis of fluorescence and neurophysin immunocytochemistry revealed the following. The ventral portion of the supraoptic nucleus, which has been shown to contain a majority of vasopressin neurons, appeared to contain a dense pattern of catecholamine varicosities in the normal rat. In the Brattleboro rat however, this same region of the nucleus contained a less dense pattern such that it often appeared devoid of catecholamine varicosities. In contrast, neurons in the more dorsal portion of the nucleus in the Brattleboro rat appeared more heavily rimmed by catecholamine varicosities; comparator bridge microscopy revealed that these neurons stained positively for rat neurophysin and are presumed to be oxytocin-containing. This division is not strict and when neurophysin-containing neurons were found in the more ventral regions of the supraoptic nucleus, they, too appeared to be rimmed in a pericellular manner. Additionally, a zone ventral to the magnocellular perikarya, shown by McNeill & Sladek (JCN 1980 in press) to contain neuritic profiles of magnocellular neurons and a maximally dense catecholamine varicosity pattern appeared to contain a decreased varicosity pattern in the Brattleboro rat. These data suggest an alteration in the innervation patterns of oxytocin and vasopressin neurons in the Brattleboro rat in comparison to normal animals. Supported by USPHS Grants MH 14577, AG 00847, AG 01456 and NS 15816.

73.1 THE MOLLUSCAN CARDIOACTIVE NEUROPEPTIDE FMRFamide IS A NEUROSECRETORY PRODUCT. G.T. Nagle, D.A. Price and M.J. Greenberg. Dept. of Biological Sciences, Florida State Univ., Tallahassee, FL 32306.

FMRFamide (phenylalanyl-methionyl-arginyl-phenylalanine amide) is a cardioactive peptide found in the ganglia of the clam Macrocallista nimbosa (Price & Greenberg, Science 197: 670, 1977). But is FMRFamide a neurosecretory product?

Homogenates of Macrocallista ganglia were subjected to differential centrifugation, and the resulting fractions were osmotically shocked and purified by cation exchange chromatography (CM-25 Sephadex). The fractions were assayed for FMRFamide on the radula protractor muscle of the whelk, Busycon contrarium. This assay can detect less than one picomole of peptide when the muscle is isolated in a microbath (< 150 µl).

The majority of FMRFamide activity occurred in the high-speed (100,000g) microsomal fraction. If radiolabelled FMRFamide was added to the ganglion homogenate, it appeared primarily in the high-speed supernatant; only about 1-5% was associated with the microsomal pellet. The FMRFamide storage granules must now be characterized more specifically. To this end, we are now purifying the microsomal fraction by sucrose density gradient centrifugation for electron microscopy.

Isolated Macrocallista ganglia were depolarized by incubation in high potassium medium (100mM K^+ replacing part of the sodium). FMRFamide activity was extracted from the bathing medium into butanol, purified by cation exchange chromatography, and assayed. The concentration of FMRFamide in the depolarizing medium was significantly increased over controls (incubation in artificial seawater: 10mM K^+). Ten-fold reduction of the calcium concentration blocked the potassium-induced release.

The occurrence of FMRFamide in the microsomal fraction of ganglion homogenates, its release from ganglia by high potassium depolarization, and the blockade of the potassium-induced release by low calcium all suggest that FMRFamide is a neurosecretory product in Macrocallista.

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73.2 CONTROL OF THE PTTH NEUROSECRETORY CELLS IN THE TOBACCO HORNWORM, MANDUCA SEXTA. Grant M. Carrow*, Ronald L. Calabrese and Carroll M. Williams*. (Spon: Joel L. Cohen). The Biological Laboratories, Harvard University, Cambridge, MA 02138.

The cell bodies of the neurosecretory cells which are the major source of prothoracicotropic hormone (PTTH) in M. sexta have been localized to bilateral clusters in the brain (Gibbs and Riddiford, 1977, JEB 66:255; Agui et al., 1979, PNAS 76:5694). We report here on studies aimed at determining the sites and control of PTTH release by these neurons.

PTTH activity was measured using an in vitro assay involving the PTTH-stimulated secretion of α-ecdysone by prothoracic glands (PG). One PG of each pair was incubated in Grace's medium (GIBCO) with an extract of PTTH while the other was incubated in PTTH-free medium. PTTH was quantified by comparing the rates of α-ecdysone secretion by each member of the pair; the response was dose dependent. α-ecdysone activity was measured by radioimmunoassay using a rabbit anti-α-ecdysone antiserum provided by Timothy Kingan (Oregon State Univ.). PG from 3-day-old 5th instar larvae exposed to brain extracts from wandering larvae in vitro (0.5 brain equivalents or more) responded with a greater than 10-fold increase in the rate of α-ecdysone secretion.

We have measured PTTH release from intact and surgically interrupted brain-retrocerebral complexes (brain, corpora cardiaca, and corpora allata) in vitro of animals at various stages of development. Results indicate that the corpora allata (CA) contain the primary sites for release of PTTH. Isolated CA from feeding 5th instar larvae released PTTH spontaneously, indicating that the hormone may be stored in the CA throughout the feeding period. However, intact brain-retrocerebral complexes from early 5th instar larvae did not release PTTH, while those from later in the 5th instar did. Additional experiments employing a sucrose gap around the nerves between the brain and the CA suggest that electrical activity in the neurons may be involved in hormone release. The morphology, electrical properties and control of these neurons are presently being investigated with anatomical, pharmacological, and electrophysiological procedures.

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73.3 ADENYLATE CYCLASE STIMULATION BY COINCUBATION OF ALBUMEN GLAND AND BRAIN EXTRACTS OF HELIX POMATIA. Jeffrey L. Ram & Esther M. Goudsmit. Dept. of Physiology, Wayne State Univ., Detroit, Mi. 48201 & Biology Dept., Oakland Univ., Rochester, Mi. 48063.

Albumen glands of reproductively active Helix pomatia synthesize galactogen (a galactose homopolymer) in preparation for laying eggs, which contain galactogen. Albumen glands from hibernating Helix do not ordinarily synthesize galactogen, but may be made to do so in organ culture by exposure to Helix brains or extracts thereof. The substance(s) in brain that cause(s) galactogen synthesis is brain-specific, heat-stable, and protease-labile. It is released from brain in high [K^+] media by a Ca^{++}-dependent mechanism (Goudsmit, Br. Res. 151: 418, 1978) and thus has the properties expected of a polypeptide neurohormone. Activation of galactogen synthesis in organ-cultured albumen glands from hibernating Helix also occurs in response to 10^{-3} M dibutyryl cAMP and is enhanced by 10^{-3} M theophylline (Goudsmit, Soc. Neurosci. Abs. 4: 194, 1978). To test the hypothesis that the stimulatory effect of brain substance(s) on albumen gland galactogen synthesis is mediated through activation of an albumen gland adenylate cyclase, we have begun to characterize this enzyme and to look for effects of brain substance(s) on it.

Albumen gland pellet fraction (38,000 x g x 30 min resuspended in 2 mM tris-maleate, pH 8.0) was incubated in a medium containing 5 mM $MgCl_2$, 25 mM tris-maleate (pH 8.0), 0.6 mM EGTA, 1 mM isobutylmethylxanthine, 20 mM creatine phosphate, 80 U/ml creatine phosphokinase, 1 mM cAMP, and 1 mM α^{32}P-ATP in a final volume of 100 µl. Synthesis of ^{32}P-cAMP was measured by the method of Salomon et al., Anal. Bioc. 58: 541, 1974. Activity was linear with time (0-20 min) and with amount of albumen gland protein (0-100 µg protein/100 µl incubation vol.). Average activity (hibernating glands) was 5.6 ± 1.4 (S.E.M.) pmole/min/mg protein (N=4 glands). Albumen gland adenylate cyclase is stimulated 80-fold by 10 mM NaF, 3-fold by 10^{-5} M GTP, and 6-fold by 10^{-5} M GppNHp. A brain extract (supernatant from 8000 x g x 4 min centrifugation of brain homogenate) added to the incubation medium to give 10 µg brain protein (≈.01 brain)/100 µl caused a doubling of adenylate cyclase activity (2.0 ± .3[S.E.M.]-fold increase, N=4 brain extracts). Under these conditions, .01 brain itself has no detectable adenylate cyclase activity (N=2).

These data are consistent with the hypothesis that Helix brain contains factor(s) which stimulate albumen gland adenylate cyclase. Further experiments to compare biochemical properties of these factor(s) to galactogen synthesis activating factor(s) of brain are in progress.

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73.4 PHARMACOLOGICAL ANALYSIS OF THE INSECT PROCTOLIN RECEPTOR: STRUCTURE-FUNCTION RELATIONSHIPS OF AGONISTS AND ANTAGONISTS. R. E. Sullivan and R. Newcomb*. Békésy Laboratory of Neurobiology, University of Hawaii, Honolulu, HI 96822.

Recent investigations have revealed that proctolin (Arg-Tyr-Leu-Pro-Thr) or proctolin-like activity is found in semipurified tissue extracts from a number of arthropods (Brown, B.E., J. Insect Physiol., 23:861, 1977, and Sullivan, R.E., J. Exp. Zool., 210(3):543, 1979). Although it was originally suggested that proctolin was a neuromuscular transmitter in the cockroach hindgut (Brown, B.E., Life Sci., 17:1241, 1975), more recent pharmacological investigations suggest a hormonal function affecting peripheral and myocardial neuromuscular events in insects (Piek, T., and Mantel, P., J. Insect Physiol., 23:321, 1977, and Miller, T., Am. Zool., 19:77, 1979) and crustaceans (Lingle, C., Ph.D. Thesis, Univ. Ore., 1979).

Having considered the possible structural and pharmacological relationships between proctolin and a crustacean cardioexcitatory peptide (Sullivan, R.E., op. cit., 1979), we undertook a study of the pharmacology of proctolin analogues. Using solid-phase techniques we have synthesized and purified numerous deletion, addition, and substitution analogues of proctolin and assayed for agonistic and antagonistic activites on the cockroach hindgut. Active analogues (effecting contractures at ≤ 10^{-6} M) to be discussed include: tryptophan and phenylalanine substituted for tyrosine, the N-terminal addition of glycine, C-terminal addition of glycine and C-terminal substitution with alanine. Glutamate and glycine substituted-N-termini were without effect. Also without effect at 10^{-6}- 10^{-5} M were Arg-Tyr-Pro-Thr, Tyr-Leu-Thr, Arg-Tyr-Leu-Pro, Tyr-Leu-Pro-Thr, Leu-Pro-Thr, Arg-Tyr, and the cysteine addition to the C-terminus. In high concentrations (>10^{-7} M), none of the inactive peptides appear to antagonize the response to proctolin (10^{-8} - 10^{-7} M).

In our hands proctolin elicits both an increase in spontaneous contractions and a contracture from cockroach hindgut. It is worth noting that C-terminus additions and substitution appeared to qualitatively enhance only the spontaneous contractions. This observation led us to reinvestigate the antagonist role (see Brown op. cit., 1975) of octopamine and tyramine on this preparation. We found that the phenolamines selectively block the proctolin induced spontaneous contractions but not the contracture, suggesting the possibility of two proctolin receptors on the insect hindgut. Further work is in progress.

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73.5 EFFECT OF THYROTROPIN RELEASING HORMONE ON THE CYCLIC AMP CONTENT IN PARIETAL GANGLIA OF THE POND SNAIL LYMNAEA EMARGINATA. Y. Grimm-Jørgensen, Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06032.

The tripeptide thyrotropin releasing hormone (TRH) is found in the nervous system of various gastropods. The physiological role of this peptide in molluscs is poorly understood. In an attempt to determine whether TRH may serve as a neurotransmitter or neuromodulator in gastropod nervous tissue, we have studied the effect of TRH on the cAMP content of various ganglia of the pond snail Lymnaea emarginata. We have reported that TRH (10^{-8}M) causes a decrease in the cAMP content of in vitro incubated cerebral ganglia, while it causes a significant increase in the concentration of this nucleotide in pooled left and right parietal ganglia (Grimm-Jørgensen, Y., Life Sci. 26: 1211,1980). The TRH-induced increase in cAMP content in parietal ganglia was further assessed. It was found that TRH causes an increase in the cAMP content of the large right as well as the small left parietal ganglion.

Because neurotransmitters cause a transient effect on the electrical activity and a transient increase in cAMP content of gastropod neurons, while the effect of neuromodulatory peptides on these parameters is more prolonged, the time course of the TRH-induced cAMP increase was assessed in right parietal ganglia. The cAMP concentration in in vitro incubated ganglia was highest at 20 min after the addition of the peptide and remained at this level for up to 30 min, the longest incubation time measured. This long-lasting effect of TRH was not abolished when the ganglia were exposed to the peptide in medium containing a high concentration of Mg^{++} (20 mM) and a low concentration of Ca^{++} (2 mM), indicating that synaptic activity is not required for the observed TRH effect.

It is concluded that the observed effect of TRH on the cAMP content of parietal ganglia of Lymnaea emarginata is compatible with the hypothesis that the peptide acts as a neuromodulator on some neuron(s) and that it is unlikely that TRH causes the release of an endogenous molecule which in turn promotes cAMP accumulation. (Supported by grant # 5 R01 AM 20929 from NIH.)

73.6 PEPTIDE HORMONE STIMULATED PROTEIN PHOSPHORYLATION CHANGES IN INSECT CNS AND MUSCLE. Susan K. Welch*, Lawrence M. Schwartz* and James W. Truman. (SPON: J. Loeser). Dept. of Zool. NJ-15, University of Washington, Seattle, Wa. 98195.

The eclosion hormone is a peptide which triggers the stereotyped behaviors of ecdysis and eclosion in silkmoths. When the peptide is added to the isolated silkmoth CNS, the motor program corresponding to these behaviors is evoked (Truman,J.W., 1978, J. Exp. Biol. 74, 151-173). The behavioral effects of eclosion hormone apparently are mediated by an increase in cyclic GMP levels in the nervous system of the moth (Truman,J.W., Mumby,S., and Welch, S.K., J. Exp.Biol. 84, 201-212).

Alterations in the phosphorylation of proteins by cyclic nucleotide-dependent protein kinases have been implicated in the action of peptide hormones in nervous and other tissues. To determine if such protein phosphorylation changes occur in moth CNS in response to eclosion hormone, cell-free homogenates of silkmoth brains were incubated with $[\gamma\text{-}^{32}P]$-ATP in the presence and absence of hormone. The homogenates were subjected to SDS-polyacrylamide gel electrophoresis and autoradiography. A physiological dose of purified eclosion hormone selectively stimulated the phosphorylations of several proteins from the silkmoth brain. A low concentration of cyclic GMP also stimulated the phosphorylation of several brain proteins, although these proteins were not the same as those whose phosphorylation was stimulated by the hormone. Both the hormone and cyclic GMP dependent phosphorylations were Ca^{++} dependent.

The eclosion hormone also acts on the intersegmental muscles of the silkmoth to trigger their degeneration following adult eclosion. An early step in this hormone-mediated cell death is a 20-fold increase in cyclic GMP levels in the affected muscles (Schwartz, L.M. and Truman,J.W., this volume). When cell-free homogenates of silkmoth muscle were incubated with $[\gamma\text{-}^{32}P]$-ATP in the presence and absence of eclosion hormone or cyclic GMP, a 25 to 30-fold stimulation of phosphorylation of several proteins was observed. Experiments are underway to determine if any of the proteins whose phosphorylation is stimulated by eclosion hormone are common to both muscle and brain. We are also focusing on demonstrating these hormone-stimulated phosphorylation changes in intact animals and in brains and muscles in culture.

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74.1 INTRACELLULAR POTENTIAL OF MEDIAL BULBAR RETICULAR FORMATION NEURONS DURING NATURALLY OCCURRING STATES OF SLEEP AND WAKEFULNESS. M. Chase, S. Enomoto*, T. Murakami*, Y. Nakamura*, and M. Taira*. Departments of Physiology and Anatomy and the Brain Research Institute, Univ. of California, Los Angeles, CA 90024.

Since 1976 we have been studying a model of motor control which describes putative neuronal mechanisms and circuitry responsible for the suppression of somatomotor activity during active sleep (AS)(1). The key element in the model is the activation of cells in the nucleus reticularis gigantocellularis (NRG) by neurons of the nucleus reticularis pontis oralis. We have proposed that cells of the NRG serve as "premotor" inhibitory neurons which initiate, directly or through interneurons, the hyperpolarization of motoneurons during AS that we have described (2,3). In order to extend our explorations of this model, we recorded intracellularly from neurons of the NRG in the chronic cat preparation during sleep and wakefulness. The experimental paradigm which was utilized for chronic intracellular recording has been previously described (2).

Seventy neurons of the NRG were recorded from intracellularly; all neurons exhibited a membrane potential greater than -40 mV. During the transition either from wakefulness (W) to quiet sleep (QS), or from QS to W, no definite change in membrane potential level was detected. In contrast, compared to QS, the membrane potential level invariably depolarized by 3 to 14 mV (8.9±3.5) coincident with the onset of AS. This pattern contrasts with that of alpha motoneurons of the brain stem and spinal cord, which hyperpolarize during AS compared to W or QS (2,3).

Six reticular neurons, which exhibited spontaneous spike activity in QS, increased their discharge frequency coincident with the onset of AS. The tonic nature of the increased discharge pattern and decrease in polarization closely paralleled the tonic decrease in EMG activity. This pattern is different from that of other putative AS related cells which fire in bursts during AS in head-restrained cats or appear to fire, again in bursts, during W and AS in freely moving animals.

In summary, the data presented in this abstract indicate an active, functional role for NRG neurons in the process of somatic atonia during active sleep. Supported by grants from the USPHS (NS 09999) and the NSF-INT (77-22299).

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74.2 PONTINE TEGMENTAL LESIONS RELEASE SIMILAR BEHAVIORS IN BOTH WAKEFULNESS AND PARADOXICAL SLEEP. J. C. Hendricks*, A. R. Morrison, G. L. Mann*. Lab. of Anatomy, Sch. of Vet. Med. University of Pennsylvania, Philadelphia, PA 19104.

Small bilateral pontine lesions disrupt the normal atonia of paradoxical sleep (PS) in cats, which permits the expression of complex motor activity in that state (Henley and Morrison, Acta Neurobiol. Exp. 34:215-232, 1974). In the present study, the behavior seen in PS differed with lesion site and was paralled by alteration in activity in wakefulness (W).

Four different syndromes were identified. Group I (n=9) showed what could be called "PS without atonia without behavior". Abnormally vigorous proximal limb movements occurred; but the head was not raised; and coordinated behavior was not seen. No specific pontine lesion was correlated with the Group I syndrome. Group II (n=10) showed coordinated behavior involving the head, neck and forelimbs with movements resembling orienting, staring, searching and attempts to rise and walk. The smallest (1 mm diam.) lesion needed to produce such behavior was symmetrical, centered at P=3.0, H=2.0, V=-3.0. Group III (n=6) exhibited periods of violent behavior resembling directed attack interspersed with tonic periods of quiet staring or searching movements. Attack behavior resulted from damage extending rostroventrally into the midbrain at P=2.0, H=2.5, V=-4.0 (4/6) or unilateral damage (2/6) to the descending projection of the central nucleus of the amygdala (Hopkins and Holstege, Exp. Brain Res. 32:529-547, 1978). In Group IV (n=5) hindlimb activity was seen as the animals walked in PS. Walking resulted from larger lesions centered at P=3.0, H=2.5, V=-4.0.

During W, cats exhibiting PS without atonia also demonstrate a significant increase in locomotion on an open-field test as well as increased orienting and exploratory behavior (Morrison et al., Soc. Neurosci. Abstr. 5:697, 1979). Five of the six cats which attacked in PS (Group III) also attacked both the experimenters and other cats during W. Both the W and PS attacks were predatory rather than defensive in nature.

The attack (Smith & Flynn, Brain Res. 164:103-119, 1979) and locomotor (Mori et al., J. Neurophysiol. 40:284-295, 1977) systems are anatomically closely related to each other and to the lesion sites of Groups II-IV. Thus, these lesions damaged neurons which must normally modulate coordinated behaviors such as attack and locomotion as well as promote dramatic motoneuronal inhibition in normal PS. Parallel lesion effects observed in W and PS indicate a remarkable similarity in motor control in the two states.

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74.3 PGO BURST NEURONS: CORRELATIONS WITH EYE MOVEMENT POTENTIALS AND RESERPINE WAVES. John P. Nelson, Robert W. McCarley and J. Allan Hobson. Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, Boston, MA 02115.

PGO burst neurons are a class of midbrain cells which fire in stereotyped bursts of 4-6 spikes 12 msec before onset of pontogeniculo-occipital waves (PGO waves) during desynchronized, rapid eye movement sleep (REM sleep). We have shown previously that cells on one side of the midbrain fire in correlation to larger PGO waves in the lateral geniculate body (LGB) of the same side. They therefore transmit eye movement information to the visual system during REM sleep. Similar but less stereotyped waves occur in visual cortex and LGB during wakefulness (W) at the offset of saccades and also occur upon sudden noises or other startling stimuli. These waves have been called eye movement potentials (EMP). We have been unable to detect consistant changes in their waveforms with saccades in various directions.

Of 35 burst cells we have studied to date, and which could be followed from REM sleep into W, 4 cells showed clear relationship to EMP occurence. The burst cells lose their bursting pattern in W and fire single spikes in association with eye movements; they tend to be silent otherwise except for single spikes elicited by sudden stimuli (taps on the cage). Most cells did not fire before each EMP, nor was every spike followed by an EMP. One cell fired at latency (to peak of EMP) of 158±45 msec before 82% of 76 EMP's in one 8 minute period of alert W. The spike usually occurred near onset of a saccade. Another cell fired before fewer EMP's and at shorter latency (63±17 msec). This cell could be antidromically activated at fixed latency from two sites in the contralateral LGB (10 and 15 msec). Collision with spontaneous bursts could be demonstrated from both sites in REM sleep. In two cases the cell fired bursts of 4 spikes during spontaneous saccades. In one instance while testing for antidromic activation in W, a stimulus fell during a saccade and elicited a 4 spike burst followed by a large EMP suggesting that the excitability of the cell was raised during the saccade. EMP-like waves can be evoked in one LGB by stimulation of the other, possibly by antidromic activation of burst cells. When stimuli occur during saccades the resulting wave is larger than usual, which suggests that the population excitability is raised.

One cell recorded after 75 mg/kg reserpine IP fired bursts before many reserpine waves, which is evidence that such waves are also generated through burst cell activity. PGO burst cells seem to be a final path in generation of PGO waves, EMP's and reserpine waves. They transmit eye movement information to the visual system in REM sleep, and fire in association with saccades in W.

This research was supported by NIH grant 2 RO1 MH 13923-13.

74.4 THE PONTINE RETICULAR FORMATION: AN INTRACELLULAR RECORDING STUDY OF INPUTS FROM MIDBRAIN, BULBAR, AND CONTRA-LATERAL PONTINE RETICULAR FORMATION IN THE UNANESTHETIZED CAT. Robert W. McCarley and Keihachiro Ito*. Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, 74 Fenwood Road, Boston, MA 02115

The medial pontine reticular formation (gigantocellular tegmental field, FTG) is important for control of somatic and oculo-motor events during waking; its neurons are further notable for the dramatic modulation of their discharge rate over the sleep-waking cycle. Anatomical studies have demonstrated projections to FTG from medial reticular areas in midbrain (MRF), bulb (BRF) and contralateral pons (cFTG), but electrophysiological studies are needed for information about the synaptic sign and time course of these inputs. This report describes the short-latency synaptic effects on caudal FTG neurons (intracellular recordings from over 100 neurons, AP -7.5 to -3.5) of low current < 70uA) square wave stimulation (200uS duration) of ipsilateral BRF and MRF and contralateral FTG (cFTG). The head restrained, unanesthetized chronic cat preparation avoids artefacts resulting from the effects of anesthetic and paralytic agents on reticular neurons. Subsequent reports will discuss sleep-waking cycle alterations of membrane and stimulation effects.Our data support the following tentative generalizations: (1) Depolarizing (excitatory) effects predominate among short-latency, probably monosynaptic PSPs (latency < 1.2mS, following of 200 Hz stimuli). Over 90% of these PSPs were depolarizing, whether evoked from BRF, cFTG, or MRF. (2) These probably monosynaptic PSPs were elicited in a high proportion of the caudal FTG neuron population by stimulation of BRF (3/4 of neurons) and cFTG (3/5) but were less common with MRF stimulation (3/10). (3) FTG neurons projecting to a reticular area also may receive input from this same area, as indicated by the presence of monosynaptic depolarizing PSPs in the same neuron at times when the previously elicited antidromic response was absent due to collision or change of stimulation parameters. (4) Hyperpolarizing PSPs play a less prominent role than depolarizing ones. Hyperpolarizing PSPs were usually of longer latency (1.2-1.8 mS) than the monosynaptic time range,were of short duration (range: 2-5 mS) and were, in this unanesthetized preparation, usually followed by large, complex depolarizing PSPs. Chloride diffusion experiments suggest that the hyperpolarizing PSPs represent IPSPs. We conclude that reticulo-reticular connections are predominantly excitatory, and thus could provide the synaptic substrate for reticular self-excitation we have postulated to exist on the basis of extracellular recordings.

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74.5 A NEW LOOK AT SPINAL PROJECTIONS FROM BULBAR AREAS OF THE RETICULAR FORMATION. EVIDENCE FOR CONNECTIONAL HETEROGENEITY IN THE NORTH AMERICAN OPOSSUM. G.F. Martin, T. Cabana*, A.O. Humbertson, L.C. Laxson* and W.M. Panneton. Dept. Anat., Sch. Med., The Ohio State Univ., Columbus, Ohio, 43210.

Retrograde and orthograde transport techniques show that the nucleus reticularis gigantocellularis pars ventralis and the nucleus reticularis gigantocellularis project the entire length of the spinal cord. Double labelling methods show that some neurons in each area innervate both cervical and lumbar levels. There is evidence, however, that neurons in the lateral part of the nucleus reticularis gigantocellularis pars ventralis and the dorsal extreme of the nucleus reticularis gigantocellularis project to only cervical and thoracic levels. The autoradiographic method shows that the above nuclei supply direct innervation to autonomic and somatic motor columns as well as to laminae V-VIII and X. The nucleus reticularis gigantocellularis pars ventralis provides additional projections to lamina I and the outer portion of lamina II. Several areas of the medullary reticular formation project mainly and in some cases, exclusively, to cervical and thoracic levels. These areas include the nucleus reticularis parvocellularis, the nucleus reticularis lateralis, the nucleus retrofacialis, the nucleus ambiguus, the nucleus lateralis reticularis, caudal parts of the nuclei reticularis medullae oblongata dorsalis and ventralis and the nucleus supraspinalis. Neurons in the lateral medulla, particularly rostrally (the nucleus reticularis lateralis and neurons related to the nucleus lateralis reticularis) innervate sympathetic nuclei. These data suggest that spinal projections from bulbar areas of the reticular formation are more complicated than previously supposed and are in conformity with the evolving concept of reticular heterogeneity. Supported by U.S.P.H.S. Grant NS-07410.

74.6 BRAINSTEM UNITS RELATED TO MOVEMENTS OF THE PINNA. J.M. Siegel, R.L. Wheeler*, S.M. Breedlove* and D. J. McGinty. VA Medical Center, Sepulveda, California and Departments of Psychiatry and Psychology, UCLA, Los Angeles, California 90024.

Sherrington first demonstrated that structures caudal to the inferior colliculus were sufficient to control a variety of complex protective reflex movements of the pinna. However, the specific regions in the lower brainstem that might be involved in the regulation of these movements have not been further localized. The present study reports the discovery of a group of neuronal units in the cat's medial pontomedullary reticular formation that discharge in relation to pinna movement.

A total of 19 cells whose discharge was correlated with "spontaneous" movements of the pinna were observed. All cells were related to movements of the ipsilateral pinna. Detailed observation of the topography of the pinna movement correlated with unit activity was made in 13 of the cells. Of these, four related to caudal, five to ventro-caudal and four to rostral movements of the pinna. Rapid pinna rotation in the appropriate direction was accompanied by a burst of unit discharge. Five cells exhibited tonic discharge if the pinna was maintained in the displaced position but the remaining units discharged only during the movement. Pinna movement cells did not discharge during eye, head, neck or limb movements.

All cells were tested for response to somatic stimulation. When such stimulation elicited pinna movement, discharge occurred as in the case of spontaneous movements. In the absence of detectable ear movements, 16 of the 19 tested cells had no somatic field. None of the cells responded to passive movement of the pinna by the experimenter or electrical stimulation of pinna musculature. None responded to auditory or visual stimulation.

All of the units were observed during polygraphically identified slow wave and REM sleep states. All but three of the cells (84%) were silent throughout sleep. This contrasts with the sleep modulation of activity in adjacent non-pinna movement cells only 25% of which are of this type.

We have conducted behavioral analyses on a total of 300 medial reticular formation cells located between stereotaxic coordinates A3 and P13, L0.8 and 3.0. Those related to pinna movement were all localized to the area between P2 and P8 and especially between P4 and P8 in the gigantocellular tegmental field. None of the pinna movement units were histologically localized to the facial nerve or nucleus.

74.7 AFFERENTS TO THE CENTRAL SUPERIOR RAPHE NUCLEUS IN THE CAT. R. Maciewicz*, S. Ronner, E. Taber-Pierce, and W. Foote. Depts. of Neurology, Neurosurgery, and Psychiatry, Mass. Gen. Hosp., Boston, MA 02114.

The afferents to the central superior raphe nucleus (CS) in the cat were studied using retrograde transport and electrophysiological methods. Following aspiration of the posterior cerebellar vermis, HRP was iontophoresed into CS through micropipettes advanced into the brain stem under direct visual control. After a two day survival period the animals were perfused with mixed aldehydes and frozen sections from the brains were reacted to demonstrate HRP with either diaminobenzidine or tetramethylbenzidine as substrate. Retrogradely labeled neurons were localized to the dorsal tegmental region of the pons (DT), the lateral habenula (both medial and lateral parts), and the interpeduncular nucleus (IP). Within the pons, labeled cells were restricted to the DT just caudal to CS. Cells were found scattered throughout this area and were not restricted to the dorsal tegmental nucleus. Labeled cells were only rarely encountered within the adjacent locus ceruleus. Within IP, labeled cells were primarily segregated to the lateral parts of the nucleus. In cases with lateralized injections of HRP into CS this projection appears predominantly ipsilateral. A few scattered cells were also found within the substantia nigra and hypothalamus, but in contrast to the known inputs to the dorsal raphe nucleus, no labeling was seen in the medulla or other raphe nuclei. To confirm these anatomical observations, single cell recordings were made from CS in anesthetized cats. Stimulating electrodes were placed in the habenula, IP, and DT of the pons. With IP stimulation both antidromic and orthodromic excitatory effects were seen in a population of CS neurons. Similarly, DT stimulation elicited excitatory responses with monosynaptic latency (1-2msec.) in some CS neurons. The demonstration of such short latency inputs from the interpeduncular nucleus and the dorsal tegmental area to CS is in agreement with the retrograde transport evidence for direct projections from these nuclei to CS.

75.1 Physiological and histochemical properties of motor units in reinnervated triceps surae muscles in the cat. T. Gordon, R.B. Stein & M.J. Gillespie. *Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7.*

When a peripheral nerve supplying fast and slow motor units is cut and allowed to reinnervate, nerve fibres return to muscle fibres that formerly belonged to different motor nerves. As a result, the muscle fibres in early reinnervated motor units are heterogenerous in their properties, and our preliminary data showed that there was no relationship between twitch tension or contractile speed of these units with motor nerve size, as there was in that muscle before reinnervation (Gordon & Stein, 1980, in "Plasticity of Muscle", Pette, ed. Springer: Berlin, pp. 283). We have now shown with chronic recording of motor unit properties that reinnervated muscle once more obeys the size principle in terms of its motor unit properties after 3 to 5 months, i.e., twitch tension and contractile speed of reinnervated motor units become directly correlated with nerve size. The fatigue characteristics of the motor units were studied in acute experiments in which motor units were classified as slow (S), fast fatigue resistant (FR) or fast fatigueable (FF) according to the classification of Burke *et al.* (1974, J. Physiol. 234: 723). The order of mean twitch and tetanic tension remained: FF greater than FR greater than S, and the nerves that supply fast units were significantly larger than those innervating the slow, as in the normal. Also, the relative proportion of unit types in the mixed medial gastrocnemius muscle was not different from control muscles. Thus, the order of tension developed by the three unit types and their contractile speed provided further evidence for the return of the size relationships shown with chronic recording of reinnervated unit properties. The MG muscle showed no apparent preference for reinnervation by slow or fast nerves. However, soleus motor units reinnervated by LGS or MG nerves were homogeneous in their histochemical properties, showing negative staining for alkaline ATPase and positive for NADH, as in control muscles. Thus, there may be limits to the degree nerve fibres can modify muscle properties following reinnervation.

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75.2 HOMOGENEITY OF ENZYME ACTIVITIES IN SINGLE DISSECTED FIBRES OF THE MOTOR UNIT. P. Nemeth*, Dirk Pette* and G. Vrbova*† (SPON: W.F.H.M. Mommaerts). Faculty of Biology, Univ. of Konstanz, Germany, †Univ. College London, England.

Biochemical analyses of single dissected muscle fibres have shown that fibre populations are heterogeneous in their content of metabolic enzymes, even among fibre groups whose histochemical characteristic are identical (Spamer and Pette, 1977, 1979); Lowry et al., 1978). It has been suggested that this heterogeneity is due to differences in the neural input to the muscle fibres. If so, it would be expected that metabolic characteristics of a motor unit be identical, as suggested by qualitative histochemistry (Edström and Kugelberg, 1968). In the present investigation, malate dehydrogenase and fructose 1,6-diphosphatase was measured in single dissected fibres of motor units. The enzyme activities were compared to those in randomly selected fibres of the same muscles. The methodology combined the microchemical techniques (Lowry and Passonneau, 1972) with the glycogen depletion means of detecting the fibres of a motor unit (Kugelberg and Edström, 1968), and is described in detail elsewhere (Nemeth, Pette and Vrbova, 1980). The malate dehydrogenase activity in single fibres of the general population of 6 extensor digitorum longus muscles of the rat ranged between 20 and 226 u/g fresh weight, showing up to tenfold differences in a single muscle. Enzyme levels of the six motor units ranged between 31 and 106 u/g fresh weight, but differed only slightly, 0.7 to 4.2%, between fibres of the same motor unit. This appears to be negligible compared to the large variations existing within the fibre population. The experimental error was judged as the SD in enzyme activity of multiple determinations on pieces of single fibres. This error ranged between 2.2 and 6.5%. Fructose 1,6-diphosphatase activities measured in fibres of one motor unit ranged between 0.75 and 0.80 u/g fresh weight, showing only a 2.2% deviation. The experimental error of multiple determinations on pieces of single fibres was 5.1%. Thus, the activities of both of these enzymes are identical in fibres belonging to the same motor unit, well within the range of experimental error. It is suggested by this study that the motor unit represents a metabolically pure population of fibres. The high resolution of microchemical techniques has revealed how precisely the nerve can control the metabolic enzyme properties of the various fibres that it supplies.

Nemeth, P., Pette, D. and Vrbova, G. (1980). Plasticity of Muscle. Berlin New York: de Gruyter.
Spamer, C. and Pette, D. (1977). Histochem. 52, 201-216.
Spamer, C. and Pette, D. (1979). Histochem. 60, 9-19.

75.3 GENESIS OF THE NEURONS IN THE MOTOR CORTEX AND VA-VL THALAMIC COMPLEX IN RHESUS MONKEY. J.J. Dekker and P. Rakic, Sec. of Neuroanatomy, Yale Univ. School of Med., New Haven, Ct. 06510.

The sequence of genesis of neurons comprising the VA-VL complex of the thalamus and cortical areas 4 and 6 of Brodmann were determined in autoradiograms from juvenile monkeys that were exposed to a pulse of ^{3}H-thymidine on selected embryonic (E) days of the 165 day gestational period in this species. The time of origin of cells in the VA-VL thalamic complex was analysed in coronal sections cut through three levels of the rostral thalamus. Heavily labeled neurons, representing cells that had undergone final cell division at the time of ^{3}H-thymidine injection, were present only in cases exposed to the isotope over a five day period from E38 to E43. The peak of genesis was at E43 (labeling index-labeled neurons/total neurons, 44%). There was a lateral to medial spatiotemporal gradient of genesis such that the majority of neurons born at E38 eventually become situated in the lateral parts of VA and VL, whereas most of those born at E43 become situated more medially. This is consistent with an outside-to-inside pattern of neurogenesis. No pronounced caudal to rostral gradient was present and both nuclei are generated as a unitary structure.

The time of origin of the motor cortical areas that receive imput from the VA-VL thalamic complex was determined in coronal sections through the frontal cortex. Detailed analysis was performed in the upper and lower half of area 6 and in 5 different regions of area 4: the upper (trunk) and lower (face) half of the anterior part of area 4, and the upper (hindlimb), middle (forelimb) and lower (tongue) one third of the posterior part of area 4. Heavily labeled cells were present in animals injected over a forty day period from E40 and E80. As in other cortical areas of primate and nonprimate species, there was an inside-to-outside gradient such that cells generated at early stages are eventually situated in the deep layers and those generated at later stages in more superficial layers. Most neurons in lamina 6 were born between E40 and E60, those of lamina 5 (including Betz cells) and 4 between E45 and E70, those of lamina 3 between E62 and E80, and lamina 2 neurons around E80.

Thus, the neurons of the cortical motor areas in monkeys are generated slightly earlier than the more posterior cortical areas such as the visual cortex but later than its major ascending input neurons in the VA-VL thalamic complex. Contrary to expectation, the timing and pattern of neuronal genesis in regions of both areas 4 and 6 are remarkably similar regardless of whether they issue projections to the brainstem, the cervical or the lumbar spinal cord. Likewise, both deep and superficial cells in lamina 5 are born at about the same time, despite the fact that their targets in the brain stem and spinal cord are generated at different times. Supported by NS 14841.

75.4 ORIGIN OF HIGH-FREQUENCY TREMOR IN EARLY-POSTNATALLY X-IRRADIATED RATS. J. Gruner* and R. Llinás (SPON: K. Walton). Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016

Early postnatal cerebellar x-irradiation of rats, which destroys 90-95% of the granule cells and most stellate and basket cells (Altman and Anderson, J. Comp. Neurol. 149: 123, 1973) also produces ataxia and head and limb tremor. Investigation of the tremor showed it be in synchrony with high-frequency (18 Hz) EMG bursting. Hindlimb tremor was not blocked by deafferentation as determined by recording EMGs before and after section of dorsal roots L2-T13 bilaterally. Descending influences are necessary for sustained hindlimb tremor. Indeed, tremor was blocked bilaterally by transection of the spinal cord and unilaterally by hemisection at the T10 level. The characteristics of the descending signals and their distribution in the cord have yet to be determined. EMGs of irradiated rats during tremor were similar in many respects to those seen during harmaline-induced tremor (de Montigny & Lamarre, Brain Res. 53: 81, 1973; Llinás and Volkind, Exp. Brain Res. 18: 69, 1973). For instance, ipsilateral extensors were usually in phase and tended to be out of phase with flexors. Also, bursting in extensor muscles such as vastus lateralis and gastrocnemius was more pronounced than in the flexors tibialis anterior or iliacus. The semitendinosus (knee flexor, hip extensor) showed strong tremor. However, some differences between post-irradiation and harmaline tremor were evident. Thus, harmaline tremor frequency was 8-12 Hz, and when harmaline was injected into irradiated rats, the tremor frequency dropped from about 18 to 10 Hz. Chemical lesion of the inferior olive by 3AP (Llinas et al, Science 190: 1230, 1975) blocked irradiation tremor. These experiments indicate that the inferior olive is necessary for generating cerebellar-irradiation tremor. Since the cerebellum is likely to be a locus for the generation of post-irradiation tremor, chronic extracellular unit recordings were undertaken in irradiated rats. In a number of animals rhythmic synchronization of Purkinje cell unit activity was observed in the anterior lobe vermis. The synchronization was very near the frequency of ipsilateral EMG bursting, though the phase relations did not remain constant for long periods. It appears that reorganization of cerebellar circuits following the irradiation treatment leads to synchronized oscillatory activity of cerebellar, brainstem, and spinal circuits, possibly involving the olivocerebellar inputs to cerebellar cortical and nuclear structures. (Supported by USPHS grants NS05880 and NS13742 from NINCDS).

75.5 INFANT LESION EFFECT: RESULT OF NEONATAL AND ADULT HEMISECTION IN CATS. B. S. Bregman and M. E. Goldberger. Dept. of Anatomy, The Med. Coll. of Penn., Phila., PA 19129.

Adult Operates (A.O.) and Neonatal Operates (N.O.) show remarkable recovery/development of motor function following spinal hemisection (L1, dorsal columns spared). Postural reflexes (tactile placing, proprioceptive placing, monopedal hopping) and conditioned locomotion on "difficult" runways were examined in adult cats: neonatally operated (day of birth), adult operated, and unoperated. Hindlimb tactile placing is abolished ipsilaterally in A.O.'s but not in N.O.'s, and represents a sparing of function. Other postural reflexes in this limb reveal enhanced function; proprioceptive placing and hopping (all directions) recover in both groups, but have a lower threshold in N.O.'s. Quantitative study of lateral hopping shows that normals have no significant (t-test, p=0.01) interlimb difference in hops/meter. N.O.'s show a deficit ipsilaterally but are superior to A.O.'s. Contralaterally, A.O.'s show no deficit, while N.O.'s show significant impairment. Thus, in N.O.'s some motor behavior ipsilateral to hemisection is superior but contralateral deficits are more pronounced. After recovery (A.O.'s) or maturity (N.O.'s) the cats were trained/tested for accurate limb placement during locomotion on difficult (parallel bars, grids, 2" wide) runways with a 12" wide runway as control. Time to cross difficult runways is increased in both groups because they make more errors than controls (i.e. misplacements). Cinematographic analysis shows that on the parallel 1" bars and grid N.O.'s attempt bar placement at each step, whereas the A.O.'s frequently cross with minimal use of the ipsilateral hindlimb, thereby reducing the number of potential errors but increasing speed. The step cycle in the ipsilateral hindlimb in N.O.'s is altered compared with control and A.O.'s. There is a 2:1 increase in stance:swing ratio, increasing total step cycle duration. Maximum angle at ankle joint prior to initiation of swing is normal in N.O.'s but is increased in A.O.'s. Thus, the enhanced postural reflexes in N.O.'s may be useful in locomotion.

These results suggest that there is no monolithic response to neonatal CNS damage. Following neonatal lesion, some aspects of motor function are enhanced (sparing of tactile placing, enhanced recovery of proprioceptive placing and hopping). However, the N.O.'s also show more bilateral deficits. In locomotion, differences in performance are apparent, but there is no simple relationship indicating a uniform advantage for either group.

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75.6 NEURAL DEVELOPMENT IN CHILDREN. P. Bawa, Dept. of Kinesiology, Simon Fraser Univ., Burnaby, B.C. Canada.

There is no neurophysiological literature available on the post-natal development of the human nervous system. The following experiments were done to investigate the development of the reflex pathways and reaction times in children between the ages of 3 and 13 years.

Torque motor-imposed angular displacements of the adult human wrist result in reflex EMG responses recorded from the muscles stretched. The response can be separated into two components, (1) the short latency reflex (SLR, 25-50msec) and (2) longer-latency reflex (LLR, 50-90 msec). The SLR has been suggested to result from a spinal reflex pathway and LLR at least partially from supraspinal pathway.

Ten boys and eighteen girls were tested for these reflex components. The subject held the handle attached to the shaft of the torque motor at a specified position shown on the oscilloscope. Surface EMG was recorded with a pair of silver disc electrodes from wrist flexors. Square torque pulses applied to the torque motor extended the wrist and stretched the wrist flexor muscles. Those children who could follow instructions were asked to compensate for the imposed displacement by bringing the handle back. In children below 6 years, SLR component lasted up to 40msec whereas LLR component lasted from 50msec to 200msec. The duration of LLR was 100-150msec in these children as compared to about 40msec in adults. Children above 8 years of age had adult-like LLR. However, reaction times were longer in children below 10 years. Shortening of reaction times took place after the reflex components had developed.

The long duration of LLR in young children could be attributed to larger range in conduction velocities of the undeveloped axons involved in the reflex pathway and/or to longer processing times at various synapses.

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75.7 ELECTROMYOGRAPHIC STUDIES ON THE MUSCLE RELAXANT EFFECTS OF PHENYTOIN IN THE DECEREBRATE CAT. A. Raines and S.K.Swope*. Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, D.C. 20007

Previous studies in our laboratory indicate that phenytoin (PH) in doses usually employed for anticonvulsant effects, depresses muscle spindle function (Anderson and Raines, J. Pharmacol. 1974) and diminishes clinical behavioral muscle extensor hypertonus and gamma motoneuron discharges in the decerebrate cat (Hershkowitz et al., Neuroscience Abs. 1978).

The present studies were designed to evaluate PH influences on the electromyogram (EMG) of the triceps brachii of the cat. Adult cats of either sex were anesthetized with halothane and decerebrated by a mid-collicular transection. Decerebrate rigidity ensued briefly after discontinuation of halothane.

Stainless steel implantable electrodes were sutured to the exposed muscle and the forelimb was briskly displaced 90° and 180° from the full extension position, thus stretching the triceps brachii muscle. The stretch was held for 10 sec and the EMG frequency determined by means of a mini-computer at 0-1 sec as an index of phasic stretch reflex and at 7-8 sec as an index of tonic stretch reflex.

PH in doses of 10 and 20 mg/kg (i.v.; 1 mg/kg/min) depressed spontaneous background EMG in 7 of 7 cats by 71 and 96% (means) respectively. The phasic response at 90° was reduced by 28 and 58%, and at 180° by 23 and 47% by these two PH doses. The tonic response was reduced by 59 and 81% at 90° and by 54 and 94% at 180°. Afterdischarge was suppressed by 71 and 96% by 10 and 20 mg/kg PH.

These data suggest that whereas the spontaneous background EMG activity, the tonic stretch evoked activity and the afterdischarge are exceedingly sensitive to depression by PH, phasically induced muscle responses are relatively resistant.

These data lend further support to the hypothesis that PH possesses muscle relaxant properties in an animal model exhibiting muscle hypertonus secondary to an upper motor neuron lesion and provides further evidence for putative utility in spastic man (Cohan et al., Arch. Neurol. 1980).

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76.1 DIFFERENTIAL DISTRIBUTION OF HOST RETINAL AND CORTICAL AFFERENTS WITHIN TECTAL TRANSPLANTS IN RATS. A. R. Harvey* and R. D. Lund. (SPON: J. S. Lund). Dept. of Anatomy, Medical University of S. C., Charleston, S.C. 29403.

We have previously shown that both host retina and cortex project to tectal transplants. We wished to determine how these separate afferents distribute and interrelate within the transplants. Tecta were dissected from fetal rats (14 to 16 days gestation) and injected over the left superior colliculus of newborn rats. After at least one month survival, experiments were undertaken to define the host input to the transplants. In some animals, the left visual cortex was removed and the right eye injected with tritiated proline. In others, one eye was injected with tritiated proline and the other eye was removed. Brains were processed for neurofibrillar, Fink-Heimer and Nissl stains as well as for autoradiography. Most of the transplants connected to the left superior colliculus, although a number connected to the right or to both colliculi.

Retinal afferents project to discrete areas within the transplants. These regions are commonly located close to the surface of the transplants and are characterized by their lack of heavy fiber staining and by the presence within them of many small, relatively close-packed cells. In some cases, particularly when the transplants connect with both superior colliculi, there is a projection to the fiber-free areas from both eyes. In this circumstance, the afferents from each eye often project adjacent to one another, but there is little overlap between them. After a cortical lesion, fine degeneration is distributed throughout most of the fibrous regions of the transplant, but is sparse or absent in areas which receive retinal input.

Thus, despite the abnormal location of the tissue and the unusual paths taken by the host afferents, the differential distribution of the inputs found in the normal, intact superior colliculus is preserved within the tectal transplants. We are currently using electro-physiological techniques to examine whether this separation of afferents can also be recognized functionally at the single cell level.

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76.2 THE UNCROSSED RETINOTECTAL PATHWAYS IN GOLDEN HAMSTERS-AN ORTHOGRADE HRP STUDY. L. S. Jen*, H. H. Woo*, and K.-F. So*. (SPON: P. W. F. Poon) Department of Anatomy, University of Hong Kong, Hong Kong.

Previous studies using degeneration and autoradiographic techniques have shown that the uncrossed retinotectal pathway in normal hamsters is restricted primarily in the anterior third of the stratum opticum of the superior colliculus. If one eye is removed on the day of birth, the uncrossed retinotectal projections from the other eye will expand and occupy all superficial laminae in the rostral half of the colliculus. The implication of these results is that the uncrossed retinotectal projection from an eye can enlarge its terminal field to cover a larger area after early removal of the opposite eye. This phenomenon is further investigated in the present study with a more sensitive pathway tracing technique, namely the orthograde HRP method.

Three milligrams of horseradish peroxidase (Sigma type VI) dissolved in 5 µl of saline were injected into one eye of normal adult hamsters and hamsters with one eye enucleated at birth. After perfusion, the brains were stored in sucrose-buffer for several hours. Frozen sections cut coronally and sagittally were collected and processed for HRP reaction with TMB as the chromogen, and the uncrossed retinotectal projections were examined.

The results indicate that in normal hamsters the uncrossed retinotectal axons and their terminals are distributed in both stratum opticum and stratum griseum superficiale in most areas of the superior colliculus. The distribution area of the uncrossed retinotectal pathway in unilaterally enucleated animals is basically similar except the density of the uncrossed projection is considerably higher than that observed in normal animals. These results contrast with previous observations that the uncrossed retinotectal projection is normally limited in the rostral colliculus, but expands to occupy a wider area in response to eye enucleation in neonatal stage. Since it is shown in preliminary studies that the uncrossed retinotectal pathway in developing hamsters covers most areas of the superior colliculus as in the normal adults. This leads us to suggest that enucleation of the contralateral eye at birth results in enlargement of terminal field of the uncrossed retinotectal projection from the remaining eye by increasing its density without an extensive increase in distribution area.

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76.3 DIFFERENT GRADIENTS OF NEUROGENESIS IN RHESUS MONKEY SUPERIOR (SC) AND INFERIOR (IC) COLLICULUS. M.L. Cooper and P.Rakic. Sec. of Neuroanatomy, Yale Univ. School of Med., New Haven, Ct. 06510

We analyzed the spatiotemporal patterns of neurogenesis in the SC and IC by plotting the positions of heavily labeled neurons in autoradiograms from a series of monkeys that had been exposed to ^{3}H-thymidine at various embryonic (E) days and sacrificed either shortly thereafter or at 2-3 months postnatally. All tectal neurons are generated in the ventricular and subventricular zones around the Sylvian aqueduct within the first third of the 165 day gestation period. Cells in the SC undergo final mitosis from E30 to E56, with peak proliferation from E38 to E43. Neurogenesis in the central nucleus (CN) of the IC also occurs between E30 and E56, and peaks around E43. A weak rostrocaudal gradient within the tectum as a whole is indicated by the later onset of robust proliferation in the IC than in the SC.

There is a weak ventral-dorsal gradient of genesis in the SC, in that all laminae except the stratum griseum superficiale are labeled before E36, whereas labeled cells tend to lie more dorsally late in neurogenesis. However, most cells in all laminae are generated in the same period. No mediolateral or rostrocaudal gradients were evident, although proliferation appeared to subside earlier rostrally. In general, SC gradients are rather weak, particularly in comparison to those evident in frog, chick and rat. There are distinct rostrocaudal, lateromedial and ventrodorsal gradients of genesis in the CN which appear in general to be more pronounced than those in the SC. We compared quantitatively the strengths of these various gradients, both within and between the SC and CN by dividing each nucleus into thirds along each axis (anterior-posterior, lateral-medial, ventral-dorsal) and determining the density of labeled cells within each third. For a weak gradient, cells generated at the same time should be more diffusely spread throughout the nucleus than for a strong gradient. Thus, using diffuseness of labeling as a criterion for gradient strength, we compared (with a Wilcoxon test) the spread of labeled cells along pairs of axes for each injection day. This analysis revealed that neurogenesis along all three axes of the SC proceeds with about equal strength, whereas in the CN the lateral-medial gradient is statistically significantly stronger than the anterior-posterior gradient. Further, the lateral-medial and ventral-dorsal IC gradients are significantly stronger than all three SC gradients. Thus, our analysis of gradient strengths reveals significant differences in proliferative patterns between the SC and IC, suggesting that there is a mosaicism in the generative zone of the midbrain tectum, with nearby regions which give rise to the SC and IC having their own distinct developmental programs controlling the spatial patterns of neuronal proliferation.(Supported by EY-02593)

76.4 EXPANSION OF THE IPSILATERAL VISUAL CORTICOTECTAL PROJECTION IN HAMSTERS SUBJECTED TO PARTIAL POSTERIOR NEOCORTICAL ABLATIONS IN INFANCY. R. W. Rhoades. College of Medicine and Dentistry of New Jersey, N.J.S.O.M., Piscataway, NJ 08854.

Partial retinal lesions, in goldfish, anurans and neonatal rodents can result in expansion of the projection of the remaining retina in the damaged eye so that it encompasses the entire optic tectum or superior colliculus. Like the retina, the visual cortex also projects topographically to the superior colliculus and the present experiments have used autoradiographic and electrophysiological methods to demonstrate that partial cortical damage in neonatal hamsters results in an expansion of the projection of the remaining visual cortex in the damaged hemisphere to the ipsilateral superior colliculus.

A strip of posterior cortex extending from the midline to the rhinal fissure was removed under methoxyfluorane anesthesia on the 7th-10th postnatal day. Three to 10 months later the visual representation in the damaged hemisphere was delineated using standard single unit recording and receptive field mapping techniques. In animals in which visual responses could be recorded from the cortical remnant and an unequivocal visual scotoma was also noted, an electrophoretic deposit of [^{3}H]-leucine and [^{3}H]-proline was made into the approximate center of the visually responsive cortex and the resulting label in the ipsilateral superior colliculus was charted. Identical experiments were carried out in normal hamsters.

In the normals superficial layer collicular label was topographically matched with the site of the cortical amino acid deposit. In the brain damaged hamsters such labelling was seen in all portions of the colliculus. Experiments in which electrical stimulation of the visually responsive portion of the cortical remnant was combined with single unit recording in the ipsilateral colliculus showed that the topography of the collicular visual field representation in the neonatally brain damaged animals was normal and that the expanded corticotectal projection made functional synapses throughout the tectum on this side.

An additional series of electrophysiological and anatomical experiments demonstrated further that the aberrant, crossed corticocollicular pathway which has been previously shown to result from lesions similar to those carried out in this study (Mustari, M.J. and Lund, R.D., Brain Res., 112:37, 1976; Rhoades, R.W. and Chalupa, L.M., J. Neurophysiol., 41:1466, 1978) was intermingled with the expanded ipsilateral cortical projection and that some tectal cells in these animals could be activated by stimulation of either cortex.

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76.5 FIBER-TO-FIBER INTERACTIONS IN DUALLY INNERVATED TECTUM IN XENOPUS WITH RIGHT DOUBLE NASAL AND LEFT DOUBLE TEMPORAL EYES Charles Straznicky, Department of Human Morph., Sch. Med., Flinders Uni. of South Australia, Bedford Park S.A. 5042, Australia.

Compound eye projections, eyes made by the embryonic fusion of two nasal (NN), two temporal (TT) and two ventral (VV) retinal halves are abnormal in adult Xenopus in that each hemiretina projects across the whole extent of the contralateral tectum (Gaze et al., 1963, J. Physiol. 165, 484-499; Straznicky et al., 1974, JEEM 31, 123-137). Yet, during development the optic fiber outgrowth from such eyes to the tectum is selective, involving the innervation of the corresponding tectal half only (Straznicky et al., 1979 Neurosci. Abstr. 5, 181).

In order to determine whether the expansion of the compound eye projection can be prevented we introduced a second set of optic fibers to the tectum soon after metamorphosis. Compound nasal (NN) eyes on the right and compound temporal (TT) eyes on the left side of Xenopus embryos were formed and tha animals reared to metamorphosis and beyond. Two weeks after metamorphosis the right or in other animals the left optic nerve was sectioned (ONC) in order to induce bilateral tectal innervation from one eye. Three to five months after ONC ^{3}H-proline was administered either into the right NN or left TT eye. Visual projections from the eye with intact optic nerve to the contralateral and from the eye with regenerated optic nerve to both contralateral and ipsilateral (monocularly and dually innervated) tecta were mapped electrophysiologically.

The restored contralateral compound eye projections, as shown autoradiographically and electrophysiologically, confirmed previous findings in that it covered the whole extent of the tectum. In contrast, in the dually innervated tecta the rostral part was innervated exclusively by the appropriate temporal retinal halves from TT and the caudal part by the appropriate nasal retinal fibers from NN eyes. Partially superimposed projections from NN and TT eyes were found in the middle of the tectum.

These observations suggest that during regeneration nasal and temporal fibers preferentially select the corresponding tectal termination. The presence of nasal and temporal retinal fibers in the dually innervated tecta prevents the spreading of the hemiretinal projections across the whole extent of the tectum. The segregation of the hemiretinal projections indicate fiber-to-fiber interactions between the two sets of optic fibers from NN and TT eyes.

76.6 RETINAL SPECIALIZATION AND GROWTH IN THE CICHLID FISH, HAPLOCHROMIS BURTONI. Russell D. Fernald and Pamela R. Johns*. Dept. of Biol., Univ. of Oregon, Eugene, OR 97403. *Neurobiology, Harvard Medical School, Boston, MA 02115.

Behavioral experiments suggest that the African cichlid fish, Haplochromis burtoni, preferentially use a restricted retinal region while viewing behaviorally interesting stimuli. This fish has a highly evolved social system with behavioral interactions mediated by bright chromatic and spatial patterns on the fishes' bodies. We have determined the density of the different retinal cell types across the retina. The ventrotemporal retinal region has a significantly higher concentration of all cell types except rods. In young fish (8-11 weeks) three times as many ganglion cells and twice as many cone nuclei are found in that region compared with the nasal retina. This density differential is maintained in older animals although it is not as great. In order to compare differently sized animals, we use a cartographer's projection of the nearly hemispherical retinal surface onto a plane. In this way the retinal representations can be scaled and direct comparisons made.

The retina continues to enlarge throughout the life of the animal. To study this, we compare the two eyes from the same fish, one removed when the animal is small and the other after a period of growth. Labelled ^{3}H-thymidine is injected and one eye excised after incorporation. The fish is then placed in a social situation known to enhance growth rate. When the desired size is attained, the second eye is removed and compared with the first using radioautography. The thymidine-labelled cells mark the boundary between retinal tissue present in the small eye and that added later with growth. In this way we can distinguish the change in retinal dimensions due to accretion of cells from that due to expansion of existing tissue.

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76.7 THE DEVELOPMENT AND MODULATION OF AVIAN MUSCARINIC RECEPTORS. Suzanne B. Por* and Stephen C. Bondy* (SPON: Clifford L. Mitchell). Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709.

The maturation of the cholinergic muscarinic receptors has been studied in the optic lobe of the chick using ^{3}H-quinuclidinyl benzilate (QNB) as a receptor ligand. The developmental profile showed these receptors to be present in the chick embryo as early as 8 days incubation. Rapid accretion of receptor binding occurred between days 12-14 of incubation, a period of maximal synaptogenesis within the optic lobe. A subsequent phase of increased binding occurred around hatch.

Removal of the optic vesicle of 3 day old embryos, prior to major development of tectal receptors caused only a 14% reduction of QNB binding in the non-innervated optic lobes at 19 days incubation. Thus, the appearance of most of the tectal muscarinic receptors is independant of innervation by the retinal ganglion cells.

Unilateral enucleation of 2 day old chicks arrested normal increases of weight, protein, and cholinergic receptors in the denervated optic lobe contralateral to the excised eye. The overall amount of binding expressed on a protein basis was not significantly changed in 22 day old birds. However, Scatchard analysis revealed that denervated optic lobes possessed significantly more receptors (B_{max} = 0.80±0.03 pmoles/mg protein) than corresponding control lobes (B_{max} = 0.58±0.02 pmoles/mg protein); whereas the binding affinity of these receptors was reduced. The K_D of deafferented lobes was (0.79±0.06) x 10^{-9}M and control value was (0.63±0.03) x 10^{-9}M. These data imply that compensatory changes in density may accompany altered dissociation constants of receptors.

77.1 IN VITRO SYNTHESIS AND MEMBRANE INTEGRATION OF THE SUBUNITS OF TORPEDO ACETYLCHOLINE RECEPTOR. D.J. Anderson*and G. Blobel* (SPON: N. Kalderon). Rockefeller Univ., New York, N.Y. 10021

We have synthesized the four subunits of T. californica Acetylcholine Receptor (AChR) in vitro, in order to define the primary translation products of AChR mRNA(s) and tostudy the membrane insertion of this oligomeric integral membrane protein. Our results indicate that the subunits of AChR are synthesized as independent polypeptides which are co-translationally inserted into rough endoplasmic reticulum membranes, by a mechanism analagous to that first described for viral transmembrane glycoproteins.

A wheat germ cell-free protein synthesizing system was programmed with total cellular RNA extracted from electroplax tissue by conventional procedures (SDS/Phenol/Chloroform.) [^{35}S]methionine -labelled AChR polypeptides were purified from the total translation products by immunoprecipitation after SDS denaturation. We used subunit-specific antibodies prepared in rats immunized with AChR polypeptides isolated from Torpedo by affinity chromatography and preparative gel electrophoresis. The sera were characterized by immunoprecipitation of [^{125}I]-labelled purified AChR from the mature tissue (authentic AChR.) Glycosylation and membrane insertion of the in vitro synthesized subunits were studied by performing translations in a system supplemented with dog pancreas rough microsomal membranes. Translation products were analyzed by SDS polyacrylamide gel electrophoresis.

When translated in the absence of membranes, Torpedo mRNA codes for four separate AChR polypeptides, of which α, $\gamma$and δ are slightly smaller (by 1,000-3,000 Daltons) than their authentic counterparts. The lower molecular weight of these products is due to their lack of carbohydrate residues. (Glycosylation of these proteins is not expected without membranes which catalyze the linkage of oligosaccharide moieties.) Furthermore, each subunit is translated optimally at a different Mg^{++} concentration. Since eukaryotic mRNA's have a single 5' terminal initiation site, and since initiation is Mg^{++}-dependent, this result suggests that the four AChR subunits may be encoded in separate mRNA molecules.

If membranes are present during the translation, the α, γ and δ subunits synthesized therein are of slightly greater mass, exhibiting an M_r close to that of their corresponding mature chain. This is presumably due to the attachment of Asn-linked core oligosaccharides. These glycosylated forms of the AChR subunits are only partially degraded by Trypsin digestion under conditions which totally digest the non-glycosylated precursors. The β chain is also incompletely cleaved by this proteolytic digestion at the cytoplasmic surface of the membrane. These data indicate that all four AChR subunits synthesized in vitro have undergone membrane insertion and, in this system, span the lipid bilayer.

77.2 MONOCLONAL ANTIBODIES AGAINST AChR AND COMPLEMENT-DEPENDENT LYSIS OF AChR-CONTAINING VESICLES. B. A. Pollok*, D. S. Dwyer, R. J. Bradley, G. E. Kemp* and J. F. Kearney*. Dept. of Microbiology & Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

Monoclonal antibodies (MAbs) against the acetylcholine receptor (AChR) were produced by injecting BALB/c and C57BL mice with purified receptor from Narcine brasiliensis. Spleen cells were removed from immunized animals and fused with the non-producing myeloma X63-Ag8.653. The resulting hybridomas were tested for anti-AChR activity by both ELISA and immunoprecipitation assays. Thus far, only two clones have been produced which cross-react with AChR from rat muscle. These were both subclass IgG1 antibodies. Isoelectric focusing of the MAbs allowed determination of the heterogeneity of antibodies produced by separate clones and indicated that different heavy chains (e.g. μ and γ3) may share the same light chain. The MAbs and heat-inactivated sera from patients with myasthenia gravis were then tested for their ability to induce complement dependent lysis of membrane vesicles containing AChR. Homogenized electric organs from electric fish (N. brasiliensis) spontaneously formed vesicles which were then radiolabelled with ^{3}H-glucose. All monospecific clones were capable of causing 35-70% lysis of the vesicles, indicating that single antigenic determinants on the receptors are close enough in the membrane to allow for IgG-induced complement fixation to occur. Mixtures of certain MAbs created a synergistic effect for complement dependent lysis by allowing crosslinking of antigenic determinants and enhanced proximity of IgG molecules. A wide variance in the ability of myasthenic sera to cause lysis of the AChR-containing vesicles was observed, with 0-70% of the maximum releasable label liberated. Heat-treated myasthenic serum alone was incapable of initiating lysis. In addition, an excess of solubilized AChR inhibited the lytic action of MG sera. It appears that anti-AChR antibodies in myasthenic sera or in the form of MAbs can potentiate complement mediated membrane damage in vitro.

77.3 SELECTIVE SUPPRESSION OF CHOLINOCEPTIVE FUNCTION IN RAT BRAIN BY INTACT IgG AND F(ab')$_2$ PREPARATIONS OF MONOCLONAL ANTIBODY AGAINST THY-1 GLYCOPROTEIN, M. L. Cameron*, J. Roberts*, N. Schupf and C. A. Williams. Div. Nat. Sci. SUNY-Purchase and Dept. Psychol. Manhattanville College, Purchase, N.Y. 10577.

In rat and mouse the Thy-1 glycoprotein (Theta antigen) reaches adult levels on neuronal cell surfaces at about 3 weeks or age (A.N. Barclay, 1979). Experiments in other tissues suggest that Thy-1 participates in developmental events. Dulbecco et al. (1979) have reported that some but not all monoclonal antibodies against the Thy-1.1 determinant (MC antiThy-1.1) block dome formation in cultures of a rat mammary carcinoma cell line. We have reported that injection via implanted cannulae of a MC antiThy-1.1 into rat perifornical hypothalamus selectively suppresses carbachol (CC, 0.8 µg) induced drinking but is without effect on norepinephrine (NE, 6 µg) induced eating. (Williams et al. Nature 283, 82, 1980).

The objectives of the present study were to determine whether complement mediated cytotoxicity could be responsible for the "anticholinergic" effect and whether different MC antiThy-1.1 antibodies may differ quantitatively in their biological activity.

Three intact MC antiThy-1.1 immunoglobulins (IgG): T32B11 (subclass 2A), T31G10 (subclass 2B) and MRC OX-7 (subclass 1), supplied by A.F. Williams, were compared. The non-complement fixing divalent peptic fragment F(ab')$_2$ of MRC OX-7 was also tested. All preparations significantly suppressed carbachol induced drinking in an approximately linear relationship to the log dose in picomoles (pmol). T31G10 was the most active with 50% suppression at a dose (ED_{50}) of 0.5 pmol. The ED_{50} for T32B11 was 3 pmol, for MRC OX-7 was 7 pmol and for MRC OX-7 F(ab')$_2$ was 15 pmol. MRC OX-7 IgG at the highest dose employed (15 pmol) had no effect on NE induced eating. The dose response curves of intact IgG preparations and the F(ab')$_2$ fragment had similar slopes suggesting that Fc dependent supplementary processes, including C-mediated cytotoxicity, do not contribute significantly to the biological effect. Subclass differences among the MC antiThy-1.1 IgG antibodies may contribute to the observed differences in relative activity through restrictions related to affinity, avidity or microspecificity. Monoclonal antibody against cell membrane antigens with nonrepeating determinants do not crosslink sufficiently to cause aggregation (capping) which could result in nonspecific disruption of membrane function. We tentatively conclude, therefore, that antibody binding to the Thy-1 glycoprotein at the 1.1 allotypic determinant directly impairs some aspect of postsynaptic cholinergic transmission.

77.4 EXPERIMENTAL MODIFICATION OF THE MICRO-ENVIRONMENT OF THE RECEPTOR FOR ACETYLCHOLINE. Y. Lass, G.Goldberg*, E.M. Landau, Dept. of Physiol. & Pharmacol. Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Israel.

The ACh receptor is an integral membrane protein, deeply embedded in the lipid matrix of the membrane. As such it will be affected by changes in both its lipid and its aqueous micro-environment. The binding of ACh is assumed to produce a conformational change in the receptor which in turn will cause an ionic channel to open. The study of the small current fluctuations observed during the application of ACh ("ACh noise"), allows one to determine both the open-time (τ) and the average conductance (γ) of the single receptor channel. Changes in the receptor micro-environment may thus affect both γ and τ. Indeed a study of the effects of temperature on the single channel conductance in chick muscle cells in culture ("myoballs"), revealed a discontinuous relationship between γ and temperature. Thus, γ was unaffected as the bathing temperature was decreased in the range of 37°C to 20°C. Below 20°C, γ decreased sharply as the temperature was lowered. It was suggested that at this transition temperature the membrane lipid underwent a phase transition, i.e. the lipids "froze" at low temperature. The increased stiffness of the membrane around the receptor would restrict the conformational change and thus decrease the single channel conductance. It is also known that the composition of the aqueous micro-environment also profoundly affects the receptor. Of particular importance are the divalent cations and the H^+ions. Increasing the concentration of the former causes a block in the ACh receptor channel. Similarly reducing the pH causes a marked decrease in the single channel conductance. We have recently discovered that in the chick myoball, an increase in pH causes a large increase in the single channel permeability. The channel permeability is thus amenable to improvement by experimental means. If this effect is mediated by hydroxylation of a specific site, it may be possible to devise pharmacological tools to achieve the same effect. To describe the properties of such a site may thus be the first step in finding a new pharmacological approach to the improvement of the function of the neuromuscular receptor for Acetylcholine.

77.5 PURIFICATION OF A PUTATIVE CNS ACETYLCHOLINE RECEPTOR FROM RAT BRAIN. G.E. Kemp*, R.L. Furner*, and B.J. Morley. Neurosciences Program, University of Alabama Medical School, Birmingham, ALA. 35294 and Neurochemistry Laboratory, The Boys Town Institute for Communication Disorders in Children, Omaha, NE 68131

α-bungarotoxin (Butx) is a well-established marker for nicotinic acetylcholine receptors (nAChRs) found at the skeletal muscle neuromuscular junction. Butx is also known to bind to a protein from the mammalian CNS with biochemical and pharmacological properties closely resemble peripheral nAChRs, and it has been proposed that this protein is an nAChR. Using a modification of a protocol used for purification of mammalian muscle nAChR), it has been possible to purify this putative CNS nAChR from rat brain. Purification steps include subcellular fractionation, Trition-X-100 solubilization, α-cobratoxin biospecific adsorption, DEAE-Sephadex ion exchange chromatography, Con A-Sepharose 4B adsorption, and Sepharose 6B gel filtration. The product binds approximately 3 pM of ^{125}I-Butx per μg protein. Sodium dodecylsulfate polyacrylamide gel electrophoresis revealed 4 polypeptide chains of 44,000, 50,000, 56,000 and 62,000 daltons. The purified product does not detectably cross-react with antibodies directed against purified Torpedo or Electrophorus nAChR or with IgG obtained from patients with Myasthenia Gravis that cross-react strongly with rat muscle nAChR.

The affinity ligand 4-(N-maleimide) α-benzltrimethylammonium (MBTA) is known to block 1 or 2 Butx sites on Torpedo and Electrophorus nAChR, a finding we have extended to nAChR purified from Narcine brasiliensis and denervated rat skeletal muscle. In contrast, MBTA blocks 100% of Butx binding sites to the CNS protein. If Butx labels only 1 of 2 nAChR ligand sites (the MBTA sensitive site) a second site might be left available for agonist-mediated conductance responses. This explanation is consistent with reports that Butx labels nicotinic sites in several CNS and CNS-derived tissues, but does not block nicotinic conductance responses in these tissues.

77.6 DIFFERENCES BETWEEN JUNCTIONAL RECEPTOR AND EXTRAJUNCTIONAL RECEPTOR MAY BE DUE TO CARBOHYDRATE RESIDUES. D. S. Dwyer, R. J. Bradley and G. E. Kemp*. Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

It is now well established that junctional receptor (JR) and extrajunctional receptor (EJR) differ in immunological properties as judged by their interaction with antibodies from patients with myasthenia gravis. EJR appears to contain an antigenic determinant not found on JR. One possible source of these differences may be the carbohydrate moieties which are attached to the receptor protein. Purified acetylcholine receptor from denervated muscle (EJR) was exposed to a mixture of exo- and endoglycosidases to remove carbohydrates. α-Bungarotoxin binding was not affected by this procedure. The enzyme treated EJR had a decreased affinity for the lectin, Con A, indicating that some of the carbohydrates had been removed. The treated receptor was then assayed by immunoprecipitation and inhibition of toxin binding assays using serum from myasthenic patients. The conventional immunoprecipitation assay detected no differences in antibody binding to treated or untreated EJR precluding gross structural changes. However, values for the inhibition assay were decreased by as much as 20%. This finding was confirmed using a modified immunoprecipitation assay which measures immunoglobulin binding to the toxin binding site. Thus, removal of carbohydrates from EJR decreased the binding of myasthenic IgG. Enzyme treatment of JR (from normal muscle) did not alter inhibition values by those patients capable of blocking toxin binding. After enzyme treatment, EJR more closely resembles JR as defined by its immunological properties. It is concluded that carbohydrate residues on the 40,000 m.w. subunit determine some of the differences between JR and EJR. These carbohydrates may be involved in the arrangement and stabilization of AChR at the neuromuscular junction.

81.1 LOW CONCENTRATIONS OF LSD MAY BLOCK REUPTAKE OF LATERAL INHIBITORY TRANSMITTER IN LIMULUS RETINA. Leonard Kass, Peter H. Hartline, and Alan R. Adolph. Eye Research Institute, Boston, MA 02114.

Accumulated evidence implicates 5-HT as the transmitter for lateral inhibition in Limulus lateral eye. Each facet in this simple compound eye contains a dozen receptor cells electrically coupled to a single spike-generating eccentric cell. Eccentric cells in neighboring facets form (lateral) inhibitory synapses with each other. Thus, lateral inhibition can be evoked in a test eccentric cell by stimulating its neighbors with light. We have examined the effects of LSD at this putative 5-HT synapse by extra- and intracellular recordings from eccentric cells. LSD is commonly believed to agonize or antagonize 5-HT action at postsynaptic receptor sites in many vertebrates and invertebrates. Our results indicate that LSD acts presynaptically. We propose the following hypothesis for LSD action in Limulus lateral eye: In 1-5μM LSD, partial blockade of 5-HT presynaptic receptors for reuptake prolongs 5-HT lifetime in the synaptic cleft and thereby enhances inhibition; in 5-20μM LSD, more sites are blocked leading to accumulation of synaptic transmitter and consequently to postsynaptic desensitization. Evidence against postsynaptic LSD action: (1) Structural specificity of postsynaptic receptor for 5-HT appears to preclude direct LSD interaction (Adolph and Kass, J. gen. Physiol. 74:549, '79). (2) We now report that LSD, but not 5-HT, action is markedly reduced when added to a High Mg^{++}/Low Ca^{++} bathing medium which prevents transmitter release. This indicates that the predominant action of LSD is not postsynaptic. We are currently investigating whether the higher [LSD] exerts any direct action on postsynaptic receptors. Evidence favoring presynaptic uptake blockade hypothesis: (1) Presynaptic reuptake receptors appear nonspecific enough to accommodate the LSD molecule (Adolph and Kass, '79). (2) We have shown that light-evoked lateral inhibition from adjacent eccentric cells onto a single eccentric cell is enhanced in low [LSD] and suppressed in higher [LSD]. (3) A different way of activating the inhibitory synapse at a test cell is by stimulating the optic nerve axons of neighboring eccentric cells. This antidromically evoked inhibition uses a more direct neural pathway and thus serves as an important control for LSD's effect on light-evoked inhibition: antidromically evoked inhibition also exhibits enhancement at low [LSD] and suppression at higher [LSD]. In contrast, 5-HT causes desensitization and reduction of lateral inhibition at all concentrations. (4) The strength of lateral inhibition that has been enhanced by low [LSD] decays (perhaps desensitizes) more rapidly than in the normal eye. The specific hypothesis presented above can account for diverse LSD effects reported in the mammalian brain literature such as: (i) "blocking", "facilitating", "potentiating", "mimicking", and other agonistic/antagonistic effects sometimes within the same tissue; (ii) concentration dependence of LSD action; (iii) effect of LSD on 5-HT turnover rate; and (iv) LSD binding to 5-HT synaptic membrane sites.

81.2 LOCALIZATION OF DOPAMINE-STIMULATED ADENYLATE CYCLASE ACTIVITY IN RETINA. G.W. De Vries*, K.M. Campau* and J.A. Ferrendelli (SPON: A.I. Cohen). Div. of Clin. Neuropharm. and Depts. of Neurol, Pharm. and Ophthal., Washington Univ. Med. Sch., St. Louis, MO. 63110.

Dopamine has been shown to stimulate retinal adenylate cyclase activity in a number of vertebrate species. Previous studies have suggested that this dopamine-stimulated activity is found primarily in the inner retina, but its relationship to regions known to contain dopaminergic nerve terminals has not been clearly defined. Therefore, we have attempted to determine the location of this enzyme more accurately using quantitative histochemical techniques.

In preliminary studies of adenylate cyclase in homogenates of whole rabbit and mouse retina we observed that its basal activity was 45-55 pmoles/mg prot/min. This was increased 50-80% by the addition of dopamine (50 μM) alone. As in other regions of the CNS, EGTA (0.5 mM) depressed basal activity of the enzyme but not the stimulatory effects of dopamine. We also found that GPP(NH)P (30 μM), an analogue of GTP, increased enzyme rate and maximal adenylate cyclase activity (113-123 pmoles/mg prot/min) was seen when EGTA, dopamine and GPP(NH)P were all included in the assay mixture.

In both rod-dominant rabbit and cone-dominant ground squirrel retinas, basal adenylate cyclase activity (no additions) in microdissected retinas had a similar pattern of distribution. The greatest activity (150-180 pmoles/mg dry wt/min) was found in the inner plexiform layer. Lesser activities were observed throughout the remainder of the retina, with relatively high levels in the outer plexiform (30-40 pmoles/mg dry wt/min) and inner segment (60-70 pmoles/mg dry wt/min) layers. GPP(NH)P, in the presence of EGTA, stimulated enzyme activity in all retinal layers. The addition of dopamine further increased adenylate cyclase activity, but only in the inner plexiform, inner nuclear and outer plexiform layers of the rabbit retina and only in the inner plexiform layer of the ground squirrel retina.

It has been suggested (Watling et al., Nature 281: 578, 1979) that all dopamine receptors in the retina are linked to adenylate cyclase. If, in fact, this is the case, the above results indicate that dopamine receptors are found in both plexiform layers and in the inner nuclear layer of rabbit retina, but are confined to only the inner plexiform layer in ground squirrel retina. This suggests that dopamine may have different physiologic actions in various animal species.

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81.3 SPECTRAL SENSITIVITY OF LIGHT EVOKED ACH RELEASE FROM THE RABBIT RETINA. Stephen C. Massey* & Dianna A. Redburn. Dept. Neurobiol & Anat., Univ. Texas Med. Sch., Houston, Texas, 77025.

There is substantial evidence that ACh is a retinal neurotransmitter. All the cholinergic markers are localised in the inner portion of the rabbit retina, specifically with amacrine cells, and ACh may be released by light stimulation. Using an in vivo rabbit eye-cup preparation, loaded with (3H)- Ch, we have attempted to identify the peak spectral sensitivity for the light evoked release of (3H)- ACh which is calcium dependent while the efflux of (3H)-Ch is unchanged; therefore, in this series of experiments, a rapid perfusion system was set up and increments in the efflux of total radio-activity were taken to be indicative of ACh release. Light stimulation was provided by a tungsten source via an electronic shutter driven at 2 Hz (50ms for 2 min) with narrow band filters equalized with regard to intensity and attenuated with neutral density filters. Experiments were carried out under mesopic or low photopic conditions.

The intensity response curve showed the threshold for light evoked release of ACh at 520 nm to be approximately 9 log quanta/cm^2/flash; this is below the intensity required to record the ERG under these conditions. The response range from threshold to maximum covered 3-4 log units. An intensity giving a half maximal response at 520 nm was chosen to examine the spectral sensitivity curve which showed a peak at 500-520 nm. These preliminary results represent a sensitive method to monitor the activity of retinal cholinergic neurones and may be used to characterize the afferent pathways associated with this specific cell type.

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81.4 NEUROTRANSMITTER AND DRUG RECEPTOR LOCALIZATION IN THE RETINA. J.K. Wamsley, J.M. Palacios, D.L. Niehoff and M.J. Kuhar (SPON: S. Bird). Johns Hopkins School of Medicine, Departments of Pharmacology and Psychiatry, Baltimore, MD 21205.

Having developed as a neuronal outgrowth of the embryonic diencephalon, the retina has served as an easily accessible model for the study of the central nervous system. Cytochemical studies have provided evidence of many putative neurotransmitter candidates in the retina, and binding studies have indicated the presence of the appropriate neurotransmitter receptors. We have localized the distribution of neurotransmitter receptors in the retina of rat, monkey and human eyes using a new in vitro technique of autoradiography which is suitable for diffusible compounds (Young and Kuhar, Brain Res., 179:255-270). A subset of amacrine cells in the inner nuclear layer have been proposed to be GABAergic. We have, accordingly, localized high affinity GABA receptors in the inner plexiform layer of the retina. Benzodiazepines, which are thought to act in conjunction with GABAergic mechanisms in some systems, were found to have receptors in the inner plexiform layer as well. This benzodiazepine binding was enhanced in the presence of unlabeled GABA. Using ^{3}H-QNB we were also able to localize muscarinic cholinergic receptors in the inner plexiform layer. Another subset of amacrine cells are thought to be dopaminergic. In the rat retina, we have utilized ^{3}H-spiperone to label both dopamine receptors and serotonin receptors. By displacing ^{3}H-spiperone binding with unlabeled ADTN we localized substantial concentrations of dopamine receptors in the inner and outer plexiform layers as well as over the layer of photo-receptor cells. Very few of these sites appear to be displaceable with cinanserin indicating few, if any, serotonin$_2$ receptors are present in the retina. Interestingly, we were able to localize beta-adrenergic receptors in the inner plexiform layer, outer plexiform layer and over the layers of receptor cells of the retina. Very few of these sites were displaceable with 10^{-7}M zinterol, indicating there are beta$_1$ receptors in the retina. Our preliminary observations indicate the dopamine receptor and beta-adrenergic receptor distributions are the same in the monkey and human retina as well.

These receptor localizations support some of the notions of neurotransmitter-receptor interactions between amacrine cell and ganglion cell processes in the inner plexiform layer and raise some new interesting questions about possible interactions in outer retina layers.

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81.5 NEURAL LOCALIZATION OF SUBSTANCE P-LIKE IMMUNOREACTIVITY IN RABBIT RETINA. E. V. Famiglietti, Jr., N. C. Brecha and H. J. Karten. Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201 and Dept. Neurobiology, SUNY, Stony Brook, NY 11794.

Substance P-like immunoreactivity was localized in rabbit retina using immunohistochemical techniques. Retinas were fixed in 4% paraformaldehyde, with or without 0.1M D,L lysine-HCl and 0.01M sodium periodate. Frozen sections were incubated in a monoclonal antiserum to substance P, and were otherwise processed by standard FITC immunofluorescence techniques. Specific immunoreactivity was established by absorption of the antiserum with 10uM synthetic substance P.

Specific substance P-like immunoreactivity was observed within cell bodies near or at the inner border of the inner nuclear layer (INL) and in the ganglion cell layer (GCL). Beaded and smooth dendritic processes of these cells formed 3 fluorescent bands in the inner plexiform layer (IPL), roughly corresponding to strata (S) 1, 3, and 5 of a 5-tiered stratification. S5 was most prominent and S3 least prominent. Processes were occasionally seen in the INL and outer plexiform layer (OPL), and rarely in the axon layer. This laminar organization of immunoreactive cell bodies and processes was found both in central and in peripheral retina.

One population of immunoreactive cell bodies in the INL gives rise to a single, stout process that descends to and ramifies in S5 of the IPL. A second population gives rise to thinner, multistratified processes which ramify in S1, S3 and S5. Immunoreactive cell bodies in the GCL bear 1 to 3 dendrites which ramify solely in S5. Correlation with Golgi preparations suggests that the first type of immunoreactive cell may give rise to the processes seen in the INL and OPL. The second type of cell appears identical to a wide-field, multistratified amacrine cell with beaded dendrites.

These studies have demonstrated at least 2 populations of amacrine cells with substance P-like immunoreactivity in rabbit retina. In addition, many reactive cells are present in the GCL. Apparently, the great majority of these are displaced amacrine cells, while a small minority may be ganglion cells. It is noteworthy that substance P-like immunoreactive dendrites are distributed in the same 3 strata as are the dendrites of catecholamine-containing and indoleamine-accumulating amacrine cells.

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81.6 GLYCINE MEDIATES TRANSIENT IPSPs AND GABA MEDIATES SUSTAINED IPSPs IN MUDPUPPY RETINAL GANGLION CELLS. D.R. Dvorak *, J.H. Belgum * and J.S. McReynolds. Department of Physiology, University of Michigan, Ann Arbor, MI 48109.

We have shown that center illumination produces both transient and sustained IPSPs in mudpuppy retinal ganglion cells. This report examines the role of amino acids as possible neurotransmitters mediating these inhibitory responses. Intracellular recordings were obtained from neurons in the superfused eyecup preparation. When added to the superfusate in 5 mM concentrations, L-aspartate and L-glutamate depolarized ganglion cells via conductance increases, while glycine, taurine and GABA hyperpolarized the same cells, also via conductance increases. Similar results were obtained when synaptic transmission was blocked by the presence of 4 mM cobalt chloride.

Dose-response functions were obtained for glycine and GABA. Over the range 0.1 mM to 10 mM the effects (increase in membrane conductance) were dose-dependent and adequately described by a Michaelis-Menten relationship. Effects of applied glycine were selectively antagonized by strychnine (<0.1 mM) and effects of applied GABA were selectively antagonized by picrotoxin (<0.1mM). (Taurine effects were in all respects indistinguishable from those of glycine and were consistent with it acting as a glycine analogue.)

Transient IPSPs were reversibly eliminated by strychnine (0.01 mM). Sustained IPSPs were reversibly eliminated by picrotoxin (0.01 mM). Recordings from bipolar and amacrine cells show that these effects can not be attributed to action of the blockers on presynaptic cells.

These results strongly imply that glycine mediates transient IPSPs and that GABA mediates sustained IPSPs in ganglion cells. If, as we suspect, amacrine cells are the source of these inhibitory inputs, then transient amacrines ought to release glycine and sustained amacrines ought to release GABA.

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81.7 CENTER STIMULI ELICIT TRANSIENT AND SUSTAINED IPSPs IN MUDPUPPY RETINAL GANGLION CELLS. J.H. Belgum *, D.R. Dvorak * and J.S. McReynolds. (SPON: T.J. Morrow) Department of Physiology, University of Michigan, Ann Arbor, MI 48109.

Illumination of the receptive field center of retinal ganglion cells elicits two distinct types of inhibition. Transient IPSPs occur at on and off in all classes of ganglion cells. Current-voltage relationships show that the transient IPSPs are due to a large conductance increase. This transient input is evident even with the smallest spots tested (40 µm diameter) and at intensities within two log units of threshold.

The second form of inhibition is a sustained IPSP which occurs in all off-center and certain on-off ganglion cells. This sustained IPSP is also produced by small, centered spots, but is maintained for the duration of the stimulus. Like the transient IPSP,this response is also due to a large increase in membrane conductance. Sustained inhibition is a clear exception to the widely held idea that sustained responses of ganglion cells are due entirely to modulation of excitatory input from bipolar cells.

These results show that inhibition in ganglion cells is not restricted to surround pathways. We also suggest that at least two different types of presynaptic neurons provide these inhibitory inputs: 1) cells which depolarize transiently at on and off, probably transient amacrines, and 2) cells which have a maintained depolarizing light response, probably sustained amacrines.

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81.8 SECONDARY LABELING OF RETINAL NEURONS BY A RETROGRADELY TRANSPORTED DYE. R. H. Masland and U. C. Dräger. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

The fluorescent dye 4,6,diamidino-2-phenylindole (DAPI) is avidly transported by retinal ganglion cells. In the course of some other experiments we noticed that it also labels retinal neurons other than the ganglion cells. When we applied the dye to the cut optic tract of the rabbit, we found that two to three days later the majority of cells in the ganglion cell layer was brightly labeled, but a distinct second population was very faintly labeled. Taken together, the labeled cells accounted for all of the neurons present in the ganglion-cell layer. Since at least 20% of the cells of the rabbit's ganglion-cell layer are displaced amacrines (Hayden et al., '80) this indicated transneuronal movement of the dye. When we waited 5-7 days after application of the dye, fluorescence was seen in the inner nuclear layer, and by 10 days the majority of the cells there was labeled.

These phenomena were seen even more clearly in the mouse, where the distance over which the dye must be transported is shorter than in the rabbit. One day after application of DAPI often observed brightly labeled ganglion cells surrounded by halos of faintly labeled cells. When we applied a mixture of horseradish peroxidase and DAPI to the optic tract we found HRP to be present in the brightly labeled cells but not in the surrounding, faintly labeled cells.

The mechanism of the retrograde transneuronal spread of DAPI is unclear. It could be due simply to leakage from the ganglion cell bodies, but we do not observe such leakage in the brain proper: When various thalamic nuclei were labeled by cortical application of the dye, the nuclei were sharply delineated, with labeled cells immediately adjacent to unlabeled ones. Focal injection of DAPI into the mouse tectum produced secondary labeling of retinal neurons, but in the same animals cortical neurons projecting to the tectum were surrounded by unlabeled neighbors. Alternatively, the ganglion cells and other neurons of the inner retina might make contact in some manner that allows the cell-to-cell passage of the DAPI molecule. Gap junctions among amacrine cells and between amacrine and bipolar cells have been reported (Famiglietti and Kolb, '75; Kolb, '79), but such contacts by ganglion cells have apparently not been observed.

81.9 LARGE RETINAL GANGLION CELLS: THEIR ISOLATION AND ANTIGENIC PROPERTIES. S.E. Kornguth and E. Langer*. Depts. of Neurology and Physiol. Chem., University of Wisconsin, Madison, WI 53706

Antisera were prepared against large ganglion cells isolated from ox retina. In retina, only the large ganglion cells specifically bound the fluorescein labelled γ-globulins. Cone cells reacted equally with immune and control (preimmune) γ-globulin. In other brain regions the immune γ-globulin bound granule, stellate and Purkinje cells in the cerebellum; neurons of the lateral geniculate nucleus, superior colliculus, superior olive and substantia nigra; ependymal cells surrounding the fourth ventricle. The reaction with other organs including kidney, adrenal, lung, heart and liver was examined; only renal tubule cells reacted specifically. The retinal antigens isolated by affinity chromatography consisted of two proteins or subunits as determined by electrophoresis on polyacrylamide gels containing SDS. The predominant protein had a molecular weight of 49,000 and a minor component had a weight of 52,000 daltons. The retinal protein is similar to benzodiazepine receptor in molecular weight, solubility characteristics, tissue distribution and cellular localization. The cells from ox retina were dispersed in a solution containing 4% polyvinyl pyrrolidone, 5 mM $CaCl_2$, 1% rabbit serum albumin, mM HEPES buffer (pH 7.1) and 0.4% formaldehyde. Dispersion was accomplished by repeated passage of tissue through a stainless steel cytosieve and nylon cloth. The dispersed cells were separated by centrifugation through a discontinuous sucrose gradient. Greater than 95% of the cell bodies recovered at the 1.75-1.9 M sucrose interface had a diameter exceeding 28 μm. Recovered cell bodies retained their plasmalemmas. To develop antibodies against the isolated cells, rabbits were injected subcutaneously with the cells suspended in Freund adjuvant and then intravenously with the cells suspended in a sucrose solution. Precipitin lines were obtained between immune sera and triton X-100 solubilized ganglion cells placed in Ouchterlony plates. IgG fractions of normal and control sera were prepared by $(NH_4)_2SO_4$ fractionation and subsequently by chromatography on DEAE cellulose. The γ-globulins were labelled with fluorescein and incubated with 6 μm thick sections of ethanol fixed tissue. The immune γ-globulins reacted specifically with the large ganglion cells in ox, rabbit, cat, macaque, rat and goldfish retinas. Affinity columns were prepared by coupling immune and control γ-globulins to Sepharose 4B. Two proteins were eluted with 3 M sodium thiocyanate from the column containing immune γ-globulins. Immunoelectrophoresis revealed that the eluate contained two antigens: one was neutral at pH 8.6 and the other was cationic. Supported in part by a gift from Mr. and Mrs. L. Weiss and a grant from the U.W. Med. School.

81.10 MORPHOLOGICAL CLASSIFICATION OF RABBIT RETINAL GANGLION CELLS. Franklin R. Amthor*, Clyde W. Oyster and Ellen S. Takahashi. School of Optometry, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

In addition to conventional Golgi methods, intraretinal injections of horseradish peroxidase (HRP) have been used to stain ganglion cells (and other neurons) in rabbit retina. Although the HRP method is selective in that not all cells are stained, we have stained cells in visual streak and numerous peripheral locations. Moreover, the stained cells represent the full range of soma sizes observed in conventional Nissl stained preparations and the dendritic field sizes are comparable to those observed in Golgi material.

It is clear that a number of different morphological classes are represented in the ganglion cell population. The classification is based on consideration of stratification in the inner plexiform layer (evaluated by three-dimensional reconstruction and/or serial sections), soma and dendritic field size, retinal location, complexity of branching pattern, and special features such as dendritic spines and dendritic flare. Both qualitative (subjective) and quantitative evaluation methods are being used.

Some of the results lead to indirect correlations of morphology with the known functional classes of rabbit ganglion cells and these will be considered. This work was supported, in part, by USPHS Grants EY02207, EY00033, and EY7033.

81.11 CHANGES IN THE SPATIAL AND TEMPORAL DISTRIBUTION OF CENTER AND SURROUND RESPONSES IN X- AND Y- CELLS WITH VARYING STIMULUS ON TIMES. Alan Stein* and John K. Stevens (SPON: T. DAVIS). Dept. of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 and Playfair Neuroscience Unit, University of Toronto, Toronto, Ontario M5T2S8.

Using the response plane method (Stevens and Gerstein, 1976), we have investigated the spatiotemporal organization of the center and surround of X- and Y- retinal ganglion cells in the cat. The response plane graphically represents the firing probability of a cell as a function of space and time. By varying the stimulus on-time from 10-500 msec, the spatial distribution of the receptive field components could be directly demonstrated.

For Y- cells it was found that with short stimulus on-times (10-80 msec) the sustained component of the center response (excitation and inhibition) disappears. The surround response (excitation and inhibition) can then be seen to extend into the center of the field, and importantly it is temporally delayed with respect to the center response. There is very little variation in the spatial extent of the field with different stimulus on-times.

For X- cells studied with short stimulus on-times (10-30 msec) it was found that the surround response (excitation and inhibition) also extends into the center of the field but in contrast to Y-cells, it has the same latency as the center response (excitation and inhibition). The spatial extent of X- receptive fields was found to decrease progressively with shorter stimulus on-times. That is, the organization of the field components and their relationship in space and time remains constant, but they are all proportionately smaller.

We have shown that the surround response in both X- and Y-cells is present in the center of the field. For Y- cells the surround response is temporally delayed with respect to the center response while for X- cells the center and surround responses have essentially the same latency. We interpret the decrease in the spatial extent of X- receptive fields with short stimulus on-times as evidence for a systematic decrease in the sensitivity of the receptive field components from the center to the periphery of the field. In contrast, the fact that the spatial extent of Y-fields is nearly constant with decreasing stimulus on-times appears to reflect a relatively flat sensitivity distribution of the receptive field components across space.

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81.12 THE SYNAPTIC BASIS OF CENTER-SURROUND ANTAGONISM IN THE MUDPUPPY RETINA. T. E. Frumkes and P. A. Coleman*. Dept. Psych., Queens College of CUNY, Flushing, New York 11367.

The synaptic mechanisms underlying the receptive field (RF) organization of mudpuppy retinal neurons was assessed by means of intracellular recording and current injection. Under our cone-dominated conditions, photic stimulation of the RF center of hyperpolarizing bipolar cells (HPBCs) produces a hyperpolarization accompanied by a resistance increase: if the RF center is continuously adapted, surround stimulation produces a depolarization accompanied by a resistance decrease (commonly, diffuse stimulation produces little measured change in input impedance). Previous evidence suggests 1) photoreceptors (Rs) release neurotransmitters maximally in the dark, 2) horizontal cells (HCs) mediate bipolar cell receptive fields, and 3) that there is no feedback onto mudpuppy Rs. Collectively, the foregoing indicates that the R and HC released neurotransmitters cause, respectively, a conductance increase and decrease in HPBCs. In contrast, photic stimulation of the RF center of depolarizing bipolar cells (DPBCs) produces a depolarization accompanied by a resistance decrease: if the RF center is continuously adapted, surround RF stimulation produces a hyperpolarization accompanied by a resistance increase. These results suggest that the R and HC released neurotransmitters cause, respectively, a conductance decrease and increase in DPBCs.

Most ganglion cells (GCs) demonstrate transient IPSPs at light onset and offset which can be attributed to neurons with no obvious center-surround antagonism (i.e., amacrine cells). Receptive fields obtained with sustained photic stimuli, however, suggest that the spatial organization of "on" and "off" responses within GCs can be attributed respectively to the DPBC and HPBC, both of which release excitatory neurotransmitters when depolarized. Thus, the depolarization and decrease in resistance produced by stimulating the RF center of "on" GCs becomes smaller if the surround is additionally stimulated. In "off" GCs, the disfacilitation (hyperpolarizing response component involving a resistance increase and a positive reversal potential) produced by stimulating the RF center becomes smaller with additional stimulation of the surround. Similarly, the RF organization of excitatory responses of some on-off GCs can be attributed to an excitatory input from both bipolar types.

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82.1 LEYDIG CELLS: AN ELECTRICAL NETWORK WITHIN THE SEGMENTAL C.N.S. OF LEECH. K.T. Keyser* and C.M. Lent. Neurobiol. SUNY, Stony Brook 11794 and Neurosci. Brown Univ., Providence, RI 02912.

Each of the 21 segmental ganglia in the leech Macrobdella decora contains two Leydig cells which can be identified by their position and electrophysiological characteristics. They exhibit "spontaneous" action potentials (1 to 10 per sec) and the two cells within each ganglion are electrically-coupled ($V_2/V_1 = 0.3$). Leydig cells in adjacent ganglia are mutually-excitatory, such that an impulse in one causes an impulse in both ipsilateral Leydig cells in adjacent ganglia. Interganglionic latency is constant for any cell pair and is unchanged by 20 mM Mg^{++} saline. This interganglionic latency is constant regardless of the direction of impulse conduction, and when assessed with results of "collision" experiments, we conclude that a common axonal pathway mediates these ipsilateral interactions. If a Leydig cell is driven, with intracellular currents, to fire impulses at frequencies of six to ten per sec, ipsilateral cells in adjacent ganglia are unable to follow in a one to one fashion and instead exhibit impulse failures.

These intra- and inter-ganglionic properties are consistent with the observed branching patterns of Leydig cells injected with HRP. Each cell projects to adjacent ganglia via its ipsilateral connectives and there sends processes to the periphery via lateral roots. Furthermore, the injection of Lucifer Yellow reveals dye coupling between paired ganglionic Leydig cells as well as between ipsilateral interganglionic cells. The combination of spontaneous activity, intraganglionic coupling and interganglionic interactions appears to generate a constant, low frequency of impulse activity within the Leydig cell network, and therefore within their processes in the periphery. The efferent impulse activity of these neurons is always conducted by the roots of adjacent ganglia and it has some unexpected properties. We have not been able to discern a sensory or motor function for these identifiable neurons. (Supported by NIH grant NS-14482 to C.M.L.)

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82.2 HYDROSTATIC PRESSURE REDUCES SYNAPTIC EFFICIENCY BY BLOCKING PRESYNAPTIC CALCIUM INFLUX. J.L. Parmentier, L.A. Orr* and P.B. Bennett. F.G. Hall Environmental Laboratory, Duke Univ. Medical Center, Durham, North Carolina, 27710.

Hydrostatic pressure has been studied on the naturally evoked responses of an identified synapse in the abdominal ganglion of Aplysia californica while iontophoresing acetylcholine onto the soma of the postsynaptic cell. 100 ATM of pressure reduced by 66.5% (S.D. 4.5%, n=10) the amplitude of the EPSP recorded in neuron R15 after en passant stimulation of the right visceropleural connective. There was no change in the synaptic latency, the time-to-peak or the time constant of decay of the postsynaptic response. The same pressure did not alter either the amplitude or the kinetics of the excitatory ACh iontophoretic response on R15 and did not affect either Cl- or K+ dependent inhibitory responses to applied ACh on other ganglion cells. These results strongly suggest that the effect of pressure is on some presynaptic mechanism.

In order to determine if pressure is altering aspects of transmitter storage or mobilization the RCl-R15 synapse was stimulated for 60 sec at 0.5, 1 and 2 Hz. Compression to 100 ATM reduced the size of all stimulated EPSPs but did not affect the relative heights of synaptic depression, frequency facilitation and posttetanic potentiation which occur at this synapse. However, when the amplitude of the EPSP was increased by raising the level of calcium in the bathing sea water from 10mM to 15 mM the application of pressure reduced the height of the EPSP back to control values. In six experiments 100 ATM of pressure was sufficient to return the amplitude from 208% due to high calcium to 110% of control. Previous studies have shown that pressure reduces the amplitude of the slow inward current in R15 which underlies burst behavior in this cell and is known to be carried predominantly by calcium ions (Parmentier et.al. Undersea Biomed. Res. 6:75-91, 1979). Similar studies on other Aplysia neurons and the squid giant axon have shown only slight effects of pressure on resting membrane potential. We conclude that pressure exerts its effects on synaptic efficiency by interfering with the presynaptic calcium influx in RCl-R15 required for synaptic transmission. Pressure also reduces the amplitude of the response at the inhibitory synapse RCl-L11. Inhibition by pressure of inhibitory synapses in the central nervous system could explain the pressure induced hyperexcitability syndrome experienced by whole animals, including man, during deep diving.

82.3 EFFECT OF LITHIUM ON CONDUCTANCE INCREASES TO SEVERAL TRANSMITTERS IN APLYSIA. A. M. Williamson*, D. O. Carpenter and T. C. Pellmar. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Lithium is an element important in treatment of manic-depressive illness, and its mode of action is of considerable interest. It is also an important tool for understanding the physiology of ion conducting channels and their receptors, since lithium shares the same chemistry as sodium (Na) and potassium (K) while being physically smaller. It is against that background that we have investigated the effect of lithium on conductance increase mechanisms in Aplysia californica.

In these studies lithium was used as a total replacement for Na in artificial seawater (Li-seawater). In most of the experiments the cells were never exposed to Li-seawater for more than 30 min because irreversible changes in the current-voltage relationship occurred with prolonged exposure. Li-seawater reduced the rapid increase in Na conductance elicited by the iontophoretic application of ACh in RB cells of the abdominal ganglion and of DA and serotonin in neurons of the buccal ganglion. Chloride conductance increases in medial pleural cells resulting from iontophoretic application of ACh, glutamate, and GABA were also reduced in Li-seawater. The change in amplitude of K conductance increase to ACh in medial cells generally paralleled changes in the input resistance as measured by injection of hyperpolarizing current pulses.

$MgCl_2$·mannitol was also used as a substitute for Na, and its effects on iontophoretic responses were compared with those of Li-seawater. $MgCl_2$·mannitol seawater completely abolished Na responses and effected a transient decrease in chloride responses.

These results reveal a difference between the effects of Na replacement by lithium and replacement by $MgCl_2$·mannitol. Those differences may reflect the pharmacological action of lithium.

82.4 SUBTHRESHOLD OSCILLATIONS UNDERLIE THE cAMP-INDUCED DISCHARGE OF APLYSIA PEPTIDERGIC NEURONS. L. K. Kaczmarek and F. Strumwasser. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The peptidergic bag cell neurons in the abdominal ganglion of Aplysia californica respond to brief synaptic stimulation or to certain cAMP analogs by generating a long lasting afterdischarge (30 min) of synchronous action potentials. We have used bag cells that have been dissociated from one another and maintained in primary culture to investigate the mechanism of this repetitive discharge (Strumwasser et al. Soc. Neurosci. Abstr. 4, 207, 1978; Kaczmarek et al. Soc. Neurosci. Abstr. 5, 249, 1979). We have found by intracellular recording from such isolated cells that sustained subthreshold oscillations, enduring for several hours, can be generated in response to the cAMP analog 8-benzylthio cAMP (8BT-cAMP). The two methods that have been used for the application of 8BT-cAMP to bag cells are extracellular addition to the culture medium (0.5 mM) and intracellular application by pressure injection from microelectrodes (tips 0.5-1.5 µm) containing 20 mM 8BT-cAMP in .3 M KCl. The mean latency to the onset of oscillations following extracellular application of 8BT-cAMP was 27 min (N=14) and their occurrence was associated with the onset of a repetitive discharge and enhanced spike broadening. The oscillations, however, persisted following the end of the discharge and reached peak-to-peak amplitudes about 10 mV. They remained unaffected by 5 x 10^{-5} M tetrodotoxin but could be abolished by extracellular addition of $CoCl_2$ (12 mM). In two experiments in which bag cells were penetrated with 8BT-cAMP-containing-micropipettes the oscillations reached a steady amplitude (4.5 mV) and frequency (~1/sec) shortly following penetration which endured for over an hour, presumably due to 8BT-cAMP leakage from the micropipette. These cells were used to examine the voltage sensitivity of the oscillations. Small hyperpolarizing currents diminished the amplitude and lowered the frequency while larger hyperpolarizing currents abolished the oscillations. Depolarizing currents increased both the amplitude and frequency. When repetitive firing was induced by depolarizing currents the frequency of firing was the same as that of the underlying oscillations which persisted even after adaptation of the firing rate. It is likely, therefore, that these oscillations underlie the mechanism of discharge induced by 8BT-cAMP in bag cells. In experiments in collaboration with U. Walter, F. D. Wilson and P. Greengard we are injecting the catalytic subunit of cAMP dependent protein kinase into cultured bag cells to determine if subthreshold oscillations, spike broadening and other membrane effects of 8BT-cAMP are mediated by protein phosphorylation. [This work was supported by NIH grant NS 13896.]

82.5 INVOLVEMENT OF CALCIUM CHANNELS IN SHORT-TERM DESENSITIZATION OF RECEPTOR-MEDIATED CYCLIC GMP FORMATION IN MOUSE NEUROBLASTOMA CELLS. E. El-Fakahany* and E. Richelson. Dept. of Psychiatry and of Pharmacology, Mayo Foundation, Rochester, MN 55901.

Mouse neuroblastoma clone N1E-115 cells have muscarinic and histamine H_1 receptors that mediate cyclic GMP formation, an effect which is dependent upon extracellular Ca^{2+}. This cyclic GMP response undergoes rapid and largely specific desensitization upon exposure of cells to high concentrations of agonist (Richelson, E., Nature, 272:366, 1978; Taylor, J. E. and Richelson, E., Molec. Pharmacol., 15:462, 1979), without any significant loss in receptor sites (Taylor, J. E., El-Fakahany, E., and Richelson, E., Life Sci., 25:2181, 1979), or guanylate cyclase specific activity (El-Fakahany, E. and Richelson, E., unpublished data). Incubation of cells with manganous chloride resulted in a rapid and transient increase in cyclic GMP formation. This effect was dependent upon the concentration of Mn(II) with an EC_{50} of about 100 μM. The manganese response progressively increased with increasing external Ca^{2+} concentration and reached its maximum at 6-8 mM Ca^{2+}. In the absence of Ca^{2+}, the effect of manganese was abolished. The Mn(II) effect was antagonized by verapamil but not by atropine or pyrilamine, suggesting that Mn(II) increases Ca^{2+} influx without interacting with the muscarinic or histamine receptors. When cells were desensitized to carbachol, histamine or both agonists there was a marked decrease in the ability of Mn(II) to stimulate cyclic GMP formation (see table). We conclude that short-term desensitization of the receptor-mediated cyclic GMP responses in these cells is associated with a state in which the calcium entry mechanisms are inactivated.

TABLE

Condition	% Control Cyclic GMP Formation Stimulated By: Carbachol (1 mM)	Histamine (1 mM)	Mn(II) (1 mM)
Cells desensitized with 1 mM carbachol (30 min)	0	82	48
Cells desensitized with 1 mM histamine (30 min)	87	0	56
Cells desensitized with both agonists (1 mM each, 30 min)	0	0	17

(Supported by Mayo Foundation and USPHS Grants MH 27692 and DA 1490.)

82.6 METHYLATION OF MEMBRANE PHOSPHOLIPIDS IN STRIATAL SYNAPTOSOMES R. E. Boehme* and R. D. Ciaranello (SPON: S. M. Stahl). Lab. of Developmental Neurochemistry, Dept. of Psychiatry, Stanford Univ. Sch. Med., Stanford, CA 94305.

The relative concentrations of several membrane phospholipids are important determinants of erythrocyte membrane fluidity and beta-adrenergic receptor levels. Alterations of phospholipid concentrations can be induced by beta-adrenergic agonists, and are mediated by S-adenosylmethionine-dependent methyltransferase reactions. The present studies were undertaken to determine if similar processes exist in dopaminergic systems of rat brain.

Purified synaptosomes were rapidly prepared from rat striata and preincubated 30 minutes at 4° with 0.2 mM [3H]S-adenosylmethionine (SAM). During this period, SAM was rapidly and actively taken up to produce a stable level of free intrasynaptosomal SAM. Subsequent incubation of the synaptosomes at 37° induced the incorporation of tritium into phospholipids. This reaction proceeded linearly for 30 to 40 minutes.

Inclusion of dopamine in the incubation medium resulted in a dose-dependent stimulation of phospholipid methylation. A maximal stimulation of 4-fold was achieved with 0.3 mM dopamine. Neither norepinephrine, epinephrine, nor acetylcholine stimulated methylation at any concentration. Following dopamine stimulation under the above conditions, the major methylated phospholipid was phosphatidylcholine. Levels of tritiated phosphatidylmonomethylethanolamine and phosphatidyldimethylethanolamine were not significantly altered by dopamine. Stimulation by dopamine was only partially blocked by either haloperidol or cis-flupenthixol. This suggests that non-receptor-mediated processes are at least partially responsible for the observed dopamine-stimulated phospholipid methylation.

82.7 THE ROLE OF RNA POLYMERASE IN THE β-ADRENERGIC RECEPTOR-MEDIATED INDUCTION OF CYCLIC AMP PHOSPHODIESTERASE IN C6 GLIOMA CELLS. J. P. Schwartz, P. Onali* and E. Costa. Lab. Preclin. Pharmacol., NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

In C6 glioma cells, the activation of the β-adrenergic receptors causes a sequelae of events, including the rise in cyclic AMP content, the activation of cytosolic cAMP-dependent protein kinase and the translocation of the catalytic subunit of protein kinase into the nucleus. This cascade of events causes several long-term changes including the increase in activity of a molecular form of cyclic nucleotide phosphodiesterase. Chromatography of C6 glioma cell extracts on a DEAE-Sephacel column separates two forms of phosphodiesterase, which differ in their substrate specificity and Ca^{2+} activation characteristics. The activity of the form which hydrolyzes only cyclic AMP is specifically increased following the activation of β-adrenergic receptors.

This increase of phosphodiesterase is blocked by cycloheximide, and by actinomycin D, suggesting that new synthesis of protein and mRNA is required. RNA polymerase II, the enzyme responsible for mRNA synthesis, can be measured in nuclei prepared from C6 glioma cells. This enzyme requires Mn++ (150-200 mM). Its activity is proportional to the amount of nuclear protein (from 25-200 μg protein) added, and is linear with time for 20 to 30 min. The polymerase activity is inhibited in vitro by either actinomycin D (53% inhibition at 24 μM) or α-amanitin (78% inhibition at 1.1 μM). Incubation of intact cells with either actinomycin D (3.2 μM) or α-amanitin (2.7 μM) blocks the isoproterenol-stimulated induction of the phosphodiesterase without affecting basal activity. Neither of these drugs blocks protein kinase translocation into the nucleus, further supporting the idea that both actinomycin D and α-amanitin block phosphodiesterase induction by inhibiting RNA polymerase II in the cell nucleus. An isoproterenol treatment of the cells which induces phosphodiesterase fails to increase the RNA polymerase II activity of nuclei, presumably because isoproterenol treatment induces a small number of proteins and the limited increase in the RNA polymerase II activity required is below the limits of detection.

82.8 A QUANTITATIVE STUDY OF DOPAMINE IN SUPERIOR CERVICAL GANGLIA OF THE RABBIT. A.G. Karczmar, A. Ingerson* and N.J. Dun. Dept. of Pharmacol. Loyola Univ. Stritch Sch. Med. Maywood, IL 60153.

The content of dopamine (DA) in superior cervical ganglia (SCG) of the rabbit was measured quantitatively under various experimental conditions by means of a sensitive HPLC method. The mean DA content of ganglia freshly removed from the animals and frozen immediately in dry ice was 2.08 ± 0.53 μg/g tissue (n=7, mean ± S.D.). When ganglia were placed in oxygenated Krebs solution for 1-2 hr, the level of DA decreased to about 20% as compared with the immediately frozen ganglia. Unilateral preganglionic denervation after 7-14 days reduced the DA content to about 25% compared to that of the contralateral intact ganglia. Exposure of the ganglia for 5-10 min to high KCl (50-100 mM) Krebs solution did not cause any apparent change in the DA content as compared with ganglia exposed to Krebs solution for the same duration. The DA content of the ganglia removed from animals injected with reserpine, 2 mg/kg for 1 day, was not statistically different from ganglia removed from animals injected with saline. Pretreatment of the rabbits with an irreversible inhibitor of aromatic amino acid decarboxylase, α-monofluoromethyldopa, 250 mg/kg for 1 day or 50 mg/kg for 3 days, did not significantly lower the DA content in the SCG (MFMD, courtesy of Centre de Recherche Merrell International). As measured by the sucrose gap method, the amplitude of positive (P) potential recorded from the surface of ganglia removed from rabbits treated with reserpine or MFMD was not statistically different from that of the ganglia removed from animals injected with saline. The DA level of the ganglia superfused with bethanechol (0.6 mM) for 20-25 min, which abolished the P as well as the late negative potential as demonstrated in the sucrose gap recording, showed no statistical difference from that of the ganglia superfused with Krebs solution. Our results show that the ganglionic content of DA is not altered significantly by amine depletors, reserpine or MFMD, and is resistant to a muscarinic agonist, but is markedly reduced by preganglionic denervation or superfusing the ganglia with Krebs solution for 1-2 hr, and that there is no close correlation between ganglionic content of DA and the amplitude of P potential. (Supported in part by NINCDS Grants NS15848 and NS06455).

82.9 A NEUROCHEMICAL AND NEUROPHYSIOLOGICAL STUDY OF SEROTONIN IN THE SUPERIOR CERVICAL GANGLIA OF THE RABBIT. N.J. Dun, A. Ingerson* and A.G. Karczmar. Dept. of Pharmacol., Loyola Univ. Stritch School Med. Maywood, IL 60153.

Serotonin (5-HT) was determined quantitatively in rabbit superior cervical ganglia (SCG) by means of a sensitive HPLC method. The mean content of 5-HT in immediately frozen ganglia was 0.216 ± 0.08 μg/g tissue (n=6, mean ± S.D.). Superfusing the ganglia with Krebs solution for 1-2 hr, or sectioning of the preganglionic sympathetic trunk for 7-14 days did not significantly alter the content of 5-HT. Exposure of the ganglia to high KCl (50-100 mM) Krebs solution for 5-10 min caused a 34% decrease of the 5-HT content as compared with ganglia exposed to Krebs solution for the same duration. Reserpine (1 mg/kg for 1 day) reduced ganglionic content of 5-HT to about 50% of that of the ganglia removed from animals injected with saline. Perfusing the ganglia with a muscarinic agonist, bethanechol (0.6 mM) for 30 min did not appreciably affect the 5-HT content. The electrophysiological effects of 5-HT on single neurons were studied using intracellular recording techniques. Superfusing the ganglia with 5-HT (1-100 μM) caused, in the majority of neurons, a membrane depolarization of low amplitude and short duration; the depolarization was accompanied in most cases by a reduction in input resistance. The ganglion cells showed rapid desensitization to the depolarizing effect of 5-HT. In the concentrations of 10-100 μM, 5-HT effectively suppressed and blocked the fast-epsp or the synaptic action potential even in neurons where depolarization and/or change in membrane resistance was small and/or absent. In several neurons, 5-HT (10 μM) reduced the amplitude of fast-epsp without altering appreciably the resting membrane potential or the response of ACh potential elicited by iontophoretic application of ACh to the surface of the ganglion cells. The present study shows that a substantial quantity of 5-HT is present in the SCG of the rabbit, and that it is most likely to be postsynaptic in origin. The action of 5-HT on sympathetic neurons appears to be complex, in addition to the postsynaptic depolarizing effect, 5-HT may inhibit synaptic transmission by reducing the amount of ACh release from preganglionic nerve fibers. (Supported in part by NINCDS Grants NS15848 and NS06455).

82.10 EFFECTS OF TRH, NE, AND ACh ON RAT HYPOTHALAMIC THERMOSENSITIVE NEURONS. Steven K. Salzman* and Alexander L. Beckman (SPON: T.L. Stanton). Alfred I. duPont Institute, Wilmington, DE 19899.

Thyrotropin releasing hormone (TRH) has been shown to reverse the hypothermia induced by a variety of centrally-acting agents, to exert direct hyperthermic effects on body temperature (T_b) following intracranial application, and to alter the firing rate of neurons in several regions of the CNS. In view of this, we have examined the effects of TRH on thermosensitive neurons in order to determine if TRH-induced changes in T_b can be attributed to its action on these functionally-identified cells. The responses of these neurons to TRH were compared to those obtained with norepinephrine (NE) and acetylcholine (ACh).

Sprague-Dawley rats were anesthetized with urethane (1gm/kg, i.p.) and placed in a stereotaxic instrument. Two bilateral, water-perfused, stainless steel thermodes, implanted rostral to the preoptic/anterior hypothalamus (POA), were employed to vary hypothalamic temperature (T_h) from 32-42°C. T_h was monitored by a bead thermistor inserted into a stainless steel re-entrant tube implanted in the POA to the left of midline, symmetrical with the contralateral POA recording site. Recordings of extracellular single unit discharges and iontophoretic application of test compounds were made with 5-barrel glass micropipettes.

Of 107 neurons that were tested for their response to changes in local temperature, 31 of 66 temperature-insensitive (TIS), 12 of 29 warm-sensitive (WS), and 9 of 12 cold-sensitive (CS) neurons were tested with at least one of the three compounds and are described here.

WS neurons were inhibited by TRH (6 of 11), inhibited by NE (8 of 10), and excited by ACh (6 of 12). CS neurons were mildly inhibited by TRH (5 of 7), excited (1 of 6) or inhibited (4 of 6) by NE, and inhibited by ACh (2 of 5). TIS neurons were inhibited by TRH (16 of 31), excited (3 of 22) or inhibited (15 of 22) by NE, and excited (5 of 20) or inhibited (1 of 20) by ACh. Only inhibitory effects of TRH were observed on each class of neurons. In addition, 4 of 6 WS, 3 of 3 CS, and 11 of 11 TIS neurons that were inhibited by TRH were also inhibited by NE. By contrast, no such parallel was noted between the effects of TRH and ACh.

Considering the higher proportion of WS versus CS cells commonly reported in the POA and the relatively more pronounced inhibitory effect of TRH on WS versus CS cells reported here, these data suggest that increases in T_b following intraventricular or intrahypothalamic administration of TRH are mediated by its inhibitory action on warm-sensitive neurons. (Supported by the A.I. duPont Institute and NSF grant BNS 78-19002.)

83.1 STRETCH REFLEXES IN NORMAL HUMAN TRICEPS SURAE. C. Kaufman*, A. Berardelli*, M. Hallett*, E.J. Fine*, B.J. Linder*, D. Loo*, P. Kamitsuka*, J. Lazarre*, S.R. Simon* and W. Berenberg*. (SPON: H.R. Tyler). Gait Lab., Children's Hosp. Med. Ctr., Boston, MA 02115.

Stretch of an actively performing muscle usually produces several reflex responses. Previous studies of the triceps surae have revealed only a single significant response, presumably the monosynaptic reflex, prior to the onset of voluntary activity (Gottlieb, G.L. and Agarwal, G.C., J. Neurophysiol. 42:91, 1979). We have studied this muscle group further with slower rates of stretch and have identified additional longer-latency reflex components. Subjects sat in a chair and pressed their foot against a pedal attached to a torque motor. The ankle was dorsiflexed at various velocities and EMG from the triceps surae was recorded. The motor produced perturbations with maximal velocities of 100 to 200 °/sec at 50-100 msec into the movement.

When the ankle was at rest only a single response was seen. This response increases in amplitude with increasing velocity of stretch, is not altered by voluntary attempts to reduce or augment it, but is significantly reduced by vibration of the Achilles tendon.

When the ankle is stretched while the foot is pressing down on the pedal, an early response is followed by one and sometimes two later responses. The early response is similar in latency and behavior to the single response seen without background force. The first of the two long-latency responses has a stable latency, is often larger in magnitude than the early response and increases in amplitude with increasing velocity of stretch, often showing less pronounced increase at the faster velocities. The second of the long latency responses was present in 30% of subjects, had a greater amplitude and probability of occurrence at slower velocities and a shorter latency with faster velocities. The three responses were not modifiable by intent, but were suppressed by vibration of the Achilles tendon.

The behavior of these long-latency responses is different from that of long-latency responses in upper extremity muscles and indicates that there are a variety of mechanisms for long-latency stretch reflexes.

83.2 FURTHER EVIDENCE THAT THE GOLGI TENDON ORGAN MONITORS THE ACTIVITY OF A DISCRETE SET OF MOTOR UNITS WITHIN A MUSCLE. Marc D. Binder. Dept. of Physiol. & Biophys., Univ. of Wash, Seattle, Washington 98195.

Direct confirmation of the Houk and Henneman (J. Neurophysiol. 30: 466-481, 1967) model of tendon organ activation requires studies in which both a tendon organ and the muscle fibers of a motor unit which excites or unloads it are "marked" and their anatomical relationship reconstructed histologically. However, in the absence of these data the model can still be tested by considering its implicit predictions. If it is true that the motor units which excite a tendon organ do so because one or more of their fibers insert directly into the receptor capsule and that only such "in-series" fibers can provide an adequate stimulus, then the combined contraction of all the remaining motor units should be incapable of exciting the receptor regardless of the amount of force generated in the muscle. Moreover, since there are some motor units which can unload a given tendon organ, the receptor's response to whole muscle contraction should be less vigorous than its response to contraction of just the in-series units alone. The present study was designed to test these predictions by comparing the responses of tendon organs in cat soleus to stimulation of different groups of subdivided ventral root filaments. In general, the results support the Houk and Henneman model. It was found that when all the ventral root filaments containing motor units which excited a given tendon organ were separated out, concommitant stimulation of all those remaining failed to excite the receptor, even when these filaments accounted for over 90% of the muscle's peak tetanic tension (10/11 experiments). Moreover, a tendon organ's response to stimulation of just those filaments which contained excitatory motor units often exceeded that to whole muscle stimulation. Exceptions to the latter result indicate that the discharge of some tendon organs may be influenced by "off-line" forces. (Supported by NINCDS Grants NS 15404 and NS 00345, BSR Grant RR 05432 and a GSRF Grant from U. of Washington)

83.3 MODULATION OF LATE STRETCH REFLEXES DURING A CYCLIC ELBOW MOVEMENT. W. A. MacKay, H. C. Kwan, J. T. Murphy and Y. C. Wong*. Dept. of Physiology, Univ. of Toronto, Toronto Ont. M5S 1A8 Canada

Human subjects, with right forearm fixed in a manipulandum, performed a continuously repeated horizontal elbow movement between two target zones. The movement cycle consisted of EXTENSION-HOLD-FLEXION-HOLD over a period of 2 seconds. During the cycle, stretch reflex efficacy was tested by delivering controlled ramp displacements to the forearm with a torque motor, and monitoring EMG responses. Each 50 msec phase of the movement was tested with small pulses in both directions. Biceps and triceps EMG were recorded with skin electrodes, then rectified and integrated. No observable monosynaptic reflexes were elicited by the stimulus. Prominent late reflexes appeared in certain phases of the cycle, at latencies of 35-65 msec (M2) or 65-120 msec (M3). Neither was usually present at rest. The M3 reflex appeared in the agonist muscles about 150-350 msec prior to active contraction. An M2 response appeared just before onset of the active contraction, leading it by only 50-150 msec. Under static conditions, reflex amplitudes were simply related to background EMG levels in the tested muscle up to a saturation level. But during the movement cycle, reflex amplitudes were uncorrelated to the simultaneous level of EMG activity. They were greatest in the preparatory period and dropped once the muscle started actively contracting. During the deceleratory phase they were briefly restored, then disappeared. A measure of relative change in central gain of the stretch reflexes exclusive of motoneuronal excitability was calculated by dividing reflex responses by concurrent EMG background (obtained in trials with no torque pulses) and comparing the quotients to those obtained at similar EMG levels but under static conditions. The results, as a function of cycle phase, suggested that channels utilizing proprioceptive information are important for the initiation of dynamic phases of movement.

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83.4 MYOTATIC REFLEX CONTRIBUTIONS TO LOAD COMPENSATION AND STABILITY. Gerald L. Gottlieb, Gyan C. Agarwal, and Robert J. Jaeger, Department of Physiology, Rush College of Health Sciences, 1753 W. Congress Parkway, Chicago, Illinois. 60612.

A blood pressure cuff at the thigh was used to occlude blood flow to the leg while torque pulses dorsiflexed the foot of normal human subjects. The subjects were instructed to resist the pulses and restore the foot to its original position as rapidly as possible. (For details see Gottlieb and Agarwal, J. Neurophysiol., 1979 & 1980).

About 20 minutes after inflation of the cuff to 150 mm hg, the soleus myotatic reflex (latency of about 40 ms) vanishes. (Independent measurements of the Hoffmann reflex show it to disappear after the same delay). Associated with this disappearance, we observe a reduction in amplitude of the earliest portions of the post-myotatic EMG (latency of about 120 ms) and a slowing of the corrective movement which brings the foot back to its original position. Analysis of the distribution of post-myotatic latencies shows an increase in both its variance and mean; not a simple shift to longer latencies. The fastest post-myotatic responses occur at the same latency, even in the absence of the myotatic reflex.

Linear systems analysis of the mechanical behavior of the foot suggests that the myotatic reflex may not act to stabilize the joint. Computation of the transfer function between torque and angular rotation leads to the conclusion that the joint has less damping when the reflex is intact than when it is not. This, and the finding that ultimate correction of foot position is slowed, leads to a hypothesis that local feedback loops provide fast, fine control to the final common pathway to quicken a response, even at the cost of stability under some circumstances. (Supported by NIH Grants NS-12877 NS-12877 and NSF Grant ENG-7608754)

83.5 LONG-LATENCY STRETCH REFLEX OF THE HUMAN THUMB PRIOR TO THE INITIATION OF VOLUNTARY MOVEMENT. M.Hallett* and C.D.Marsden*. (SPON: E.T.Hedley-Whyte). Department of Neurology, Institute of Psychiatry, London, England.

Electroencephalographic events such as the contingent negative variation and bereitschaftspotential have suggested that there are changes in cortical neuronal firing during the second prior to the initiation of voluntary movement. Recordings of single cell activity in monkey cortex have apparently confirmed this suggestion. Long-latency stretch reflexes in the long thumb flexor can be produced when the muscle is engaged in voluntary movement, but not usually when the muscle is relaxed. Experiments were undertaken to see if the long-latency stretch reflex would be enhanced in this one-second period just prior to voluntary movement.

Subjects placed the distal phalanx of one thumb on a lever attached to the spindle of a torque motor. They were told to relax the thumb until they heard a high tone, after which they were to make a rapid flexion movement. One second (or in some experiments 0.5 sec.) prior to the high tone they heard a low tone which was a reliable warning about the coming of the high tone. In the first experiment responses to stretches were compared at various times during the interval between the low and high tones. In 3 of 5 subjects the stretch reflex appeared to increase in amplitude as the stretch got closer to the voluntary movement. Because results were not consistent a second experiment was done carefully comparing stretch between a low and high tone and stretch with thumb relaxed without tones (along with controls for each of these two conditions without stretches). In only 5 of 17 subjects was the reflex before voluntary movement larger than the reflex without preparation for voluntary movement. These subjects could not relax completely and baseline EMG activity was often greater with the tones than without them. Subjects who relaxed well had no reflex in either circumstance, while subjects who relaxed poorly had a large reflex which did not vary significantly. A gradual increase in baseline EMG activity between the tones would appear to be responsible in those trials in the first experiment which exhibited growth of the stretch reflex.

In this period prior to the initiation of voluntary movement there appears to be no stretch reflex unless there is background EMG activity and no change of the stretch reflex other than that which can be related to subtle changes in background EMG activity. Either the long-latency stretch reflex does not mirror the cortical changes prior to movement or the cortical changes themselves are a direct correlate of the background EMG changes.

83.6 SPINDLE PRIMARY RESPONSES TO SUDDEN DISPLACEMENTS OF THE HUMAN WRIST. K-E. Hagbarth*,R.R. Young,J.V. Hägglund* and E.U. Wallin* Department of Clinical Neurophysiology, University Hospital, Uppsala, Sweden, and Clinical Neurophysiology Laboratory, Massachusetts General Hospital, Boston, MA 02114

An abrupt angular displacement at a joint, stretching voluntarily contracting muscles, produces segmentation of the EMG. Three discrete EMG bursts are usually seen, the 1st of which represents the spinal stretch reflex. The 2nd and 3rd have been considered "longloop reflexes" passing through cerebral structures including motor cortex. A single afferent input at the start of stretch was assumed to produce all 3 EMG bursts. In order to record the muscle afferent response to such displacements, intraneural tungsten microelectrode recordings (microneurography, Vallbo et al., Physiol. Rev., 59: 919-957, 1979) of single or multi unit activity were made from median or radial nerve fascicles in 17 experiments in 3 normal human subjects. Flexion or extension movements at the wrist were produced by several devices including a torque motor.

With angular displacements exceeding 200 deg/sec and amplitudes 10-20 deg, segmentation of muscle afferent activity was always recorded. In addition to bursts of primary spindle activity following the sudden start and stop of abrupt angular displacements, one or two more bursts of spindle impulses occurred during the stretch movement itself. This segmented afferent response,from primary endings which are remarkably sensitive to mechanical perturbations during muscle stretch, may be due to fluctuations in the velocity of stretch resulting from previously described non-linear viscous properties of muscle. Such irregularities appear inevitable even when smooth torque pulses produce the stretch.

This segmented afferent input following abrupt limb displacements is followed, at latencies compatible with operation of segmental stretch reflex mechanisms, by segmented EMG activity. Even with much smaller displacements than noted above, it is difficult to produce only one burst of afferent activity. When that is accomplished, only one burst of EMG activity is seen. Though they do not disprove the presence of "longloop reflexes", our observations document difficulties inherent in assumptions about reflex pathways which are based only on timing of EMG responses to a presumably simple stretch stimulus. These results provide evidence in man for Ghez and Shinoda's hypothesis, derived from studies of spinal cats, (Exp. Brain Res., 32: 55-68, 1978) that "receptor properties and/or spinal mechanisms involved in the stretch reflex are sufficient to produce a segmented (EMG) response".

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83.7 EFFECT OF DANTROLENE SODIUM ON THE DISCHARGE OF PRIMARY SPINDLE AFFERENTS DURING FUSIMOTOR STIMULATION. Petit, J.E.*, Cameron, W.E., and Murthy, K.S.K., Division of Neurosurgery, University of Texas Medical School, Houston,Texas 77030.

The excitation-contraction coupling process in a muscle may be affected by the drug dantrolene sodium (Dantrium^{R}, Norwich-Eaton) which has been suggested to interfere with the release of calcium from the sarcoplasmic reticulum (Desmedt and Hainaut, J. Physiol. 265: 565-585, 1977). In a recent study (Murthy, Jami, Petit and Zytnicki, Fed. Proc. 39: 579, 1980), it was shown that the action of dantrolene is predominantly on the faster contracting muscle fibres of the FF and FR motor units. The muscle fibres of the slow twitch type S motor units were comparatively resistant to the action of this drug. The present experiments extend the observations to effects on intrafusal muscle fibres as inferred from a study of muscle spindle Ia afferent responses to stimulation of gamma fusimotor axons.

Experiments were performed in 9 cats (2.0 - 4.0 kg) anesthetized with sodium pentobarbital. The left hindlimb was completely denervated except for the nerve to the peroneous tertius muscle. The dorsal and ventral roots of L6, L7 and S1 were sectioned. Single spindle Ia afferents were functionally isolated from split dorsal root filaments and single fusimotor efferents were likewise isolated from split ventral root filaments. The discharge of Ia afferents was recorded in response to the tetanic stimulation (75 pps for 3 seconds) of single gamma efferents before and after the administration of the drug. A 3mg/kg dose of dantrolene sodium (dissolved in propylene glycol 3mg/ml) was injected I.V. at a rate of 2ml/min.

A total of 113 fusimotor effects due to 61 gamma axons acting on 59 Ia afferents were studied. Effects of the drug consisted of (1) a small reduction in the mean level of discharge during maintained tetani, (2) a reduction in the variability of the mean discharge, and (3) a slowing of the rise of the spindle response at the onset of fusimotor stimulation.

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83.8 THE NECK MUSCLE AFFERENT PROJECTION TO THE SUPERIOR COLLICULUS IS FROM THE GROUP III AFFERENTS. V. C. Abrahams and C. J. Turner*. Dept. of Physiology, Queen's University, Kingston, Ontario. K7L 3N6

Two systems of muscle afferents are known to be the origin of substantial projections to the superior colliculus. One system takes origin in extraocular muscle and the other in the large dorsal muscles of the cat neck. In experiments on chloralose anaesthetised cats, it has been possible to show that a signal is conveyed from extraocular muscle receptors indicating the onset of a saccade and also the position of the eye in the orbit during a saccade. Experiments in chloralose anaesthetised cats were undertaken to see what information could be recorded in the superior colliculus during controlled stretch of neck muscles at parameters known to excite the large population of muscle spindles present in these muscles. Although evidence from electrical stimulation was used to identify units in the superior colliculus receiving neck muscle afferent input, only a very small number of units (8 of 69) were found to respond during passive stretch of neck muscle. Careful examination was therefore made of the threshold of trains of electrical impulses applied to neck muscle nerves which led to unit activity in the superior colliculus. Units were rarely excited by stimuli adequate only to stimulate Group I and II fibres. Almost invariably units in the superior colliculus could only be activated by stimuli in excess of 2 x threshold. At this stimulus strength many Group III muscle afferents are excited. The assumption from these experiments is that it is the Group III afferents (which constitute an unusually high proportion of the myelinated fibres of neck muscle) which give rise to the projection to the superior colliculus. This would explain why controlled neck muscle stretch is an ineffective stimulus to unit discharge. Early analysis of receptor properties of Group III afferents from large dorsal neck muscles suggest that only a minority are sensitive to algesic chemicals. The most effective stimulus so far found is pinch of the muscles.

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83.9 DETERMINATION OF CHANGES IN REFLEX BEHAVIOR DURING TRANSITIONS FROM ONE TASK TO ANOTHER. J.F. Soechting and F. Lacquaniti*. Lab. of Neurophysiology, Univ. of Minnesota, Minneapolis, MN 55455.

A method has been developed which permits the identification of linear systems whose parameters are varying with time. Pseudo- random binary m- sequences of length n are used as the input. The manner in which the system's parameters vary is assumed to be unknown except that the time course of the variations is fixed relative to the onset of the sequence. Impulse responses for the output at each interval of the sequence are obtained by cross- correlations using n trials, in each of which the sequence has been shifted by one interval.

This method has been used to study changes in reflex behavior of biceps and triceps in response to torque perturbations applied to the forearm of human subjects when the motor task changed. In particular, we asked the subjects to switch from "resist the applied perturbation" to "do not resist", or vice versa, as quickly as possible upon command. Perturbations were applied for one second prior to and two seconds following the command. It was found that changes in reflex amplitude followed an approximately exponential time course, with a time constant of 140 ms for the transition from "resist" to "do not resist" and one substantially longer (220 ms) for a transition in the other direction. The method is also being used to study changes in reflex behavior during the initiation, control and arrest of simple movements.

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83.10 NONLINEAR INTERACTIONS OF STRETCH REFLEXES IN THE DECEREBRATE CAT. J.W. Aldridge and R.B. Stein. Dept. of Physiology, Univ. of Alta., Edmonton, Alberta, Canada. T6G 2H7

Previous studies from this laboratory have described nonlinear properties for contractions of soleus and plantaris muscles[1]. The following experiment extends the analysis to stretch reflexes. Reflexes were elicited in decerebrate cats with two brief muscle stretches separated by a variable interval (30-600 ms). Tension output, EMG activity and neural activity were recorded. In a linear system the response to the second stretch would be equal to the response to the first. The contribution of this second stretch to the total output was extracted by subtracting the response to the first stretch from the total response. A greater output to the second stretch compared to the first indicates a nonlinear potentiation. A smaller output signifies a depression.

Three phases of nonlinearity during the reflex were observed in the tension output. For short intervals (< 100 ms) following a stretch, the contribution of the second reflex to the total tension was depressed. This was followed by a period of potentiation (100-200 ms) and then a longer period of depression (200-600 ms). At intervals of 80 msec or less in soleus, the second stretch actually diminished the tension from the first stretch. This occlusion probably resulted from disrupting bonds already formed.

The pattern of nonlinearity for EMG output was qualitatively similar to that of tension. This suggests that the nonlinearity of reflexes cannot be attributed to muscle properties alone. Unlike tension and EMG, the neural activity from the second stretch was relatively unchanged. The neural responses mainly represent afferent activity[2] implying that the nonlinear reflex properties are not due to altered sensory input. Confirmation of these nonlinear reflex properties was obtained using electrical stimulation to elicit H-waves. All nonlinearities other than the early occlusion were seen with the nerve shocks.

In conclusion, an occlusion of stretch reflexes due to muscle properties has been described. Other nonlinearities are not due to afferent changes and the qualitatively similar behaviour of EMG activity and tension output points to the motoneuron as a possible site for these effects.

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J.W.A. is a postdoctoral fellow of MDAC.

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84.1 RESPONSE PROPERTIES OF IDENTIFIED SENSORY INTERNEURONS IN THE SIXTH ABDOMINAL GANGLION OF THE CRAYFISH. K. A. Sigvardt, G. Hagiwara* and J. J. Wine. Dept. of Psychology, Stanford Univ., Stanford, Ca. 94305.

We are characterizing a group of approximately 50 sensory interneurons in the sixth abdominal ganglion of the crayfish whose axons are the exclusive pathway for the transmission of processed sensory information from the tailfan to the rest of the animal's nervous system.

To date we have described 107 interneurons by intracellular injection of Lucifer yellow and have determined many of their response properties. Using the combined physiological and anatomical profile of each cell we have tentatively determined that these 107 represent 53 individual neurons. Most of these neurons fall into five relatively homogeneous classes.

Type A interneurons (n=20) fire to whole field water movement. They are directionally selective; half fire only to headward water movement and half fire only to tailward movement. All neurons are non-adapting, spontaneously active, and all have non-electrogenic cell bodies.

Type B interneurons (n=6) fire only when the tailfan or the water surface near the tailfan is touched. They are not directionally selective. They are not spontaneously active and the cell bodies are non-electrogenic. All Type B neurons are inhibited by roots outside the receptive field or by the giant axons.

Type C interneurons (n=4) include both nearfield and tactile cells, with response properties much like Type B cells. They are distinctive in having bilateral, multisegmental receptive fields, and electrogenic cell bodies. Unlike Type A and B cells, these neurons produce a long barrage of spikes in response to strong root shocks. All adapt to repetitive stimulation.

Type D interneurons (n=7) are proprioceptive interneurons. They are spontaneously active and the somata are non-electrogenic. Two of the three have multisegmental, ipsilateral receptive fields.

Type E interneurons (n=9) are proprioceptive interneurons with no spontaneous activity, electrogenic cell bodies, and bilateral, multisegmental receptive fields. The cells respond to strong root shocks with a long barrage of spikes.

Our analysis may eventually provide the first case in which all members of a sensory interneuron pool have been indentified. The interneurons are of special interest because they are afferent to several well-described motor systems.

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84.2 CERCAL SENSORY PROJECTIONS IN DERMAPTERA AND NOTOPTERA. John S. Edwards and Eldon Ball. Zoology Dept., Univ. of Wash., Seattle, WA 98195 and Dept. of Neurobiol. Australian Nat. Univ., Canberra, Australia.

Of the several orthopteroid insect orders, the cercal sensory system and associated giant interneurons are known in detail from only two, represented by the blattid Periplaneta americana and the gryllid Acheta domesticus. This study describes cercal projections and giant interneurons in two further orders, the Dermaptera represented by Forficula auricularia and the Notoptera by Grylloblatta sp.

In Dermaptera the cerci are modified to form forceps and thus assume a motor rather than sensory function. In Notoptera the annulate cerci are sensory and are intermediate in form between gryllids and blattids. Cercal sensory projections to the terminal ganglion are closely similar in Dermaptera and Notoptera, and distinctly different from those of blattids and gryllids. The major postero-lateral glomerulus characteristic of the latter two groups is absent. Four submedian projections in ipsilateral anterior, mid, posterior and far posterior positions. A loosely organised glomerulus occupies the sub-dorsal mid region from which some fibres extend into the connectives. A minor ventral projection also extends forward to the connectives.

The distinctive giant interneurons of Periplaneta and Acheta have no comparable counterparts in Forficula or Grylloblatta, but a group of relatively large axons, about 10 μm in diameter may be their homologs, for the largest spikes recorded from the ventral nerve cord of Grylloblatta are evoked by stimulation of cercal mechanoreceptors.

This work was supported in part by NIH grant NB 07778.

84.3 CONNECTIVITY BETWEEN IDENTIFIED SENSORY CELLS AND IDENTIFIED GIANT INTERNEURONS OF THE COCKROACH PERIPLANETA AMERICANA. Darryl L. Daley and Jeffrey M. Camhi. Sect. Neurobiology and Behavior Cornell Univ., Ithaca, NY 14853

The giant interneurons (GI's) of the ventral nerve cord of the cockroach are thought to be involved in initiating and orienting the directional escape response to wind puffs. The wind-sensitive receptors are filiform hairs located on the ventral surface of each of the two cerci (peg-like abdominal appendages). Each hair is innervated by a single sensory cell. Each sensory cell responds maximally to one direction of wind, but gives some excitatory response to wind over a range of about 180°. The hairs are arranged on the cerci in longitudinal columns. For 9 of these columns, it is known that all the hairs of a given column have the same best excitatory direction. The best excitatory direction is different for different columns. We have investigated the relative contribution that single columns of sensory hairs make to the response of individual GI's.

Synaptic input from single columns of hairs was recorded from the somata of both directionally selective and omnidirectional GI's. Single columns of filiform hairs were isolated from neighboring columns of hairs by first plucking out the unwanted hairs and then covering their bases with glue. (Generally all but six hairs in one column were inactivated in this way.) In response to controlled puffs of wind, synaptic potentials and sometimes action potentials could be recorded over approximately 180° of wind angle. In general, the maximal response in the GI occurred at the best angle for the column of hairs being stimulated.

GI 1, a somewhat directionally selective interneuron, receives excitatory synaptic input from columns of hairs on both the ipsilateral and contralateral cerci. Some of the columns that excite GI 1 have their best excitatory direction within the range of wind angles to which GI 1 is most responsive. However, synaptic input from at least one column of hairs whose best excitatory direction falls within the range of wind angles to which GI 1 is least responsive also excite GI 1. GI 2, an omnidirectional cell, receives excitatory synaptic input from columns of hairs with their best excitatory directions in all four quadrants surrounding the animal. We are currently examining interactions among other hair columns and the GI's to reveal further excitatory and possible inhibitory connections, and how these inputs shape the directionality of the GI's.

This work was supported by NIH grant NS 09083-10.

84.4 ELECTRON MICROSCOPY OF PUTATIVE CHEMOSENSORY STRUCTURES IN PLEUROBRANCHAEA CALIFORNICA. Gene Matera*and W.J. Davis, Thimann Labs, Univ. of Calif. at Santa Cruz, Santa Cruz, CA 95064

We have examined histologically a number of areas of the body of Pleurobranchaea californica with special emphasis on the known chemosensory areas -- the rhinophores, tentacles, oral veil, mouth and lips. Our studies indicate that one type of structure occurs in the sensory epithelium of all chemosensory areas, and nowhere else. These putative chemoreceptive sites are found at a very high density in the sensory epithelium of the lumen of both the rhinophore and tentacle, and at a lesser density in the oral veil, mouth and lips. Scanning electron microscopy has shown ciliated structures protruding through the epithelium, bearing many (200 or more) long cilia. Each receptor is a discreet entity about 10 micrometers in diameter. They appear, in the rhinophore and tentacles, evenly spaced in a dense array, at a density of approximately 5-10 X 10^3 per square millimeter. These receptors are complex in structure, bearing at least two distinct types of cilia. These include long, straight cilia resembling the presumably motile cilia of the foot, gill and elsewhere. Most of the cilia, however, have characteristic round spatula-like tips. These spatulate cilia are morphologically very similar to the long immotile (or very slow) olfactory cilia found in the frog (Reese 1965). Extracellular nerve recordings have shown that the rhinophore and oral veil tentacle are sensitive not only to food substances, but also to pH, salinity, osmolarity, and touch. These receptors may be multimodal, a possibility that is consistent with their morphological complexity. Transmission electron microscopy and light microscopy sections have shown that these putative chemoreceptors send projections into the peripheral ganglia associated with the sensory epithelium of both the rhinophore and tentacle.

The oral veil sensory apparatus, unlike that of the rhinophore and tentacle, is more spatially segregated, occurring only at the tips of specialized papillae which are everted or retracted in response to chemical stimulation. The sensory epithelium is organized into large (hundreds of micrometers), highly innervated floral-like regions which include centrally located putative chemoreceptors morphologically similar to those found in the rhinophore and tentacle. Similar structures have also been found in the mouth and lip regions. Other regions of the body also bear innervated sensory epithelium, but no other area examined to date has shown these putative chemoreceptors bearing spatulate cilia other than the areas known to have chemosensory capacity. We therefore hypothesize that these receptors underlie chemosensation in Pleurobranchaea.

84.5 ODOR AND PUFF SENSITIVE NEURONS IN THE SNAIL TENTACLE. Ronald Chase. Dept. Biology, McGill Univ., Montreal, Que., H3A 1B1.

Behavioral evidence indicates that the snail tentacle houses a sensitive and functionally complex olfactory system. In order to study this system physiologically, electrical recordings have been obtained from the tentacular ganglion and its digitate extensions into the sensory epithelium. The experiments utilized excised posterior tentacles of the terrestrial snail Achatina fulica. The tip of the tentacle was pinned out and exposed to clean air puffs or odors. A stimulus delivery system was arranged to provide precision control of air flow rate and duration. The use of solenoid valves permitted the insertion of odor-laden "air plugs" into a continuous airstream, with negligible distrubance of flow.

Extracellular recordings were obtained with suction electrodes. The signal:noise ratio was typically 3:1. These experiments demonstrated a sensitivity to air puffs with velocities as low as 25 mm/sec. Responses were of the ON-OFF type at low velocities, but sustained at velocities of several hundred mm/sec. Odor sensitivity was tested at 27 recording sites in 13 different preparations; each site yielded a response to air puffs. Four of the sites produced an unambiguous olfactory response to a mixture of fresh vegetable odors. Afferent responses to air puffs and vegetable odors were also recorded from the tentacular nerve using the sucrose gap method.

Intracellular recordings have been obtained only in the digitate extensions of the tentacular ganglion. The current sample consists of 44 cells, each with a minimal holding time of two min. Sixteen of the cells were held for more than 10 min. The resting potentials ranged from 45 mV to 80 mV with a mean of 57 mV. Two cell types have been encountered. Type I cells generally show no spontaneous activity, although some units produce undershooting spikes, especially during electrode penetration. EPSPs are reliably evoked by electrical stimulation of the tentacular nerve (centrifugal effect). Olfactory stimulation produced a slow depolarizing potential in a minority of type I cells (1 mV to 3 mV). The sensitive units responded reliably to three natural food odors, and one showed a preference for food odors over either amyl acetate or benzaldehyde. Type II cells constitute about 15% of the sample. They are characterized by frequent spontaneous EPSPs (up to 7 mV) and overshooting action potentials. No olfactory responses have yet been recorded from Type II cells.

The physiological data are consistent with previously published anatomical descriptions of the tentacle, which also indicate the presence of two cell types in the digitate extensions. Thus, following the anatomical classification, Type I cells are tentatively identified as receptors, and Type II cells are tentatively identified as interneurons.

84.6 LOCALIZATION OF DENDRITIC FIELDS AND TERMINAL ARBORIZATIONS IN SUSTAINING FIBERS OF THE CRAYFISH VISUAL SYSTEM. B. Waldrop and R.M. Glantz. Dept. of Biology, Rice Univ., Houston, Tx 77001

Sustaining fibers (SF's), tonic light-on cells, are functionally identified interneurons in the crayfish that transmit information from the optic ganglia to the brain via axons in the optic tracts. Fourteen SF's have been identified in each optic tract on the basis of their functional receptive fields.

We have impaled SF's and filled them with the fluorescent dye Lucifer Yellow (supplied by Dr. Walter Stewart of NIH), to visualize their morphological features. The major dendritic structures consist of a large transverse process, located along the proximal edge of the medulla externa, which gives rise to small fibers parallel to the long axis of the eyestalk. The small fibers project into the neuropile of the medulla externa. The terminal arborizations in the brain consist of tightly clustered varicosities located bilaterally at the bases of the optic tracts and in the optic neuropiles. Some fibers appear to project a fine axonal process to the contralateral optic tract. Electrophysiological studies of the same cells show that intracellularly injected current interacts with light-mediated synaptic input to determine the spike output frequency.

A major goal of this work is to correlate the dendritic distributions of identified interneurons with their visual receptive fields.

84.7 PATTERNS OF SYNAPTIC INTERCONNECTION IN THE DRAGONFLY LAMINA. Christine Armett-Kibel* and I.A. Meinertzhagen, Dept. of Biology, Univ. of Massachusetts-Boston, Boston, MA and Life Sciences Centre, Dalhousie University, Halifax, N.S. Canada.

Description of the connectivity between identified neurons is a major motivation for the analysis of simple nervous systems. A favourable system for that analysis is provided in the visual system of arthropods by the many parallel pathways between the compound eye and optic neuropiles. The modular synaptic composition of these neuropiles is analysed in the dragonfly (Sympetrum rubicundulum) in the unit module, or cartridge, of the outermost neuropile, or lamina. An ultimate goal is to arrive at a matrix of synaptic interconnections between the small, fixed number of uniquely identifiable elements constituting the lamina cartridge. A previous study (Meinertzhagen, Armett-Kibel and Frizzell: Cell Tiss. Res. 206: 395, 1980) reports the number of these elements as 16: 8 receptor axons, 2 of which (the long visual fibres) pass through the lamina; 5 monopolar cells and three additional unidentified cells all with axons. The cartridge thus receives an input from 8 receptor axons and has an output of up to ten axons which connect to a corresponding cartridge in the next most central neuropile, the medulla.

Synaptic connectivities of the five monopolar cells (the larger, MI & MII, and the smaller, hitherto undocumented MIII-MV) and two long visual fibres (R6, R7) have been analysed from extended series of electron micrographs. The analysis also uncovered the existence of another element (or elements) with widespread processes, and connectivities suggesting a role in intracartridge local circuit interactions.

Of the two long visual fibres, R6 is presynaptic only to the monopolar cell MII while R7 is presynaptic to both MII and MV. Thus, of the five monopolar cells, two (MI, MII) receive a general receptor input while a third (MV) has a receptor input exclusively from R7. MIV receives a small input from both MI and MIII. In addition, MIII and MIV are both postsynaptic to the unknown element of possible local circuit function. This element not only insinuates itself at a location postsynaptic to the chief receptor synapses upon MI and MII but also forms dyadic synapses upon receptors and other processes of its own kind (thus constructing possible reciprocal synaptic interconnections and serial synapses, respectively). MIII and MIV may substitute postsynaptically at either of these dyadic locations. There are similarities between the connectivities of the processes of this unidentified element and those of the α-β system in the fly's lamina.

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84.8 IONIC MECHANISMS UNDERLYING LIGHT-EVOKED DEPOLARIZATIONS IN THE RETINA OF STROMBUS, A MARINE GASTROPOD. K.S. Chinn* and H.L. Gillary. (SPON: D. Russell). Bekesy Laboratory for Neurobiology, University of Hawaii, Honolulu, HI 96822.

The retinal type 2 cells of Strombus luhuanus described by Quandt and Gillary (J. Exp. Biol. 1979, 80, 287) show characteristic light-evoked depolarizations (LED). The responses to brief flashes of white light exhibited two peaks or phases (amplitudes ca. 15mV). The latency to the first peak was generally ca. 0.2 sec while that to the second was 0.5-1.0 sec. The ionic mechanisms underlying the LED were studied by examining the effects on intracellularly recorded potentials of perfusion media (modified ASW) having different ionic compositions or pharmacological agents. The experiments indicate: 1) The resting potential of these cells is governed primarily by $[K^+]$; the resting potential changed approximately 50 mV/10X $[K^+]_o$. 2) The LED was tetrodotoxin-insensitive, remaining unchanged at concentrations of up to $6x10^{-6}$M TTX. 3) Lowering $[Na^+]_o$ by replacement with choline or tetramethylammonium ions decreased both phases of the response. This decrease, approximately a linear function of log $[Na^+]_o$, was greater for the second phase than the first. 4) Total replacement of Na^+ with Li^+ markedly reduced phase 1 (in some cases to zero); this also reduced phase 2, but less than phase 1. 5) Lowering pH from 7.7 (normal) to 5.5 reversibly abolished phase 2 while phase 1 was only reduced after an initial increase. 6) The results of application of 4-amino pyridine and tetraethylammonium chloride suggest the occurrence during the LED of a K^+ current. The onset of this current occurs sometime during phase 1 and lasts throughout phase 2. 7) Input resistance decreased in two phases, corresponding to the respective phases of the LED. 8) Changing $[Ca^{++}]_o$, substituting Sr^{++} for $[Ca^{++}]_o$, or adding Mn^{++} markedly affected the waveform of the LED. Lowering $[Ca^{++}]_o$ increased the response amplitude. The results indicate that the LED arises from two separate Na^+ conductance increases as well as an increase in K^+ conductance. These conductance changes may be modified by divalent cations. (Supported by NIH Grant EY 01531).

84.9 GRADED NEURAL INTERACTION BETWEEN AXONS FROM THREE SIMPLE EYES OF AN INSECT. Charles P. Taylor* (SPON: C.H.F. Rowell). Grad. Group in Neurobiology, Univ. of California, Berkeley, CA 94720.

Axons in the nerve leading from the retina of one of the three dorsal ocelli (simple eyes) of a locust (Orthoptera, Acrididae) were studied by intracellular recording and dye injection with Lucifer Yellow. The three ocelli were stimulated independently by ON steps of light to investigate interactions between the three eyes.

Seven (10-20 μm diam.) anatomically identifiable large interneurons (LIs) lead from the retinal neuropile of the median ocellus to the brain (Goodman, 1976, Cell Tiss. Res. 175:183). In response to a flash of light(which depolarizes receptor cells) LIs hyperpolarize with a characteristic waveform which is similar to that observed in other invertebrate visual systems.

Three physiological categories of response to light ON were recorded from axons of the median ocellar nerve: 1) Hyperpolarization from median stimulus only; small amplitude depolarization from stimulation of either lateral ocellus. 2) Hyperpolarization from stimulation of either the median or one lateral ocellus (lateral stimulus produced smaller response); small amplitude depolarization from stimulation of the remaining lateral ocellus. 3) Hyperpolarization from stimulation of the median ocellus; smaller amplitude hyperpolarization from stimulation of either lateral ocellus. These categories could not be distinguished by stimulation of the median ocellus alone. Simultaneous stimulation of two or more ocelli caused summation of the responses.

Subsequent examination of dye fills made through the recording electrode revealed that category 1 corresponds anatomically to the two Ml-cells of Goodman which have a single axon leading from the median ocellar retina to the brain. Category 2 corresponds to the four ML-cells of Goodman which each have a large axon communicating between the retinas of the median and one lateral ocellus. Category 3 has tentatively been identified with the single M2-cell of Goodman.

The depolarizing responses were shown to accompany a decrease in membrane time constant, and were reversibly eliminated by bath application of picrotoxin (1 mg/ml) (which left the hyperpolarizing responses relatively unchanged).

One hypothesis which accounts for these results is that the ML-cells inhibit the response of the Ml-cells via a picrotoxin-sensitive mechanism. This would serve the behavioral function of the ocelli in registering changes of the animal's orientation with respect to the horizon (Taylor, in preparation). Responses caused by changes in illumination of the entire visual world would be inhibited, while stimuli to the median ocellus only would not.

85.1 FUNCTIONS OF OTOCONIAL AND AUDITORY ORGANS OF THE BULLFROG INNER EAR IDENTIFIED WITH INTRACELLULAR DYE. R. A. Baird, H. Koyama*, E. L. Leverenz*, and E. R. Lewis. Electronics Research Laboratory, University of California, Berkeley, CA 94720.

After functional identification, individual afferent fibers in the VIIIth nerve were iontophoretically filled with the intracellular fluorescent dye Lucifer Yellow and subsequently traced to their peripheral origins. To date, this study has provided direct corroboration of organ to organ functional divisions proposed as early as 1925 for the otoconial organs and papillae of the frog inner ear (McNally, W. & Tait, J., Am. J. Physiol. *75*: 155-174, 1925; Ashcroft, D.W. & Hallpike, C.S., J. Laryng. *49*: 450-458, 1934; Ross, D., J. Physiol. *86*: 117-146, 1936; MacNaughton, I. & McNally, W., J. Laryng. *61*: 204-214, 1946; Frischkopf, L., Capranica, R.R. & Goldstein, M.H., Proc. IEEE *56*: 969-980, 1968). In addition, it has demonstrated heretofore unsubstantiated functional divisions within three of those organs (Lewis, E.R. & Li, C.W., Brain Research *83*: 35-50, 1975; Lewis, E.R., Scanning Electr. Microsc. *9(2)*: 4-9-304, 1977). Approximately 100 fibers have been identified functionally and traced, and the results are summarized in the following table:

FUNCTIONAL DIVISIONS OF THE INNER EAR

FUNCTION	PERIPHERAL ORIGIN
Auditory (c.f. 1 kHz - 2 kHz)	Basilar papilla
Auditory (c.f. 400 Hz - 1 kHz)	Amphibian papilla (posterior)
Auditory (c.f. below 400 Hz)	Amphibian papilla (anterior)
Vibratory	Sacculus
	Lagena (striolar region)
Vestibular: responses to head position/linear acceleration	
Tonic only	Lagena (extrastriolar region)
	Utricle (extrastriolar region)
Phasic and Phasic/Tonic	Lagena (striolar region)
	Utricle (striolar region)

Some lagenar vibratory units also exhibited tonic response to head position. A few units in each of the two major branches of the VIIIth nerve exhibited both auditory and vibratory sensitivity, but were insufficiently filled with dye to be traced. The functional distributions listed in the table show marked correspondence to distributions of hair-cell morphological types observed by Lewis & Li (1975).

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85.2 RECEPTOR ORGANIZATION IN THE UTRICLE OF THE GUITARFISH INNER EAR. Christopher Platt. Dept. Biological Sciences, Univ. Southern California, Los Angeles, California 90007.

The utricle of the inner ear is an otolith organ that often is considered to be gravistatic. But among elasmobranchs, some rays have a part of the utricular macula, the lacinia, which is covered only by otolithic membrane and responds to vibration (Lowenstein & Roberts, J. Physiol. 114:471, 1951). Recently, the guitarfish Rhinobatos productus, a more primitive elasmobranch, also has shown both gravistatic and vibrational sensitivity in the utricle (Budelli & Macadar, J. Neurophysiol. 42:1749, 1979).

It seemed pertinent to see whether the epithelial surface of the guitarfish utricular macula has regional structural differentiation that might relate to these functional distinctions. Fresh tissues were fixed in situ with buffered glutaraldehyde, removed and post-fixed with osmium tetroxide, and dried by the critical-point method for scanning electron microscopic study.

The utricle of small adults (ca. 1 Kg) contains roughly 100,000 hair cells in a rostro-caudally elongated macula of roughly 4 mm^2. Medially, the macular margin is quite sharply defined, but the lateral margin has an unusual fringed appearance, where narrow groups of hair cells interdigitate with narrow wedges of peripheral supporting cell groups. Cellular orientation is based on the asymmetric location of the single kinocilium in the apical ciliary bundle of each hair cell. Usually in vertebrates the utricular pattern contains only two groups of oppositely oriented cells, separable by a single dividing line. The guitarfish pattern instead confirms earlier TEM reports of "patchy" distributions in the ray; a local clump of cells sharing the same orientation may be surrounded by a population of the opposite orientation. The utricular floor contains large areas of similarly oriented cells, with a few boundary lines that can be drawn; but the lacinia, extending caudally up the utricular wall and roof and covered only by otolithic membrane as in the ray, has a much finer grain of patchiness. The predominant orientation over the whole macula is transverse to the long axis. Hair cell bundles show a variety of forms similar to those in teleosts. Short kino- and stereocilia occur at the macular edge, long kinocilia with short stereocilia occur near the margins, but over most of the macula the cells have kinocilia 5-8 μm long and tallest stereocilia of 3-7 μm. There is not as clearly distinct a striolar band of prominent hair cells as in many teleosts.

The difference in otolithic covering and the difference in patchiness between utricle and lacinia are not accompanied by other surface features distinctly different enough to suggest regional differentiation of gravistatic and vibrational reception in the guitarfish.

85.3 VESTIBULOTOXICITY IN THE CHICK CAUSED BY STREPTOMYCIN. J. C. Park and G. M. Cohen. Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida 32901.

We are using the chick as a model system for auditory and vestibular studies. In the present experiments, 7 day-old male white leghorn chicks were injected daily for as many as 30 days in different subcutaneous sites on the breast with streptomycin sulfate at one of three dosages: 400, 800, or 1200 mg/kg. The birds were weighed daily to determine rates of growth and tested for impaired equilibrium. Birds were sacrificed daily to follow cytopathic changes in the cristae ampullares using light and electron microscopy.

Bodily growth and maturation continued normally but growth lagged slightly at 800 mg/kg before catching up by the second week. The birds began to tremble by the third day at the two higher dosages and by the fifth at the lowest. By the 14th injection, one-third of the birds trembled at least mildly and two-thirds trembled moderately or severely. However, the tremor began to subside shortly thereafter, possibly because of visual and proprioceptive compensation. The birds perched on wooden dowels for tests of vestibular function. When hooded, birds displayed moderate to severe dysequilibular symptoms. In some instances, the birds could not maintain their balance on the dowel even when unhooded. Controls showed no disabilities under either condition.

In the ampulla, dark cells were damaged earlier than other cell types. The first intoxicatory symptoms appeared regularly by the third injection. Cytopathic changes involved retraction of basal processes, vacuolization of cytoplasm, mitochondrial damage, and rearrangement of organelles. Resistant dark cells showed little, if any, intoxicatory damage but they accumulated glycogen, in both the somata and basal processes. Severely damaged dark cells often abutted intact ones. The small, cuboidal dark cells, located on the ampullary periphery, were most sensitive, whereas the larger, columnar cells, flanking the crest, were most resistant. (Supported in part by NIH grant RR09032)

85.4 DIFFERENCES BETWEEN PROPERTIES OF AFFERENTS INNERVATING HORIZONTAL AND VERTICAL CANALS OF THE BULLFROG. Robert J. Peterka* (SPON: David L. Tomko). Electrical-Biomedical Eng. Dept., Carnegie-Mellon Univ. and Dept. Otolaryngology, Univ. of Pittsburgh, School of Medicine, Pittsburgh, PA 15213.

The amplitude of displacement of a point within the bullfrog's horizontal canal (HC) cupula was shown to depend on the location of that point (McLaren and Hillman, Neuroscience 4: 2001-2008, 1979). McLaren also found differences in response properties of afferents innervating different portions of the HC crista (doctoral thesis, 1977). It was postulated that these differences may result from local variations in the displacement of the cupula. Since the shapes of vertical canal (VC) critae and cupulae resemble one another but differ somewhat from the configuration of the HC crista and cupula (Hillman, Brain Behav. Evol. 10: 52-68, 1974), one might expect that properties of VC afferents would also resemble one another and differ from those of the HC afferents.

To test this, resting discharge and dynamic response properties of eighth nerve afferents innervating all three canals of adult bullfrogs were characterized using data from intracranial, extracellular single unit recordings. Response parameters were obtained using transfer function curve fits to gain and phase data determined from responses to pseudorandom stimulation. Properties of VC afferents were similar to one another but differed from those of the HC afferents in certain aspects. Compared to the VC afferents, a larger proportion of HC cells had 1) high resting discharge rates (greater than 10 spikes/sec), 2) low coefficients of variation (less than 0.4), 3) large gain constants (greater than 20 spikes-sec^{-1}/deg-sec^{-2}), and 4) longer time constants (greater than 5 sec). Afferents which did not show any evidence of response adaption were also more prevalent in the HC afferent population than in the VC cells. Finally, there was no correlation between gain constant and time constant parameters from VC cells, whereas the relationship between these parameters for HC afferents indicated that the population could be clearly divided into two groups. One group consisted of high gain, short time constant cells; the other contained a wide range of time constants but had lower gains.

Most of the average differences between HC and VC afferent response parameters and resting discharge properties were not large since the differences usually occurred in the tails of the distributions of these values. However, taking the data as a whole, the conclusion that the population of HC afferents had certain properties which differed from those of the VC's appeared to be reasonable. Whether or not these differences are directly correlated with cupula structure remains to be determined.

85.5 CORRELATION OF SEMICIRCULAR CANAL AFFERENT RESPONSE DYNAMICS WITH SWIMMING MOVEMENTS IN THE GUITARFISH. D.P. O'Leary and R.F. Dunn, Div. Vestibular Disorders, Dept. Otolaryngology, Univ. of Pittsburgh School of Medicine, Eye and Ear Hospital, Pittsburgh, PA 15213.

Dynamic response characteristics of vertical semicircular canals are thought to be similar to those from horizontal canals of the same mammalian species. But analyses of different canal diameters in fish showed significant differences, that were considered, on the basis of biophysical modeling, to be correlated with the different dynamic ranges of swimming movements that stimulate vertical as opposed to horizontal canal planes (Howland and Mascii, J. Embryol. exp. Morph., 29, 721, 1973). Our data from first-order canal afferents of the guitarfish show directly that the response dynamics of vertical and horizontal canals differ in ways that match the swimming dynamics of this animal.

Isolated labyrinths of the guitarfish (Rhinobatos productus) were aligned at the center a computer-controlled turn table for maximal stimulation of either the horizontal or anterior canal planes. Bandlimited white noise rotational acceleration stimuli were used to characterize the response dynamics of each afferent unit, through cross-correlation and cross-spectral analyses. Two major classes of response dynamics were observed from horizontal canal afferents, those maximally sensitive to fast head movements versus those sensitive to slow movements (O'Leary, Dunn & Honrubia, J. Neurophysiol., 39, 631, 1976). In contrast, only responses from the slow movement class were observed from anterior canal afferents, although systematic recordings were made from afferents shown to innervate specific regions of both cristae (Dunn, J. Comp. Neurol. 183, 779, 1979; Dunn and O'Leary, these abstracts 1980). Plots of gain versus frequency of afferent responses showed average gains from anterior canal cells that were 1/10 those of the fast class of horizontal canal cells, at frequencies above 1 Hz.

Profile drag measurements on the cartilaginous head were made to determine differences in drag coefficients during slow underwater rotations about the guitarfish's three principle axes. The arrowhead shape of the head, with broad dorsal and ventral surfaces, results in side-to-side swimming movements with drag coefficients that are at most 1/12 those of movements about roll or pitch axes. These theoretical predictions were confirmed by observations of 16 mm films and a computer simulation of swimming guitarfish. Swimming head movements that occur in planes that stimulate horizontal canals are faster, because of profile drag, than those that occur in planes that stimulate vertical canals. This behavioral difference is correlated with our finding that a fast class of afferent responses was present in horizontal canal fibers that was not observed in anterior canal fibers.

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85.6 SPATIAL DISTRIBUTION OF NERVE FIBERS THROUGH THE ANTERIOR AMPULLARY NERVE IN GUITARFISH. R.F. Dunn and D.P. O'Leary, Div. Vestibular Disorders, Dept. Otolaryngology, Univ. of Pittsburgh School of Medicine, Eye & Ear Hospital, Pittsburgh, PA 15213.

The anterior ampullary nerve (AAN) approaches the anterior ampulla as a single nerve trunk which branches close to the ampulla. All nerve fibers approach the ampulla at right angles to the longitudinal axis of the anterior crista. The nerve bundles form a fan in a medial-lateral plane relative to the head. The medial and lateral bundles are quite large; each containing 35-40% of the nerve fibers contained in the AAN. The remaining 20-30% of the AAN fibers are divided into 9-11 small central bundles. Projection patterns made by the nerve bundles onto the anterior crista are: medial bundle to the medial crista bulbus; the small central bundles to the crista angustarum; and the lateral bundle to the lateral crista bulbus.

Taking advantage of this projection pattern to the anterior crista, it is possible to record from specific regions of the crista (i.e. each crista bulbus and the crista angustarum), and to compare the impulse responses between these areas. Further, the AAN responses can be correlated to those previously observed in the horizontal ampullary nerve (O'Leary, D.P., et al, J. Neurophysiol., 39: 631, 1976). It is therefore relevant to determine the course of nerve fibers comprising each nerve bundle group within the AAN since the recordings are generally obtained from nerve fibers in the AAN trunk.

Consecutive serial one micron cross sections were prepared from Araldite 502 embedded AAN. The series began at the anterior crista, proceeded centralward through the bundles to include 1.5 mm of the AAN trunk. Every sixth section was photographed, and the nerve fibers were color-coded on each photograph. When the small central bundles were considered collectively, they retained their central position within the AAN with some displacement caudally by the medial bundle nerve fibers, and rostrally by the lateral bundle fibers. Based upon these preliminary findings, it appears that the AAN trunk is organized such that nerve fibers in the medial portion of the AAN trunk project to the slopes and crest of the medial crista bulbus; the central fibers innervate the slopes and crest of the crista angustarum; and the lateral fibers project to the slopes and crest of the lateral crista bulbus.

85.7 EFFERENT VESTIBULAR NEURONS PROJECT TO VARIOUS SEMICIRCULAR CANAL CRISTAE IN BOTH LABYRINTHS WITH AXON COLLATERALS. D.W.F. Schwarz, Sat Bir Khalsa*, I.E. Schwarz*, and J.P. Landolt. Lab. of Otoneurology, Univ. of Toronto, Toronto, Ont. Canada.

We attempted to assess the specificity of efferent vestibular control by determining how discrete efferent projection to individual semicircular canals is organized, using morphological and electrophysiological techniques in pigeons. A large proportion of efferent neurons recorded extracellularly in the caudal pontine reticular nucleus responded with antidromic spikes to discrete electrical stimuli of more than one semicircular canal nerve in the same labyrinth. A number of cells could be so excited by such stimuli in either labyrinth. Many efferent neurons can be retrogradely labelled by HRP injected in one crista as well as by [^{3}H]- Adenosine in another crista. A smaller number of cells can be retrogradely labelled by injection of Evan's Blue into all cristae of one side and also by Bisbenzimide into all cristae of the other side. The extensive collateralization of individual efferent axons within the labyrinths demonstrated by these experiments explains the lack of a topographical separation of efferent neurons projecting to different cristae. It is also explained why there is no systematic increase in the number of retrogradely labelled cells when an increasing number of cristae is injected. It must be concluded, that efferent control can hardly be specific for directions of head rotation.

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85.8 MECHANISMS DETERMINING STEADY-STATE DISCHARGE PATTERNS AND RESPONSE DYNAMICS OF VESTIBULAR AFFERENTS. J.M. Goldberg, C.E. Smith* and C. Fernández*. Univ. Chicago, Chicago, IL 60637

Vestibular afferents characterized as regularly or irregularly discharging differ in several other respects, including fiber caliber, afferent response dynamics and response to efferent stimulation. We were interested in examining the mechanisms responsible for differences in discharge regularity. At least two possibilities need to be considered. Afferents could differ in (1) the "noisiness" of their synaptic input or (2) their post-spike recovery times. The two mechanisms lead to different predictions concerning the response to galvanic currents. If post-spike recovery were involved in determining variations in discharge regularity, then irregular afferents should be more sensitive to galvanic currents than are regular afferents. In contrast, variations in synaptic noise should not influence galvanic sensitivity.

Vestibular-nerve recordings were made in barbiturate-anesthetized squirrel monkeys. Electric currents of 5-sec duration were delivered via an electrode implanted in the perilymphatic vestibule. Excitatory (cathodal) and inhibitory (anodal) responses were similar in magnitude and both were linearly related to current strength. Galvanic sensitivities were 5-10x greater in irregular, as compared to regular, units. A parallel difference in sensitivities was observed when 50-μsec shocks were used. Shock-response times were brief (ca. 0.3 msec) so as to preclude the involvement of synaptic mechanisms. Galvanic responses can be silenced by inhibitory head rotations, suggesting that the currents act on the afferent terminal, rather than on the parent axon. Post-spike recovery was measured by determining short-shock thresholds at several fixed times after naturally occuring action potentials. Irregular units had faster recovery times than did regular units, showing that differences in galvanic responses cannot be solely explained by variations in the afferents' passive electrical properties. The faster recovery of irregular afferents should make them more sensitive to synaptic, as well as to artificial, currents. Irregular discharge, it may be suggested, offers no functional advantage in its own right. Rather it is a necessary consequence of the mechanisms leading to an enhanced sensitivity.

Comparisons were made of the afferents' responses to sinusoidal galvanic currents and sinusoidal head rotations. Velocity-sensitive response dynamics, including a high-frequency gain enhancement and phase lead, were seen during natural, but not during galvanic, stimulation. The velocity-sensitive response dynamics would thus appear to arise at a relatively early stage of the transduction process.

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85.9 AFFERENTS TO THE VESTIBULAR COMPLEX IN RAT. A HORSERADISH PEROXIDASE STUDY. J. A. Rubertone* and W. R. Mehler (Spon. J. Norvell.) NASA-Ames Research Center, Biomedical Research Division, Moffett Field, Ca. 94035.

Rats are used extensively in biomedical studies related to the rigors of space flight. Most of this experimentation is concerned with various physiological responses to labyrinthine mediated stimuli. An accurate interpretation ofsuch data relies on a complete understanding of the vestibular system in rat. A review of the literature, however, reveals a relative paucity of data on vestibular connectivity in rat.

The present study is the first in a series of experiments designed to illucidate the afferent and efferent connections of the rat vestibular complex. Vestibular nuclei and subgroups were injected stereotactically with horseradish peroxidase (Sigma VI) in 18 cases to date. Iontophoretic injections were performed at a current of 1.0-3.0 μa using micropipettes ranging from 20-60 μm in tip diameter. Following survival times of 23-48 hours animals were perfused with 0.9% heparinized saline followed by a buffered 1.5% glutaraldehyde, 1.0% paraformaldehyde solution. Following immersion in a buffered 30% sucrose solution for 15-24 hours brains were frozen and sectioned at 50 μm in either transverse, parasagittal or horizontal planes. Sections were collected and processed according to the diaminobenzidine (Graham, R. C. and M. J. Karnovsky, J. Histochem. Cytochem., 14:291, 1966), benzidine dihydrochloride (Mesulam, M-M., J. Histochem. Cytochem., 24:1273, 1976), and tetramethyl benzidine techniques (Mesulam, M-M., J. Histochem. Cytochem., 26:106, 1978). All sections were examined under both light and dark field microscopy. Injections of the spinal vestibular nucleus (SpVN) resulted in HRP-labeled neurons in the central cervical nucleus and superior vestibular nucleus (SVN) bilaterally. Contralateral labeling also occurred in the SpVN, medial vestibular nucleus (MVN) and fastigial nucleus. HRP-positive cells were present in similar locations following injections of MVN. Injections of SVN resulted in labeled somata in the contralateral SpVN, MVN, SVN, and subgroup y. The connectivity of the lateral vestibular nucleus as well as the question of vestibular afferents originating in the reticular formation and regions other than the cerebellum will be discussed.

*National Research Council Research Associate. Supported by NASA TASK - 199-05-02-07.

85.10 VISUAL AND GRAVICEPTIVE INFLUENCES ON LOWER LEG EMG ACTIVITY DURING BRIEF FALLS Roger W. Wicke* and Charles M. Oman
Man Vehicle Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139

Human subjects were suspended in a safety harness 17 inches above the floor by a steel cable connected to a computer controlled force generator (magnetic particle brake). After the subjects were unexpectedly released, various controlled patterns of downward acceleration (less than 1 G) could be produced. During the falls, EMG activity was recorded simultaneously from the gastrocnemius, soleus, tibialis anterior, rectus femoris, and biceps femoris, along with knee and ankle joint angle in one leg. Subjects were tested eyes closed and also eyes open, both in darkness and in light using a wide field visual display. The display scene could be moved downwards at exactly the same velocity as the moving subject, left fixed with respect to the laboratory ("normal visual field"), or moved upwards at a speed equal to the subject's falling velocity ("upward moving visual field"). Ten vestibularly normal subjects each underwent a total of 45 drops, experiencing 3 replications of each vision/motion combination used. Under normal visual field conditions, both short and long latency EMG responses were seen, which were dependent on the magnitude of the acceleration stimulus. Certain of the visual conditions significantly altered both the short and the long latency responses in most of the muscles tested. Effects were particularly prominent in the gastrocnemius and soleus, and were also more pronounced during slow (0.5 G) falls. The upward moving visual field condition increased the short latency EMG reaction in gastrocnemius and soleus. A preliminary model for visual-vestibular interaction in short latency EMG responses is presented.

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85.11 RESPONSE CHARACTERISTICS OF VESTIBULOSPINAL NEURONS OF DIFFERENT SIZES TO SINUSOIDAL STIMULATION OF MACULAR LABYRINTH RECEPTORS. R.Boyle* and O.Pompeiano. Ist.Fisiologia Umana, Catt.I, Univ. di Pisa, 56100 Pisa, Italy.

The functional significance of cell size within the lateral vestibular nucleus of Deiters (LVN) has been investigated in decerebrate, cerebellectomized cats by recording the responses of vestibulospinal neurons to natural stimulation of macular labyrinth receptors and correlating these responses with the conduction velocity (CV) of the corresponding axons. Only antidromically identified neurons projecting to the lumbosacral segments of the spinal cord (lLVN) were tested during slow sinusoidal tilt around the longitudinal axis at 0.026 Hz (10° peak amplitude).

CV of the recorded units varied from 45.0 - 118.7 m/sec (mean: 84.3 ± 17.1, S.D., m/sec). An inverse correlation was found between CV and resting discharge rate, which ranged in different units from 1.7 - 134.4 imp./sec (mean: 44.5 ± 22.7, S.D., imp./sec). 80/136 (i.e. 58.8%) units responded with a periodic modulation of their firing rate to the sinusoidal input. The higher the CV, the greater was the sensitivity of the first harmonic of the response, expressed in percentage change of the mean discharge rate per degree of displacement; this was a general finding, which indicates that the pool of lLVN neurons represents a rather homogeneous population of cells, differing only in size and in certain size-dependent properties. However, within individual experiments units were encountered which had comparable CV but large variations in resting discharge rate and response sensitivity, or the converse. These observations suggest that size-independent differences in both firing rate and sensitivity depend either or intrinsic neuronal properties or on differences in connectivity of the macular labyrinth input on cells of similar size.

Most of the responses of lLVN neurons to tilt were related to the direction of stimulus orientation, 48/80 (i.e., 60.0%) units excited during side-up tilt and 21/80 (i.e., 26.2%) units excited during side-down tilt. The units of the former group had a significant slower CV, higher resting discharge rate and lower response sensitivity than those of the latter group. However, exceptions to this rule were observed in individual experiments.

85.12 CORTICO-CORTICAL CONNECTIONS TO THE VESTIBULAR CORTICAL AREAS IN THE CAT. R. Ramírez-Camacho*, C. Avendaño and F. Reinoso-Suárez. Dep. Morfología, Fac. Medicina, Univ. Autónoma, Madrid 34, Spain.

Two areas have been defined in the cat's cerebral cortex from which short-latency potentials are recorded after vestibular stimulation: one situated in the anterior suprasylvian sulcus (ASSS) and another in the region of the postcruciate dimple (PCD), in the posterior sigmoid gyrus. Both areas are topographically included within Woolsey's area SI. Although there is a good deal of information regarding the cortico-cortical connectivity of SI, both by morphological and electrophysiological methods, only brief comments are found on the cortical input to these particular areas.

We have made unilateral single or multiple injections of a 50% solution of HRP either in ASSS or PCD of adult cats. Total volumes injected ranged between .06 and 1.0 μl. Controls received similar injections in other portions of SI and in SII. After 48 h survival animals were perfused and brains processed according basically to Graham and Karnovsky (1966) and Mesulam (1978) procedures.

Area PCD receives a fairly large number of projections from the rest of the posterior sigmoid gyrus (all layers, mainly III and VI), the anterior portion of the lateral gyrus (layer V), the posterior portion of the coronal gyrus (predominantly layers II and III) and the infero-posterior portion of the anterior ectosylvian gyrus (layers II-VI). The connections to the vestibular area in ASSS have a similar origin, although showing a distinctive topography: the anterior portion of ASSS receives connections from the posterior sigmoid gyrus; the middle portion from the anterior portion of the lateral gyrus and the posterior portion of the coronal gyrus, and the caudal portion from most of the anterior ectosylvian gyrus, except from that region projecting on PCD. Further, a consistent projection was found from the bottom and lateral bank of the posterior rhinal sulcus (layer V) to the upper bank of ASSS.

Contralaterally, fewer projections were observed, although their topographical arrangement was essentially the same.

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86.1 POSSIBLE INVOLVEMENT OF BRAIN EPINEPHRINE IN CONTROL OF LUTEINIZING HORMONE SECRETION IN RATS. W.R. Crowley and L.C. Terry, Depts. of Pharmacol. and Neurol., Univ. of Tenn. Cntr Hlth Sci., Memphis, TN 38163.

Norepinephrine has been proposed as a stimulatory neurotransmitter for the secretion of Luteinizing Hormone (LH) because noradrenergic receptor blockers and synthesis inhibitors interrupt episodic LH release, block ovulation and prevent the stimulatory effects of ovarian hormones. However, these drugs should also interfere with central epinephrine neurotransmission. The purpose of the present study was to test whether brain epinephrine (EPI) systems may also be involved in control of LH secretion. Sixty day old female rats were ovariectomized and allowed 4 week's recovery. One group of females received 50 ug estradiol benzoate subcutaneously and were sacrificed 2 hr later (acute inhibitory feedback). A second group received 50 ug estradiol, followed 72 hr later by 2 mg progesterone and were killed 6 hr later (stimulatory feedback). A third control group were given oil vehicle only. Thirty minutes prior to these injections, one half of the animals in these 3 groups were treated with 50 mg/kg of 7,8 dichloro-1,2,3,4-tetrahydroisoquinoline (SKF64139), an agent that blocks epinephrine synthesis by inhibiting the activity of phenethanolamine-N-methyltransferase. Animals treated with estrogen plus progesterone also received a second injection of this drug 2.5 hr after progesterone. The remaining rats received saline vehicle. After decapitation, trunk blood was collected and LH concentrations were determined by a double antibody radioimmunoassay, using NIAMDD procedures. Levels of catecholamines in the hypothalamus were measured with a radioenzymatic assay.

In the ovariectomized, hormonally untreated rats, SKF64139 significantly decreased plasma LH levels (200 $\pm$ 22 ng/ml vs 288 $\pm$ 18 ng/ml), and completely suppressed the surge of LH induced by estrogen plus progesterone (151 $\pm$ 26 ng/ml vs 671 $\pm$ 118 ng/ml). The drug also tended to potentiate the acute inhibitory effects of estradiol alone (115 $\pm$ 13 ng/ml vs 162 $\pm$ 14 ng/ml). The concentrations of EPI in the hypothalamus were significantly reduced by SKF64139 in oil vehicle-(-33%)and in estrogen plus progesterone-treated rats (-35%), but there were no significant changes in hypothalamic concentrations of norepinephrine or dopamine. These results suggest (1) that EPI may participate in the physiological regulation of LH secretion and (2) that the inhibitory effects on on LH release observed with adrenergic antagonists may be due to an action on brain epinephrine systems.

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86.2 OPIATES AND AGGRESSION IN FEMALE DEERMICE. H. Monder, N. Yasukawa* and J. J. Christian*. Dept. of Biological Sciences, State Univ. of New York at Binghamton, NY 13901.

Female deermice (Peromyscus leucopus) show increased levels of aggression against other females during proestrus, late in pregnancy, and during the first week of lactation. These periods are associated with increased serum levels of prolactin (PRL). Injections of PRL produce increased aggressive behavior in female deermice against other female mice (Gleason et al., submitted).

Morphine produces increased levels of serum PRL, while naloxone reduces PRL levels in rodents. To determine if pharmacological manipulation of PRL levels affects aggression, female P. leucopus were injected with either 1.0 or 2.0 mg/kg morphine sulfate, 1.0 mg naloxone HCl, or the drug vehicle. Injected animals were observed, under red light, during the dark part of the illumination cycle. Injections were given ½ an hour before testing. Immediately after injection animals were placed in a 'neutral arena'; a plastic cage with an opaque central divider. A noninjected naive female was placed on the other side of the divider. The central divider was removed and the animals were observed for 10 minutes.

Control animals in diestrus did not show aggressive behavior against the noninjected opponent. Those animals treated with the lower dose of morphine did not differ from controls. All the animals treated with 2 mg/kg morphine sulfate attacked their opponents. However, attacks were initiated only if the opponents approached the treated animal. Morphine treated animals were not aggressive, but also did not show any submissive behavior. They approached and attempted to sniff and groom their opponent.

These studies indicate that morphine and naloxone affect aggressive behavior in mice. Further studies are underway to determine if these behavioral changes are due to the effects of the drugs on hormonal levels.

This research was supported by a grant from the Harry Frank Guggenheim Foundation.

86.3 MORPHINE EXERTS TESTOSTERONE-LIKE EFFECTS IN THE HYPOTHALAMUS OF THE CASTRATED MALE RAT. T. J. Cicero, E. R. Meyer*, S. M. Gabriel*, R. D. Bell*, and C. E. Wilcox*. Dept. of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110

Previous research has indicated that endogenous opioids participate in the regulation of activity in the hypothalamic-pituitary-luteinizing hormone (LH) axis and mediate the negative feedback control exerted by testosterone. If this assumption is correct, then two predictions can be made. First, the effects of testosterone should be competitively inhibited by narcotic antagonists; and, second, opiates should mimic the acute and chronic effects of testosterone in the castrated male rat. The results of the present investigations support both of these predictions. We found that naloxone competitively antagonized the depressive effects of testosterone on serum LH in the castrated rat and, conversely, that testosterone competitively antagonized the LH-releasing properties of naloxone. In addition, morphine and testosterone both depressed serum LH levels in a dose-dependent fashion in the acutely castrated animal. Moreover, morphine was just as effective as testosterone in reversing the castration-induced fall in hypothalamic-LH-releasing hormone (LH-RH), which occurs in the chronically castrated male rat. On the other hand, morphine failed to reverse the long-term changes in pituitary LH content and increase in serum LH, which is consistent with prior observations that morphine affects only the hypothalamic aspect of the hypothalamic-pituitary-LH axis in the male rat. These results thus support the concept that an as yet unidentified opioid-containing neuronal system regulates activity in the hypothalamic-pituitary-LH axis and mediates the effects of testosterone on this axis.

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86.4 FURTHER EVIDENCE THAT REPEATED ADMINISTRATION OF 5-METHOXY-N,N-DIMETHYLTRYPTAMINE TO MALE RATS INCREASES THE SENSITIVITY OF POSTSYNAPTIC SEROTONIN RECEPTORS INVOLVED IN PROLACTIN SECRETION. M.Simonovic and M.Y.Meltzer, Depts. of Psychiatry and Pharmacological and Physiological Sciences, University of Chicago Pritzker School of Medicine, Chicago,Illinois 60637.

Rat prolactin (PRL) secretion can be stimulated through a serotonin(5HT)-dependent process. A potent 5HT agonist, 5-methoxy-N,N-dimethyltryptamine (5DMT) elevates serum PRL levels in male rats. We have recently reported (Eur.J.Pharmacol.58:399,1979) that repeated administration of 5DMT (5mg/kg,ip, every 3 hours for a total of 4 injections) has no effect on resting serum PRL levels 3 hrs after the last pretreatment dose, but it greatly potentiates PRL-releasing effect of a number of 5HT agonists including 5DMT. Stimulation of presynaptic 5HT receptors by 5DMT inhibits the activity of central 5HT neurons. We have hypothesized that repeated 5DMT administration, according to the schedule outlined above, may, by virtue of its greater and longer-lasting presynaptic effects, produce prolonged disruption of central 5HT transmission and lead to increased sensitivity of postsynaptic 5HT receptors.To further test this hypothesis we have studied the effect of this drug treatment on the hypothalamic 5HT levels and turnover. Repeated administration of 5DMT had no effect on the steady-state 5HT levels, but it significantly decreased the rate of hypothalamic 5HT accumulation after the inhibition of brain monoamine oxidase with pargyline (75 mg/kg,ip). These results are consistent with the hypothesis that repeated 5DMT administration decreases the activity of central 5HT neurons. Inhibition of 5HT synthesis with p-chlorophenylalanine (PCPA, 300 mg/kg,ip, 24 hrs earlier) markedly lowered (-70%) hypothalamic 5HT levels. PCPA treatment, like the repeated 5DMT administration, potentiates PRL response to serotonergic stimulation. Repeated administration of 5DMT to PCPA-treated rats did not further potentiate PRL stimulation by 5DMT. Enhanced PRL response to 5HT stimulation was evident after one or four days of repeated 5DMT administration. Similar results were obtained 24 hrs after acute or chronic (200 mg/kg,ip, daily, 4 days) administration of PCPA. These results show that enhanced PRL response to 5HT agonists persists as long as the interference with the serotonergic transmission.

These results are consistent with the hypothesis that repeated 5DMT administration increases sensitivity of postsynaptic 5HT receptors involved in the regulation of rat PRL secretion. The importance of 5DMT dose and inter-dose interval will also be discussed.

86.5 ACUTE ADMINISTRATION OF CLONIDINE ELEVATES PLASMA CONCENTRATIONS OF IMMUNOREACTIVE BETA-ENDORPHIN IN RATS. D.J. Pettibone and G. P. Mueller. Department of Physiology, Uniformed Services University of the Health Sciences, Bethesda, MD. 20014.

Several studies have demonstrated recently that the systemic administration of the alpha-adrenergic agonist, clonidine ($CATAPRES_R$), produces analgesia in rats and mice. Since the release of pituitary opiate peptides has been implicated in the mechanisms of certain forms of analgesia (i.e. stress-induced, acupuncture), we have studied the effects of clonidine administration on plasma levels of beta-endorphin (B-END) as measured by radioimmunoassay. This RIA detects less than 10 pg B-END immunoreactivity and exhibits negligeable cross-reactivity with related peptides including beta-lipotropin (<3%)(Mueller, Endo., A518, 1979).

The subcutaneous (s.c.) administration of clonidine·HCL (0.5 mg/kg) to male, Sprague-Dawley rats (150-200g) maximally raised ($p<0.05$) plasma B-END concentrations from a control value of 0.22 ± 0.04 ng/ml ($\bar{x}$ ± SEM) to 0.59 ± 0.05 ng/ml by 15 minutes post-injection. This rise in plasma B-END, which returned to control levels within 60 minutes after injection, was dose-dependent up to 0.5 mg/kg; larger doses (1 or 2.5 mg/kg, s.c.) had no further effect. The administration of alpha-adrenergic blocking agents, phentolamine (1, 3, or 10 mg/kg,i.p.)or yohimbine (1 mg/kg,i.p.), 30 or 60 minutes, respectively, before clonidine (0.5 mg/kg,s.c.) produced a 50% inhibition of the clonidine-induced elevation in plasma B-END concentrations. In contrast, the rise in plasma B-END produced by clonidine was not altered by a beta-receptor antagonist, propranolol (1 or 5 mg/kg, i.p.), when given 45 minutes before clonidine. None of the adrenergic blocking agents alone significantly influenced basal levels of plasma B-END.

These data suggest that the acute administration of clonidine to rats enhances the release of pituitary B-END partly through the activation of alpha-adrenergic receptors. It is presently unknown whether clonidine is acting directly at the level of the pituitary or at sites within the central nervous system. The extent to which the effects of clonidine on pituitary B-END release may be related to the drug's analgesic properties remains to be determined.

86.6 A POSSIBLE INHIBITION BY ENDOGENOUS OPIATES ON THE PROGESTERONE-INDUCED LH SURGE IN LONG-TERM OVARIECTOMIZED FEMALE RATS. E. Terasawa, L.V. Rubens*, W.E. Bridson* and D.W. Lincoln Wisc. Reg. Primate Res. Ctr., Univ. of Wisc., Madison, WI 53706

It has been suggested that endogenous opiates are involved in controlling the release of oxytocin by a presynaptic inhibitory mechanism (Clarke et al., Nature, 282:746-748, 1979). In order to determine the possible involvement of an opioid mechanism in LHRH release, the effect of naloxone on the progesterone-induced LH surge was examined in ovariectomized estrogen primed rats. Normal cyclic female rats of Sprague-Dawley strain were ovariectomized at one of two times prior to the experiments either 4-6 weeks (long-term OVX) or 2-3 days (short-term OVX). They were housed under controlled illumination, 14 h light (500-1900) and 10 h darkness. All animals were injected s.c. with 5 µg estradiol benzoate at 1200 (day 0) and received an indwelling catheter implantation in the external jugular vein under ether anesthesia between 1000-1200 on day 1. On day 2, 1.0 mg progesterone was injected s.c. at 1200, and naloxone was administered at 1400, 1600 and 1800. Serial blood samples (0.5 ml) were obtained through the catheters at 1130, 1300, 1430, 1630, 1830, and 2030, and plasma LH was measured by means of RIA. Baseline LH values before progesterone injection in long-term OVX animals were higher than those in short-term OVX animals. A progesterone-induced LH surge attained its peak at 1630 in both long-term OVX and short-term OVX controls. The magnitude of the LH surge in both control groups did not differ. Naloxone injections, however, greatly enhanced the magnitude of the progesterone-induced LH surge in long-term OVX animals ($df = 1,15$, $F = 6.25$, $p < 0.025$, ANOVA2), whereas, it did not induce significant effects in short term OVX animals ($df = 1,13$, $F = 1.87$, $p > 0.10$).

Therefore, it is suggested that endogenous opiates inhibit the progesterone-induced LH surge in long-term ovariectomized estrogen-primed rats. However, the reason for the lack of response to naloxone in short-term ovariectomized animals, remains unknown.
(Supported by NIH Grants RR-00167 and HD-11355.)

86.7 EFFECTS OF DIAZEPAM WITH XYLAZINE ON GONADOTROPIN RELEASE IN THE FEMALE RAT. J.A. Keller-Halbe, B.K. Jenkin *, D.W. Lincoln and E. Terasawa. Wisc. Reg. Primate Res. Ctr., Univ. of Wisc., Madison, WI 53706

Because most anesthetic drugs, such as barbiturates and urethane, interact with neurosecretory cells in the medial preoptic area and the hypothalamus, suitable anesthetics for neuroendocrine research have yet to be established. Since the combination of diazepam, a sedative, and xylazine (α_2 - adrenergic agonist), a nonnarcotic analgesic, does not block suckling-induced oxytocin release (Lincoln, unpublished), an attempt was made in the present study to determine whether this anesthetic combination would be suitable for research in reproductive neuroendocrinology. In the 1st experiment, effects on spontaneous ovulation of the combination of xylazine and diazepam were compared to the effects of diazepam in combination with fentanyl, a narcotic analgesic. Regular 4 and 5 day cyclic rats, confirmed by daily vaginal smears, were housed in a room lighted 14 h (500-1900 h) and determined for critical period of ovulation on the afternoon of proestrus by pentobarbital injections. Administrations of 6 mg/kg diazepam and 6 mg/kg xylazine before the critical period induced anesthesia for 179 ± 3 min (N=11 ataxia and no withdrawal reflex), but did not block spontaneous ovulation (9 of 11 animals ovulated, 12.6 ± 0.6 ova/ovulating animal were counted). In contrast, injections of 12 mg/kg diazepam and 60 µg/kg fentanyl induced a similar period of anesthesia, 189 ± 12 min (N=8), and blocked spontaneous ovulation (1 of 8 animals ovulated). In the 2nd experiment, effects of diazepam and xylazine on the release of LH were investigated in intact proestrous animals. Intact rats were injected with both drugs before the critical period, decapitated at 1730, 1930 and 2130, and trunk blood was collected. For controls, animals were decapitated at 1300, 1730, 1930 and 2130. Serum LH was measured by RIA. The LH surge occurred in controls at 1730 and 1930, while it did not occur until 1930 or later (2130) in drug injected animals. Diazepam and xylazine thus induce a delayed LH surge in intact rats.

Therefore, neither the combination of diazepam and fentanyl nor diazepam and xylazine are useful for the study of hypothalamic control of gonadotropin release. However, the present experiment indicates that diazepam and xylazine anesthesia suppresses hypothalamic neurosecretory cells selectively, e.g. the LHRH neuronal system.
(Supported by NIH Grants RR-00167 and HD-11355.)

86.8 MELATONIN MODULATES RAT PROLACTIN SECRETION BY STIMULATING CENTRAL SEROTONIN RECEPTORS. M.Y.Meltzer, M. Simonovic and V.S. Fang*, Depts. of Psychiatry and Medicine, University of Chicago Pritzker School of Medicine, Chicago, Illinois 60637.

A number of serotonin (5HT) analogs known to stimulate central 5HT receptors elevate rat serum prolactin (PRL) levels. Melatonin (M), an O-methoxylated 5HT derivative synthesized by the pineal gland, stimulates rat PRL secretion. In order to assess possible 5HT agonist properties of M we have compared its effects on PRL secretion in male rats with those of 5-methoxy-N,N-dimethyltryptamine (5DMT), a known 5HT agonist. M(50 mg/kg,ip) or 5DMT (10 mg/kg,ip) produced marked but short-lasting rise in serum PRL levels. PRL levels were maximally elevated 15 min and returned to baseline 30 min after the treatment. M (10,25 or 50 mg/kg,ip) or 5DMT(1, 5 or 10 mg/kg,ip) stimulate PRL secretion in a dose-related manner. The D-R curves for the two compounds were parallel; 5DMT is about five times more potent than M. Pretreatment with a 5HT antagonist,mianserin (5 mg/kg,ip, 30 min earlier), blocked the PRL-releasing effect of both drugs. Inhibition of 5HT synthesis with p-chlorophenylalanine (300 mg/kg,ip, 24 hrs earlier) markedly lowered (-70%) hypothalamic 5HT levels and greatly enhanced PRL response to M or 5DMT. This effect is probably due to increased sensitivity of 5HT receptors secondary to 5HT depletion. These results suggest that M, like 5DMT, increases PRL secretion by direct stimulation of postsynaptic 5HT receptors.

Repeated administration of 5DMT or M (5 mg/kg,ip, every 3 hrs for a total of 4 injections) had no effect on resting serum PRL levels in male rats 3 hrs after the last pretreatment dose, but it greatly potentiated PRL stimulation by 5DMT, a potent 5HT agonist. Stimulation of presynaptic 5HT receptors by 5DMT inhibits the activity of 5HT neurons in the brain.We have recently hypothesized (Eur.J.Pharmacol.58:399,1979) that repeated 5DMT administration, according to the schedule outlined above, may, by virtue of its greater and longer-lasting presynaptic effects, produce prolonged disruption of 5HT transmission and lead to increased sensitivity of postsynaptic 5HT receptors. The effect of M on the activity of central 5HT neurons is not known. M-induced increase in midbrain 5HT levels may reflect its ability to decrease the activity of 5HT neurons. If the above hypothesis is correct, the fact that much lower dose of M is needed to alter PRL response to 5HT agonists than to stimulate PRL release suggests that M may have greater affinity for pre- than for postsynaptic 5HT receptors. These findings also suggest that endogenous M rhythm may participate in the regulation of postsynaptic 5HT receptors by altering the activity of central 5HT neurons.

86.9 THE EFFECTS OF INTRAVENTRICULAR AND INTRAVENOUS P-CHLOROAMPHETAMINE ON URINE VOLUMES AND ELECTROLYTE BALANCE. J. M. Stein, R. W. Lind, & A. K. Johnson. Department of Psychology and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242

Systemic administration of any of several aralkylamines (phenylisopropylamines) initiates changes in fluid and electrolyte balance. Diuresis and natriuresis has been shown to occur in rats following amphetamine, methamphetamine, fenfluramine and phentermine and following amphetamine in humans. Whether these effects are exclusively the result of peripheral effects or due to CNS actions is unclear. P-chloroamphetamine (PCA) is an amphetamine derivative, the acute and chronic actions of which on brain monoamines, and food and water intake have been well documented. However, the effects of PCA on fluid and electrolyte balance has received little attention.

The following experiments were performed in order (a) to determine if PCA shares these same diuretic and natriuretic properties, and (b) to determine the relative importance of the CNS vs. the periphery in initiating these effects.

In Experiment 1, nonhydrated unanesthetized rats were systemically administered (i.p.) either PCA (5 mg/kg), fenfluramine (20 mg/kg), pargyline and tryptophan (75 and 180 mg/kg), pargyline (75 mg/kg), tryptophan (180 mg/kg) or saline. Urine volumes, urine Na and K concentrations, fecal weights, and body weights were recorded during the 4 hr post-injection period. A diuresis and a natriuresis was seen following both PCA and fenfluramine.

In Experiment 2, rats were implanted with intraventricular cannulae, jugular catheters, and urinary bladder catheters. A hydrating solution (10 mM NaCl; 4 mM $NaHCO_3$; 130 mM Glucose) was continuously infused into the jugular catheter during the experiment. Following a baseline equilibration period, the unanesthetized rats were administered PCA either intraventricularly (600 µg) or intravenous (i.v.; 5 mg/kg). Intraventricular administration produced an antidiuresis and concomitant natriuresis. In contrast, i.v. administration of PCA produced a diuresis and natriuresis similar to that seen following i.p. administration. The results of Experiment 1 and 2 will be discussed with respect to the biogenic amine and hormonal basis of these effects. (USPHS NIH HLP-14388 & 1 RO1-H124102; NIMH 1-KO2-MH00064)

86.10 BEHAVIORAL EFFECTS OF TRICYCLIC ANTIDEPRESSANTS IN THE RAT AND THEIR MODULATION BY GONADAL HORMONES. A. Biegon* and D. Samuel. (SPON: B.S. McEwen). Weizmann Institute of Science, Rehovot, Israel.

The direct effects of tricyclic antidepressants (TAD) on several behavioral and physiological parameters were studied in male and female rats, in an attempt to correlate these with brain uptake of TAD, which is higher in females than in males, and maximal on the day of estrus (Biegon, A. and Samuel, D., Psychopharmacol. 65:259, 1979). Desipramine and amitriptyline were injected i.p. (10 or 15mg/kg/day) for 14 days. 30min. after the first injection, the animals were observed for 5min. in an 80x80x40cm open field, or subjected to electric footshock of increasing intensity to determine their pain threshold. Total food and water intake was measured the following morning. Both drugs caused decreased locomotion and grooming in an open field: to 60% of control values in males, to 40% in proestrous and diestrous females and to 25% of control in estrous females. This correlates well with brain uptake of TAD. The pain thresholds (in mamp) of the control groups were 1.4 in males, 1.7 in diestrous and 2.0-2.2 in proestrous-estrous. TAD caused a 25-35% increase in pain threshold (comparable to the effect of 10mg/kg morphine in the same paradigm) in all groups. This may mean that the females' pain thresholds, already elevated by circulating hormones, could not be much further elevated by TAD. However, the duration of analgesia was longer in females. Food intake was decreased by TAD; in males by 50%, in diestrous females by 20-30% and in estrous females the decrease was less than 10% and not significant. Thus, the effects of TAD on food intake do not correlate with their brain uptake, but, rather with pretreatment levels of this behavior. On estrous, food intake is already reduced by 50% as compared to males, and it seems that the effects of TAD and circulating gonadal hormones are not additive. The course of the estrous cycle was not affected by a single injection of TAD. However, after 5-7 days of chronic treatment, prolonged diestrous appeared in 10/21 treated females and persistent estrous in 5/21. We conclude that the magnitude and duration of TAD effects are modulated by gonadal hormones, which influence the drugs' metabolism in the liver, but also affect the neurotransmitter systems in the brain at which TAD exert their central action (e.g. Crowley,W.E., O'Donohue,T.L. and Jacobovitz,D.M. Act. Endocrin.89:20,1978). Modulation of drug response by gonadal steroids seems to be a general phenomenon (e.g. Chiodo,L.A., Caggiula,A.R. and Saller,C.F. BRES 172:360,1979) and has to be considered when testing new drugs.

86.11 PHARMACOLOGICAL ISOLATION OF HYPOTHALAMIC DOPAMINE NEURONS. Susan R. George* and Glen R. Van Loon, Departments of Medicine and Physiology, University of Toronto, Toronto, Canada.

Study of hypothalamic(HYP) dopamine(DA) neurons involved in neuroendocrine regulation is complicated by the fact that they constitute only a small percentage of the HYP aminergic neurons. Also, it is not possible to clearly differentiate in hypothalamus precursor DA in NE neurons from neurotransmitter DA in DA neurons. Furthermore, uptake of DA occurs not only into DA neurons, but also into the more predominant population of NE neurons in hypothalamus. Study of median eminence alone compared to whole hypothalamus improves localization with regard to study of HYP DA neurons, but does not obviate the above problems and introduces the additional technical problem of requiring dissection of many animals for each study. To circumvent these difficulties, we have attempted to isolate HYP DA neurons pharmacologically in order to provide a means by which the effects of physiological alterations of neuroendocrine parameters on HYP DA neurons may be studied. We have characterized the uptake of ^{3}H-DA into a HYP synaptosomal preparation. Desmethylimipramine(DMI) $5x10^{-7}$M, was added to block DA uptake into NE nerve terminals. This concentration of DMI had no effect on ^{3}H-DA uptake into synaptosomes(SYN) from striatum which contains very few NE neurons and a large number of DA neurons. In contrast, DMI produced a profound inhibitory effect on ^{3}H-DA uptake into cerebellum which contains a significant number of NE neurons and almost no DA neurons. SYN from hypothalamus were intermediate in showing inhibition of ^{3}H-DA uptake by DMI. The DMI-insensitive ^{3}H-DA uptake into HYP SYN showed a further dose-related reduction with addition of benztropine, a drug known to block catecholamine uptake into DA neurons. Thus, DMI-insensitive ^{3}H-DA uptake into HYP SYN probably represents DA uptake into HYP DA neurons. Although HYP DA neurons include a variety of DA neuronal systems (A12,A13,A14),a large percentage of these neurons appears to be comprised of the tuberoinfundibular (A12) DA neurons. DMI-insensitive ^{3}H-DA uptake into HYP SYN may then provide an index of DA uptake into tuberoinfundibular DA neurons. Lineweaver-Burke analysis yielded an affinity constant (Km) of $0.79\pm.07x10^{-6}$M for DMI-insensitive ^{3}H-DA uptake into HYP SYN compared with $0.26\pm.02x10^{-6}$M for striatal SYN. These data showing a 3-fold lower affinity constant for ^{3}H-DA uptake into HYP DA neurons are similar to findings of Demarest and Moore (Brain Res.171:545,1979). However, they do not support the conclusion that these neurons lack a high affinity amine transport mechanism but rather suggest that the efficiency of their DA transport mechanism is lower than that of other brain DA neuronal systems. CONCLUSION: DMI-insensitive HYP DA uptake appears to provide an index of DA uptake into tuberoinfundibular DA neurons.

87.1 THE EFFECT OF CHRONIC UNDERNUTRITION FOR 10 GENERATIONS ON BRAIN DEVELOPMENT IN YOUNG AND AGING RATS. Stephen Zamenhof and Edith van Marthens*. Mental Retardation Research Cntr., and Brain Research Inst., UCLA School of Medicine, Los Angeles, CA., 90024.

In the previous communication (Zamenhof and van Marthens, J. Nutr., 108, 1719, 1978) we have reported the effects of chronic undernutrition over five generations on newborn rats. In the present work we have studied the effect of chronic undernutrition over ten generations on rat brain development in neonatal, adolescent (30 days) and aging (150 days) rats. The animals and their nutrition regimes were described previously: undernutrition (2/3 of normal (control) ad libitum diet) started at the time of mating of Fo (maternal) generation, and continued throughout pregnancy and post-weaning.

In the newborns, chronic undernutrition resulted in highly significant reduction of brain parameters in almost all generations. The reduction in the weight of cerebral hemispheres was up to 21% (F_4 generation) and decreased to 13% in F10. In contrast, the reduction in cerebral DNA (cell number) was severe (16 to 19%) only in the first two generations and disappeared in F8 and F10. The reduction in cerebral protein was severe in almost all generations, and most severe in F10 (26%).

In adolescent animals (30 days old males) individual brain parts could be analyzed separately. The reductions in cortical weight were moderate (up to 10%) as compared with the reduction in cortical DNA (up to 29%) and cortical protein (up to 32%); all reductions persisted through F10. Cerebellar and brain stem reductions were, in general, less pronounced, but the reductions in diencephalon were up to 18% in weight, up to 27% (F_4) in DNA and up to 39% (F_{10}) in protein.

In aging animals (150 day old females) increases rather than decreases were found in cortical weight and DNA (in most generations); they may represent multiplication of glial cells in an attempt to compensate for permanent neuron deficit caused by prenatal undernutrition. On the other hand, diencephalon still shows full reductions in weight (up to 33%), DNA (up to 33%) and protein (up to 30%)

If one adds, for comparison, the deficits in individual brain parts, one finds that the deficits in brain weight and protein essentially do not change between 30 and 150 days, but DNA deficit decreases by 68%. There was no consistent pattern of changes of deficits through generations; in general the deficits were lower in the last five generations than in the first five for brain weight and DNA ("adaptations"), but the reverse was true for protein. There was no cumulative effect of this undernutrition on offspring parameters over generations.

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87.2 Behavioral and neurochemical effects of prenatal imipramine exposure. K.M. Jason and E. Friedman*, Dept. of Psychiatry, New York Univ. Med. Ctr., New York, N.Y. 10016.

Tricyclic antidepressants (TCA's) are lipid-soluble substances which readily cross the placenta. In adult rats, chronic exposure to TCA's alters neurotransmission, and when exposure takes place throughout pregnancy, the number of live offspring is decreased. These experiments investigated effects of TCA exposure during late gestation on behavioral and neurochemical development in neonatal rats.

Control and experimental animals (8/gp) received 1 ml/100g oral doses of water or 15mg/kg imipramine-HCl (IMI) daily on days 8 through 20 of gestation. On the day following birth, pups were randomly distributed within treatment group, 9 per dam. Control females had a 52% weight gain from day 8 to day 20 of gestation compared to a 40% weight gain for IMI-treated dams ($p<.01$). Neither the number of pups born per litter nor the number of stillborn pups was affected by the IMI treatment. IMI-treated pups weighed significantly less than controls day 1 and 7 postpartum, but not on days 14, 21 or 30. Brain weights of treated offspring were also significantly lower than controls on days 7 and 14, but were equivalent at day 30.

The development of 3 reflexes was assessed in pups 3-10 days old. The appearance of surface righting was significantly delayed in IMI-treated offspring. This reflex was observed daily in 2 trials per day in which each rat was placed on its back on a smooth surface and timed until it righted itself and all 4 feet were in contact with the surface in $\leq$ 2" on both trials on a given day. Negative geotaxis, completing a 180° turn on a 25° inclined plane in $\leq$ 60", was also altered. Treated offspring took significantly longer to complete the task even after all animals reached criterion. There were no group differences in the cliff avoidance reflex, which consisted of sideward or backward movement away from a table edge in $\leq$ 30".

Offspring were examined for eye-opening as a developmental landmark. The percentage of pups with eyes open was significantly higher in the IMI-treated group at 13 and 14 days; by day 15, eye-opening was nearly complete in both groups.

Steady-state levels of dopamine (DA), noradrenaline (NA) and adrenaline (A) were measured in hypothalamus of offspring 7, 14 and 30 days old. As is the case for NA and DA in adults after chronic TCA exposure, the prenatal exposure to IMI did not alter levels of either DA or NA, nor were A levels affected at these ages. Experiments are now in progress to determine the effects of prenatal IMI exposure on specific binding to α and β-adrenergic receptors.

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87.3 DEVELOPMENT OF CATECHOLAMINERGIC SYSTEMS IN THE HUMAN FETUS. J. Pearson, L. Brandeis*, M. Goldstein and K. Markey*. New York University Medical Center, New York, NY 10016.

A highly specific antibody for human tyrosine hydroxylase(TH) is used immunocytochemically to detect the enzyme. In the developing human sympathetic chain TH is present at 5½-6 weeks of gestational age. By 9-10 weeks sympathetic ganglia are well developed and contain increased amounts of the enzyme. At this age sympathetic plexi and axon terminals can be demonstrated. The cells migrating from paraganglia into the adrenal medulla are also positive (only on reaching the adrenal do these cells acquire immunoreactivity for phenylethanolamine N-methyl transferase). The carotid body glomus cells also become positive for TH at this stage. Within the central nervous system medullary, locus coeruleus and substantia nigra neurons as well as the rich axon terminal complex in the striatum contain the enzyme. Thus the major catecholaminergic systems are developed before the end of the first trimester.

By 12-13 weeks the sympathetic axons acquire more reactivity than the perikarya; these are the staining characteristics of adult catecholaminergic neurons. The ductus venosus attains rich sympathetic innervation. Older fetuses are being studied with reference to innervation of the ductus arteriosus.

The peroxidase anti-peroxidase technique of Sternberger works well in stored, paraffin embedded, formalin fixed tissue. It is thus applicable to archival specimens. It will provide valuable information regarding biochemical ontogenesis.

87.4 DEVELOPMENT OF A DESCENDING ADRENERGIC PATHWAY IN THE CHICK SPINAL CORD. M. T. Caserta and L. L. Ross. Dept. Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Previous work from this laboratory has described the developmental sequence of the descending serotonergic and noradrenergic pathways in the chick spinal cord that originate in the medulla and terminate in the region of the preganglionic sympathetic nucleus (nucleus of Terni) and on ventral motor neurons. The serotonergic terminals first appear between 8-10 days in ovo and the noradrenergic terminals at 12-14 days in ovo.

As part of a continuing investigation of the role of the descending aminergic pathways in the development of the chick spinal cord, the possible existence of an adrenergic pathway was investigated since several studies have shown the presence of epinephrine and PNMT (phenylethanolamine-N-methyl transferase) containing neurons in the mammalian and avian central nervous systems. The methods used were: 1) in vitro uptake of ^{3}H-epinephrine in the presence or absence of various drugs; 2) a modification of the PNMT assay as described by Saavedra et al. 1974; 3) spinal cord transection at 12 days in ovo at T_1-T_2 levels followed by the above procedures after 4-6 day survival.

There is a specific, saturable uptake mechanism for epinephrine in the adult spinal cord with a Km of 6×10^{-7}M and a Vmax of .02nM/gm/min. This uptake mechanism can be abolished by ouabain (10^{-4}M) and reduced to 50% of control by desmethylimipramine (10^{-7}M). Norepinephrine at 10^{-5}M reduces uptake by 50% but at 10^{-7}M (10x the concentration of ^{3}H-epinephrine) has no effect. Serotonin (10^{-7} to 10^{-5}M) also has no effect. This uptake mechanism can first be detected at 14 days in ovo. There is a 2-3 fold increase at 18-20 days followed by a decline until 4 days posthatching at which time there is another peak which lasts until 7 days and then gradually declines to adult levels. When the spinal cord is transected at 12 days in ovo, there is a reduction of uptake to between 14-32% of control by 16-18 days in ovo below the lesion.

PNMT activity can first be measured at 14 days in ovo (1.7 pm/hr/mg protein) and gradually increases until approximately 3-4 days posthatching when maximal values are attained (26 pm/hr/mg protein) followed by a gradual decline to adult levels.

Thus, there appears to be a separate adrenergic uptake mechanism which is markedly reduced by transection and which has a difference developmental pattern from the noradrenergic and serotonergic mechanisms. The presence of PNMT in the spinal cord at the time that an adrenergic uptake mechanism is also present supports the hypothesis there is a distinct descending adrenergic pathway in the chick spinal cord.

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87.5 EFFECT OF FETAL LESION WITH METHYLAZOXYMETHANOL ACETATE ON THE DEVELOPMENTAL NEUROCHEMISTRY OF THE RAT STRIATUM AND FRONTAL CORTEX. M. Beaulieu* and J.T. Coyle. Dept. of Pharmacol. and Expt. Therapeut., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Methylazoxymethanol acetate (MAM), a potent alkylating agent, when administered to the rat fetuses at the beginning of the last third of gestation, causes a selective reduction of neurons intrinsic to forebrain . We have examined the effects of this lesion on the development of neuronal markers for GABAergic, cholinergic and dopaminergic neurons in striatum and frontal cortex following a single intraperitoneal injection of 20 mg/kg MAM to pregnant dams at 15 days of gestation. At birth and throughout maturation, the body weights of treated offspring did not differ from controls.

Striatum was dissected according to white matter landmarks. The weight of the MAM lesioned striatum was reduced by 18% in the neonate but by 37% ($p<0.001$) in the adult. Nissl-stained sections of the adult MAM striatum revealed a reduction in mass with no apparent disruption of neuronal organization. The markers for dopaminergic terminals - dopamine and tyrosine hydroxylase (TH) - were increased in concentration by 31 and 38% ($p<0.01$) respectively in the adult striatum; this increment was constant throughout postnatal development. In contrast, the presynaptic markers for GABAergic neurons - glutamate decarboxylase (GAD) and GABA - were unchanged in concentration from birth to adulthood. Whereas the specific activity (sp. ac.) of choline acetyltransferase (CAT) was consistently increased by 40%, the concentration of acetylcholine was not elevated.

The lesion resulted in a 53% ($p<0.001$) reduction in frontal cortical weight in the adult but only 37% ($p<0.01$) decrease in the neonate. Nissl-stained sections revealed loss of neurons in the intermediate layers of the frontal cortex. In the adult, the sp. ac. of TH was increased by 52% ($p<0.01$) in the neonate and by 82% ($p<0.001$) in the adult. Although CAT sp. ac. was increased by 44% ($p<0.001$) in the adult, it was slightly depressed(-13%) in the neonate; the increase in the sp. ac. of CAT became apparent by10 days after birth in the lesioned cortex. The sp. ac. of GAD was not altered by the lesion in the neonate or the adult.

These studies indicate that the development of dopaminergic projections to striatum and frontal cortex are insensitive to a fetally induced loss of neurons within these regions; regional hypoplasia results in a relative hyperinnervation by dopaminergic terminals, a phenomenon present at birth. In contrast, the intrinsic GABAergic neurons are reduced commensurate with the loss of cortical or striatal volume. Cholinergic neurons intrinsic to the striatum differ from basalis projection to frontal cortex in their response to the lesion.

87.6 SURFACE GLYCOCONJUGATES CHANGE IN DIFFERENTIATING NEUROECTODERM. J.R. Currie, M.-F. Maylié-Pfenninger* and K.H. Pfenninger. Department of Anatomy, Columbia University, College of Physicians and Surgeons, New York, New York 10032.

Different cell surface saccharide compositions have been described for neural crest and neural tube derivatives at the stage of neurite formation. We have examined the neuroectoderm of rat embryos at 9 1/2 days of gestation to determine whether these differences are present at earlier stages. Our probes are several well-characterized lectin-ferritin and lectin-HRP conjugates (Con A, WGA, RCA I and II, SBA, LTA and PA). The neuroectoderm is pretreated with collagenase and hyaluronidase or high ionic strength solutions to remove glycoconjugates not integral to the plasmalemma, incubated at 0°C with saturating concentrations of the lectins, rinsed, and processed for EM. Control tissues (incubated with the lectins and 0.2M hapten sugar) are free of the label. In some experiments, the neural tube region which has already fused is cut open before the application of the markers. The apical plasmalemma of both neural crest and neural tube cells is very rich in binding sites for 4 of the lectins: Con A, WGA, RCA I and II (monosaccharide spec.: glc/man, glcNAc, gal, galNAc, respectively). The neural crest cells bind the lectins more heavily than the neural tube cells (approx. 12,000 WGA binding sites/μm^2 of plasmalemma for crest cells versus 7000 for tube cells). These values can be compared to more mature derivatives of the crest and tube: an average of 2500 for sprouting dorsal root and superior cervical ganglion cells and 900 for sprouting spinal cord neurons, respectively. No binding sites are present on crest or tube cells for SBA, LTA or PA (monosaccharide spec.: galNAc, fuc, gal, respectively). Pre-exposure of the ectoderm to high ionic strength solutions does not change the pattern or density of the markers, indicating the integral nature of the plasmalemmal lectin receptors. A marked decrease in the number of binding sites for WGA, RCA I and II is observed to occur as fusion takes place. Few or no binding sites are observed even after artificially reopening the fused tube. Con A, however, continues to bind to the apical plasmalemma of the fused tube. Therefore, the apical surfaces of neuroectodermal cells at the onset of neurulation contain glycoconjugates rich in receptors for a variety of lectins, but not for LTA, SBA or PA. Crest and tube derivatives can already be distinguished at this stage by their surface sugar composition. As neurulation takes place, changes in membrane saccharides occur. Thus, the dissociation of the peripheral from the central nervous system may involve the development of a dichotomy in the surface properties of crest and tube cells, and subsequent developmental stages of the derivatives of the crest and tube are reflected in changes in the composition of their cell-surface saccharides. (Supported by NSF BNS78-15910.)

87.7 SYNCHRONOUS MATURATION OF SOMATOSENSORY ISOCORTEX AND SOMMER'S SECTOR OF AMMON'S HORN: QUANTITATIVE HISTOCHEMICAL STUDY OF CELL REPLICATION, DIFFERENTIATION, AND MYELINATION IN THE RAT. S.T. DeKosky and N.H. Bass, Department of Neurology, University of Kentucky, College of Medicine and Lexington VA Medical Center, Lexington, Kentucky 40536.

Frontal association isocortex and Sommer's sector of Ammon's horn have been associated with a wide variety of disorders of intellectual function in man, some of which have been attributed to selective vulnerability during critical periods of perinatal life. In an effort to explore the developmental synchrony of these regions in the albino rat brain, we have compared postnatal neural cell migration and differentiation in the six-layered somatosensory area of cerebral cortex with the three-layered CA1 region of hippocampus. Expression of biochemical components per unit volume of fresh-frozen tissue reveals that in both instances DNA initially declines (83% during the first 10 days of cortical maturation; 50% between 5 and 20 days of hippocampal maturation) due to decrement of cell packing density and cell death. Protein synthesizing capacity per cell, reflected as RNA/cell, increases 3-fold at 10 days of cortical maturation and 2-fold at 20 days of hippocampal development. This is a reflection of marked metabolic activity of neuronal perikarya associated with massive axodendritic arborization. Ganglioside sialic acid, a index of axodendritic proliferation peaks at 20 postnatal days in cortex (4-fold increase) and hippocampus (2-fold increase) paralleling maturation of Golgi-staining neurons,cortical electrical activity and behavior. Although the cerebral cortex reaches adult laminar size by 10 postnatal days, in the CA1 region maximum depth of apical and basal dendritic lamina of the pyamidal cells is attained by 20 postnatal days. Beginning on day 10 in cortex and day 20 in hippocampus, there is a continuous acquisition of small cells, probably representing replication and differentiation of oligodendrocytes. Accordingly, galactocerebroside, an index of myelination, increases 6-fold between 20 and 50 postnatal days in both brain regions. These studies show a marked synchrony in regional maturation of cerebral cortex and hippocampus with respect to neuronal differentiation and myelinogenesis associated with glial cell replication and differentiation. Intellectual deficits induced by perinatal environmental insults previously ascribed solely to cortical vulnerability, may in fact be due to abnormalities of both cortex and hippocampus and their integrated activity. (Supported in part by NIH Grant NS16009.)

87.8 Developmental changes in cerebral energy metabolism in the chick embryo. T. Gonya-Magee and R. C. Vannucci.* Dept. of Pediatrics; M. S. Hershey Medical Center, Pennsylvania State Univ.; Hershey, PA 17033

In mammals, cerebral metabolic rates are low at birth and increase with postnatal age. The low energy demands of the immature brain are thought to favorably influence its resistance to hypoxia. Rates of cerebral metabolism have not been thoroughly studied in the fetus or embryo, even though it is often quoted that the developing brain possesses a high glycolytic capacity to sustain energy requirement. We have investigated oxidative metabolism in brains of chick embryos at 9, 14, 16 and 19 days of incubation and in 1 day post-hatch peeps. Chicks were decapitated at 37°C and frozen in freon (-197°C) at 0, ½, 1, 2 and 5 minutes post-sacrifice. Brains were dissected, powdered and extracted into 3M perchloric acid, and were assayed for ATP, P-Creatine, lactate and glucose by fluorometry. Changes in these metabolites post-decapitation were used to calculate cerebral metabolic rates [$\Delta\sim P = 2\Delta ATP + \Delta PCr + 2\Delta glucose + 1.45\ (\Delta lactate - 2\Delta glucose)$] as well as rates of maximal glycolytic flux (Δlactate):

Embryonic Age (days)	Δlactate (mmole/kg/min)	$\Delta\sim P$ (mmole/kg/min)	$\frac{\Delta lactate}{\Delta\sim P}$
9	1.00	2.90	0.34
14	1.55	3.10	0.50
16	2.15	2.90	0.74
19	2.70	5.50	0.55
22 (1 day post-hatch)	4.75	8.65	0.55

The steady increases in Δlactate were different ($p < 0.05$) from each preceding age group. In contrast, $\Delta\sim P$ did not change from embryonic day 9 through 16 but thereafter increased 3-fold through the first post-hatch day ($p < 0.01$). The percent of total energy utilization ($\Delta\sim P$) that could be derived from glycolysis (Δlactate) increased from day 9 through 16 and subsequently declined. The relative peak in the potential glycolytic contribution to energy utilization at day 16 correlates temporally with the maximum resistance of the embryonic CNS to hypoxic-induced inhibition of neural activity (Gonya-Magee and Stokes, 1980). The findings also suggest that anaerobic glycolysis is never capable of totally supporting the metabolic requirements of the embryonic brain, and that a capacity for oxidative phosphorylation is required by primordial tissue.

87.9 GABA AND GLUTAMATE FLUXES IN DEVELOPING NERVE ENDING PARTICLES. R. Hitzemann, C. Mark* and A. Panini*. Lab. of Psychobiology, Dept. of Psychiatry, Univ. of Cincinnati Coll. of Med., Cincinnati, OH 45267.

It is still unresolved as to whether or not the development of the high affinity GABA transport (uptake) system can be used as a marker of neuronal and synaptic maturation. Some workers have suggested that a significant portion of the transport occurs into gliosomes which contaminate immature nerve ending preparations (Levi et al, J. Neurochem. 33, 1043, 1979). Redburn et al (Brain Res. 152, 511, 1978) have found that GABA taken up into immature nerve endings is not taken up into a functionally releasable pool. We have suggested (Hitzemann and Loh, Brain Res. 159, 29, 1978) that it is difficult to assess the competency of developing transport systems unless special precautions are taken in the preparation of the developing nerve ending particles. In the present study we have examined various aspects of GABA and glutamate transport and release in both the granular (type I) and mature (type II) appearing nerve endings found in the developing rat cortex. For type II nerve endings the IC_{50} values to inhibit GABA transport were 1.15, 3.6 and $2.3X10^{-3}M$ for B-alanine (a glial GABA transport inhibitor) and 7.1, 9.2 and $6.6X10^{-5}M$ for diaminobutyric acid (a neuronal GABA transport inhibitor) in 7 and 14 day and adult animals, respectively. Type I nerve endings had similar IC_{50} values. Compared to the adult preparation, 7 day type II nerve endings showed a greater Na^+ dependent change in the Km values for both GABA and glutamate transport. In contrast, the Vmax values for transport were less Na^+ dependent in the 7 day animals. The Km but not Vmax values for GABA and glutamate transport showed greater temperature dependent changes in 7 day animals. These effects on Km were most obvious at low (19mM) Na^+ concentrations. Many of the differences in transport between 7 day and adult nerve endings are probably related to the differences in membrane fluidity (see Hitzemann and Johnson, Fed. Proc. Abst. #2390, 1980) since it was possible to duplicate the transport properties of 7 day type II nerve endings by fluidizing adult nerve endings with cis-vaccenic acid. Type II but not type I nerve endings show a Ca^{++} dependent K^+-induced release of pre-accumulated labeled GABA. Overall it is concluded that (a) GABA transport in our preparation of type I and II nerve endings is not occurring into gliosomes, (b) many of the developmental differences on transport are related to the differences in synaptic membrane viscosity, and (c) some but not all developing nerve endings can sustain a Ca^{++} dependent release of pre-accumulated GABA. This study was supported in part by grant NS-16061.

87.10 ONTOGENETIC DEVELOPMENT OF GLUTAMATE RECEPTORS IN RAT HIPPOCAMPUS. M. Baudry*, D.S. Arst*, M. Oliver*, and G. Lynch. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717

Various groups have reported the existence in the adult rat brain of a class of Na-independent glutamate binding sites which exhibit characteristics of postsynaptic glutamate receptors. We previously showed that these sites are markedly labile and that Ca^{++} ions are able to increase the number of glutamate receptors possibly by stimulating a Ca^{++}-sensitive protease (Baudry and Lynch, Proc. Nat. Acad. Sci., 1980, in press). We now report the developmental pattern of both ^3H-glutamate binding and the Ca^{++}-induced stimulation of the binding in rat hippocampal membranes.

The amount of Na-independent glutamate binding sites (pmol/hippocampus) represents 4% of the adult level at postnatal day (PND) 4, increases very rapidly until PND 9 and then increases at a slower rate reaching 80% of the adult value at PND 23. In contrast, the density of binding sites (pmol/mg protein) exhibits a maximum at PND 9 and slowly decreases to reach the adult value at PND 23. The changes are only quantitative since the affinity and Hill coefficient of these binding sites remain constant throughout development. Ca^{++} is unable to stimulate glutamate binding until PND 8. The stimulatory effect appears and develops rapidly between PND 9 and PND 16 when it is similar to that seen in the adult rat. These changes in the Ca^{++} effect are also only quantitative since no changes in the EC_{50} for Ca^{++} (about 30 μM) nor in the Hill coefficient (n_H=2) occurred between PND 9 and adult. In addition, we determined the minimum age at which long-term potentiation (LTP) of synaptic transmission could be detected in the hippocampal slice preparation following repetitive electrical stimulation of the Schaeffer collateral-commissural system. LTP was only rarely detected at PND 8 whereas it could be reliably obtained after PND 10.

These results indicate that the development of Na-independent glutamate binding sites closely parallels synapse formation in the hippocampus, further supporting the idea that these binding sites are associated with a physiological receptor. In addition they show that the appearance of the Ca^{++} stimulation of the binding occurs at a time when several functional events take place in the hippocampus. The correlation of the development of LTP with the Ca^{++} stimulation of glutamate binding thus strengthens the previously proposed link between these two phenomena (Baudry and Lynch, Exp. Neurol., 1980, 68, 202-205).

Supported by N.I.M.H. grant MH-19793 to G.L.

88.1 IN VIVO SINGLE CELL RECORDING FROM HYPOTHALAMIC NEURONS EXHIBITING MONOAMINERGIC PROPERTIES. S. A. Rasmussen* and B. S. Bunney (SPON: J. Ebersole). Departments of Psychiatry and Pharmacology, Yale University Sch. Med., New Haven, CT 06510

Anatomical studies have demonstrated that monoaminergic neurons are present in a number of hypothalamic nuclei. In an effort to identify and electrophysiologically characterize these neurons, we have begun in vivo studies of these nuclei in the rat, using extracellular single unit recording and microiontophoretic techniques. A homogeneous group of neurons has been identified in the periventricular zone of the posterior hypothalamus. These cells are remarkably similar to norepinephrine containing neurons of the locus coeruleus. They exhibit firing rates of 0.5 to 5 spikes per second and their duration is 3.5-4.5 msec. The shape of their action potentials is biphasic with an initial positive component. The α-agonist, clonidine, induces a 50% decrease in firing rate at a dose of approximately 4 ug/kg (i.v.). This inhibition is rapidly and completely reversed by the α-antagonist, piperoxane (0.5 mg/kg i.v.). The burst-pause firing pattern following foot pinch, characteristic of noradrenergic neurons in the locus coeruleus (Korf, J. et al., Eur. J. Pharm. 25: 165, 1974) is also present in these cells, and has aided in their identification. Histologically, these cells appear to coincide with the group of norepinephrine containing neurons in the hypothalamus described by Björklund et al. (Brain Res. 51: 171, 1973).

A group of neurons has also been found that exhibit a wide, biphasic action potential, a firing rate of 0.5 to 5 spikes/second, and a 50% decrease in firing rate after i.v. administration of apomorphine (8 ug/kg). The inhibition induced by apomorphine is completely reversed by haloperidol (50 ug/kg, i.v.). These cells appear similar to the dopamine containing neurons of A9 and A10 and have all been located in the periventricular zone of the mesencephalo-diencephalic junction. Although cells with firing rates and action potentials characteristic of dopaminergic neurons have also been found in the region of the arcuate nucleus, these cells have shown no response to haloperidol or apomorphine. Further characterization, including the effects of several hypothalmic and pituitary peptides on these various cell types, will be presented. (Supported by NIMH grants MH-28849, MH-25642 and the State of Connecticut.)

88.2 INTRACELLULAR RECORDINGS FROM NIGRAL DOPAMINE NEURONS IDENTIFIED BY L-DOPA INJECTION COMBINED WITH FLUORESCENCE HISTOCHEMISTRY. A. A. Grace and B. S. Bunney. Departments of Psychiatry and Pharmacology, Yale University Sch. Med., New Haven, CT 06510

Intracellular recordings were obtained from positively identified dopamine (DA)-containing neurons in the rat substantia nigra zona compacta. DA cells were identified by intracellular injection of the DA precursor L-dihydroxyphenylacetic acid (L-DOPA) and subsequent processing of the brain for fluorescence histochemistry. DA cells injected with L-DOPA could be identified by their markedly increased fluorescence in contrast to surrounding, non-injected DA cells. Zona reticulata (non-DA) cells injected with L-DOPA did not fluoresce. As only DA cells in the area possess the enzyme necessary to convert L-DOPA to DA (aromatic amino acid decarboxylase), this was a specific direct histochemical verification of recordings from DA-containing cells in this region.

Intracellular recordings from DA neurons were judged sufficiently stable for electrophysiological measurements if the resting potential was greater than -55 mV, spike amplitude was above 50 mV and the cells fired at a rate below 8 hz. Generally, stable resting potentials centered around 60-65 mV, with further hyperpolarization occuring with recording times greater than 20 min. DA cells recorded intracellularly were antidromically activated from the ipsilateral caudate nucleus. The antidromic activation met the criteria of: (1) constant latency, (2) follow of 100 hz stimulation without spike failure, (3) one spike per stimulus, and (4) collision with directly elicited spike. Antidromic spikes exhibited a latency of 11.9 $\pm$ 1.1 msec (mean $\pm$ S.E.M.), corresponding to a conduction velocity of approximately 0.54 meters/second, which is similar to that reported previously for indirectly identified dopaminergic neurons (Guyenet, P. G. and Aghajanian, G. K., Brain Res. 150: 69, 1978). Commonly, an attenuated antidromic spike (15-30 mV) was seen to ride on an i.p.s.p.--presumably resulting from orthodromic activation of a striatonigral GABAergic inhibitory pathway. In addition, fast prepotentials, spontaneous depolarizations leading to burst firing, and 5-10 mV fast potentials were also observed. Action potentials were often seen to arise from these fast potentials.

These intracellularly recorded cells, positively identified as DA neurons, were found to possess all of the extracellular electrophysiological characteristics and pharmacological responses previously attributed to indirectly identified DA neurons (Bunney, B. S. et al., J. Pharmacol. Exptl. Therapeut. 185: 560, 1973; Guyenet, P. G. and Aghajanian, G. K., Brain Res. 150: 69, 1978). (Supported by NIMH grants MH-28849, MH-25642, MH-14276 and the State of Connecticut.)

88.3 DOPAMINERGIC NEURONS IN THE RAT VENTRAL TEGMENTAL AREA: ELECTROPHYSIOLOGICAL EVIDENCE FOR AUTOREGULATION. Rex Y. Wang, Dept. Pharmacol., St. Louis Univ., Sch. Med., St. Louis, MO 63104.

Recently Beart et al (Neurosci lett. 15:165, 1979) reported that the rat ventral tegmental area (VTA or A-10 area) contained high levels of dopamine (DA) and tyrosine hydroxylase, possessed the ability to synthetize and metabolize the transmitter and could accumulate and release ^{3}H-DA. Somadendritic DA stores of A-10 DA neurons therefore have the capacity to play a functional role in regulation of neuronal activity. German et al (Brain Res. 181:191, 1980), on the other hand, made the observation that there is a post-stimulus period of inhibition in the activity of presumed A-10 DA neurons following antidromic activation. The aim of this study was to investigate the possibility that the inhibition of A-10 DA neurons induced by antidromic activation may be mediated by DA somadendritic varicosities (or axon collaterals).

Chloral hydrate anesthetized rats were used. In addition to routine histology, histofluorescence was performed in some cases to verify locations of cells recorded at the end of experiments. Presumed A-10 DA neurons had a slow-bursting or regular firing rate (0.5-8 spikes/sec). Their action potentials were unusually wide (>2.5 msec). The majority of VTA neurons with these characteristic firing patterns and spike shapes could be antidromically activated by electrical stimulation of the nucleus accumbens (NAc). The mean latency of activation was 16.4 msec. The estimated conduction velocity was 0.47 m/sec. The firing rate of these presumed DA cells was markedly reduced following i.v. injection of apomorphine (ID 50 = 7.8 µg/kg) or by iontophoretic application of DA or γ-aminobutyric acid (GABA).

Following electrical stimulation of the NAc, DA neurons usually showed a period of suppression of firing. Some cells responded with excitation preceding the suppression. The NAc-induced suppression was either blocked or markedly reduced by iontophoresis of trifluoperazine or i.v. injection of haloperidol. The effect of these DA antagonists on DA cells is rather selective in that they antagonize the inhibition produced by iontophoresis of DA but not GABA. Little or no increment of firing rate was observed following the administration of either trifluoperazine or haloperidol. Picrotoxin (a GABA antagonist) applied either systemically or directly by microiontophoresis also produced a reversal of the post-stimulus suppression; however, there was also a marked increase in DA neuron's baseline firing rate.

These results are consistent with evidence for the existence of DA autoreceptors on A-10 DA neurons. They further suggest that release of DA from the somadendritic varicosities or axon collaterals may act upon these DA autoreceptors and serve a regulatory function. (Supported by a PMA Starter Grant and the St. Louis University Biomedical Research Support Grant.)

88.4 AMPHETAMINE ISOMERS HAVE DIFFERENT POTENCY RATIOS FOR REDUCING DORSAL VS. VENTRAL SUBSTANTIA NIGRA DA IMPULSE FLOW. S. Browder and D.C. German. Depts. of Psychiatry and Physiology, U. of Texas, Health Sci. Ctr., Dallas, Tx, 75235.

The nigro-striatal dopamine (DA) neuron has been used extensively to study the mechanisms of amphetamine (AMP) action. The d-isomer of AMP is more potent than the l-isomer in releasing and blocking DA uptake in striatal synaptosomes. The d-isomer binds to striatal synaptosomes at the DA-uptake carrier, much more than does the l-isomer. Electrophysiologically, d-AMP is more potent than l-AMP in reducing nigro-striatal-DA impulse flow. Because anatomical evidence indicates that dorsal cells in the substantia nigra zona compacta (SNC) project to nonstriatal forebrain regions and the ventral SNC cells project to the striatum, we sought to determine whether both groups of DA cells responded the same to the AMP isomers. Single unit activity was recorded from SNC-DA neurons in the chloral hydrate anesthetized rat. All drugs were administered via a jugular catheter. d-AMP produced 50% inhibition of ventral SNC-DA impulse flow at 1.0-1.5 mg/kg, while, l-AMP had little effect on impulse flow, doses as high as 21 mg/kg of l-AMP producing <50% inhibition of cell firing. However, both d- and l-AMP reduced dorsal SNC-DA impulse flow by 50% at doses of 1.0-1.5 mg/kg. These results suggest that DA neurons which project to different anatomical regions respond differently to l-AMP, but about the same to the d-isomer. The present data, along with our previous finding that d- and l-AMP are about equipotent in reducing ventral tegmental area DA impulse flow (Browder, et al, Catecholamine: Basic and Clinical Frontiers, 1979, pp. 734-736), suggest that the AMP isomers have equipotent effects on nonstriatal DA neurons. (Research supported by NIMH grants MH-30546 and MH-33513).

88.5 NOREPINEPHRINE - DOPAMINE INTERACTION: AN ELECTROPHYSIOLOGICAL DEMONSTRATION. M.K. Sanghera, S. Browder, P.A. Shore, R.S. Kiser and D.C. German. Depts. of Physiology, Psychiatry and Pharmacology, U. of Texas Health Sci. Ctr., Dallas, TX 75235.

Both anatomical and pharmacological data suggest that noradrenergic (NA) neurons communicate with dopaminergic (DA) neurons. Dopamine-β-hydroxylase-containing neuronal processes are found in the region of the substantia nigra zona compacta and ventral tegmental area (VTA), both areas which contain DA neurons. Furthermore, there is evidence of a locus coeruleus NA input to the VTA. Lesioning ascending NA axons decreases the DA turnover of VTA-DA neurons. We have previously found that systemic d- and l-amphetamine (AMP) both have very potent effects in decreasing the firing rate of VTA-DA neurons (Browder, et al, Catecholamines: Basic & Clinical Frontiers, 1979, pp. 734-736). Because both the AMP isomers also potently influence NA neuronal impulse flow, we sought to determine whether the effects of the AMP isomers on VTA-DA impulse flow were mediated via AMP's effects on NA neurons. Single unit activity was recorded from VTA-DA neurons in the chloral hydrate anesthetized rat. The firing rates of these cells were decreased by 50% or more after intravenous doses of from 0.5-1.0 mg/kg of d- or l-AMP. This decrease in impulse flow was reversed by an intravenous injection of the NA uptake inhibitor, desipramine (DMI) (0.5-1.0 mg/kg). The firing rate could then be decreased again by an intravenous injection of the DA agonist, apomorphine. These data suggest that AMP decreases DA impulse flow in part, at least, by releasing NA onto VTA-DA neurons. DMI blocks AMP's ability to release NA (shown in in vitro studies), and thereby reverses the AMP-induced decrease in DA impulse flow. These data provide electrophysiological evidence suggestive of a noradrenergic dopaminergic interaction. (Research supported by NIMH grants MH-30546 and MH-33513).

88.6 NEOSTRIATAL AND DORSAL RAPHE NEURONS: RECIPROCAL CHANGES IN FIRING RATE FOLLOWING LOCAL INFUSIONS OF D-AMPHETAMINE. George V. Rebec, Stephen D. Curtis and Kenneth S. Zimmerman. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Apart from acting on central catecholaminergic systems, amphetamine appears to produce its behavioral effects, at least in part, by altering the activity of serotonin (5-HT)-containing neurons. In fact, the anterior neostriatum (ANS), which has been implicated in the amphetamine behavioral response, receives 5-HT input from the dorsal raphe nucleus (DRN). To further elucidate the neural mechanisms of action of amphetamine, we infused the drug directly into the DRN, while simultaneously recording single unit activity in this site and in the ANS of immobilized, locally anesthetized rats. The spontaneous firing rate of neurons in both sites ranged from approximately 25-75 spikes/min. d-Amphetamine ($1x10^{-6}$-$2.75x10^{-4}$M) was infused at a rate of 1.5 µl/hr for a period of 5-9 min; single unit activity was monitored until firing rate returned to the pre-infusion baseline rate.

A local infusion of d-amphetamine into the DRN inhibited unit activity in this site but produced a mirror-image excitation in the ipsilateral ANS. It is unlikely that a catecholaminergic mechanism is involved in these effects since similar results were obtained in animals pretreated 4 hrs previously with 250 mg/kg α-methyl-para-tyrosine. Moreover, local infusions of d-amphetamine outside the DRN into the surrounding brainstem reticular formation failed to produce consistent changes in neostriatal activity. Thus, by acting locally in the DRN, amphetamine appears to release neurons in the ANS from an inhibitory serotonergic influence.

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88.7 EFFECTS OF α -ADRENERGIC DRUGS ON NIGRAL DOPAMINE CELL ACTIVITY: MEDIATION BY THE SEROTONERGIC RAPHÉ SYSTEM. S. A. DeRiemer* and B. S. Bunney (SPON: W. Huttner). Departments of Psychiatry and Pharmacology, Yale University Sch. Med., New Haven, CT 06510

Neuroanatomical studies in the rat suggest that noradrenergic projections to the substantia nigra are meager or non-existent. Consistent with these anatomical findings, we have reported previously that the α -agonist clonidine (20-800 ug/kg, i.v.) has no effect on identified dopaminergic (DA) cell activity (Svensson, T. H. et al., Brain Res. 92: 291, 1975). However, we have now found that when an imbalance is induced in basal ganglia function by pretreatment with direct or indirect acting DA agonists (i.e. apomorphine or d-amphetamine) an effect of α noradrenergic drugs on the DA system emerges. Thus, under these conditions, low doses of i.v. clonidine (< 10 ug/kg, i.v.) reverse DA agonist induced inhibition. In addition, using the same extracellular single unit recording techniques, Kim et al. (Kim, H., et al., Fed Proc. 39(3): 258, 1980), as well as ourselves, have found that α-antagonists (e.g. piperoxane), reverse DA agonist-induced inhibition of DA neuron activity. When given alone, these drugs have little effect on the spontaneous activity of identified zona compacta DA neurons.

Clonidine has been demonstrated to inhibit serotonergic raphé neurons, most likely through an action on α_2 receptors (Svensson et al., Brain Res. 92: 291, 1975). Alpha antagonists have also been shown to decrease the activity of serotonergic neurons through an action on α_1-receptors (Gallager, D. W. and G. K. Aghajanian, Eur. J. Pharm. 39: 341, 1976; Baraban, J. and G. K. Aghajanian, Neuropharm. 19: 355, 1980). As multiple lines of evidence have demonstrated a noradrenergic input to the raphé and a serotonergic input to the substantia nigra it is possible that the α-adrenergic drugs are affecting DA cell activity indirectly through an action mediated via raphe neurons. In confirmation of this hypothesis we have found that destruction of the serotonergic neurons within the dorsal raphé nucleus totally prevents α-antagonist reversal of DA cell inhibition.

Our studies provide evidence for an indirect influence of noradrenergic systems on DA cell function. In addition, they suggest that some drugs may have actions on a particular neurotransmitter system in the brain only after that system's normal functioning has been altered. (Supported by NIMH grants MH-28849, MH-25642, a NSF graduate fellowship to S.D., and the State of Connecticut.)

88.8 NORADRENERGIC NEURONS OF THE A-5 GROUP: ELECTROPHYSIOLOGICAL CHARACTERIZATION BY SINGLE CELL RECORDING TECHNIQUES. Rodrigo Andrade and George K. Aghajanian. Depts.of Pharmacology and Psychiatry. Yale Univ. School of Medicine, New Haven, CT 06510

Anatomical studies indicate that the noradrenergic innervation of the hypothalamus, brainstem, and spinal cord is derived to a considerable extent from the lower brainstem catecholamine cell groups. While substantial information has been accumulated on the more rostrally located locus coeruleus (A-6 of Dahlstrom and Fuxe), very little is known about the lower brainstem catecholamine cell groups. Among the latter, the A-5 group (Dahlstrom and Fuxe) has been recently shown to project to motor areas of the brainstem[1] and the intermediolateral cell column of the spinal cord[2] and has been implicated in the control of blood pressure[2]. Cells in this group are concentrated in the ventrolateral tegmentum between the superior olive and the exiting VIIth nerve; two thirds of all cells in this region have been reported to be catecholamine containing[2]. We have attempted to characterize electrophysiologically the cells of the A-5 group located in this area using single cell recording techniques.

In these experiments chloral hydrate anaesthetized rats were used. Recording sites were marked by iontophoresis of pontamine sky blue and localized in brain sections processed for either glyoxylic acid histofluorescence or cresyl violet staining. In the A-5 region a subpopulation of cells was found which exhibited regular spontaneous activity, wide action potentials, low to moderate rates of firing (<8/sec), and a characteristic response to noxious stimulation consisting of a brief excitation followed by a transient period of inhibition. These cells were invariably associated with the A-5 catecholamine cell cluster visualized by the histofluorescence method. The firing of these cells was suppressed by intravenous administration of low doses of the α_2-agonist clonidine; this effect was readily reversed by the α-antagonist piperoxane (0.1 mg/kg). As ℓ-amphetamine also suppressed their spontaneous activity, these cells share many of the characteristics previously reported for cells in the locus coeruleus and it is suggested they correspond to the A-5 noradrenergic cell group. These neurons could be readily distinguished from cells in immediately adjacent areas based upon their physiological and pharmacological properties. Further characterization of these presumed noradrenergic neurons in the A-5 area using antidromic stimulation and microiontophoresis is currently in progress.

[1] M. Silver et al., Anat. Record 193: 684 (1979).
[2] A.D. Loewy et al., Brain Res. 174: 309-14 (1979).
Supported by USPHS Grants MH 17871 and MH 14459 and by the State of Connecticut.

88.9 DIAZEPAM ATTENUATES SINGLE UNIT ACTIVITY IN THE RAT LOCUS COERULEUS, AND LOCUS COERULEUS-ASSOCIATED BEHAVIORS IN THE MONKEY. S.J. Grant, Y.H. Huang and D.E. Redmond, Jr. Department of Psychiatry, Yale Univ. Sch. of Med, New Haven, CT 06510.

Electrical or pharmacological stimulation of the locus coeruleus (LC) elicits a consistent set of behaviors in chair restrained monkeys. The same set of behaviors have also been observed during periods of impending danger or uncertainty. This has led to the suggestion that the LC may act as an "alarm" center and might be involved in the generation of anxiety in hymans (1). This hypothesis predicts that anxiolytic agents would 1) attenuate increases in LC single unit activity or reduce net LC function and 2) attenuate LC-stimulation associated behaviors. These predictions have been tested in single unit recordings in anesthetized and awake, paralyzed rats, and in behavioral observations of chair restrained monkeys during LC stimulation.

Following administration of yohimbine (0.5-1.0 mg/kg I.V.), the firing of LC neurons (N=12) increased from 1.6±0.23 spikes/sec (Mean±S.E.M.) to 2.75±0.35 spikes/sec through blockade of inhibitory alpha-2 adrenergic norepinephrine "autoreceptors" found on LC neurons. Subsequent administration of diazepam (0.1-0.2 mg/kg I.V.) decreased firing rates to 1.86±0.3 spikes/sec. Diazepam (0.1-0.2 mg/kg I.V.) also decreased the accelerated firing rate of LC units in rats subjected to immobilization stress (unanesthetized, paralyzed preparations) from 5.1±0.27 to 1.8±0.24 spikes/sec.

A behavioral correlate of this action was observed in chair-restrained monkeys (Macaca arctoides) (N=5). Electrical stimulation of the LC (trains of biphasic 0.5 msec pulses of 0.15-1.25 mAMP amplitude through bipolar electrodes) was given intermittently over a one hour testing period. During a non-stimulation, pre-drug baseline period, LC-stimulation associated behaviors were emitted spontaneously at a rate of 13.6±5.0 (Mean units behavior/minute (1) ± S.E.M.). Stimulation of the LC increased the rate of LC-associated behaviors to 20.5±6.2. Subsequent administration of diazepam (0.1-0.2 mg/kg I.V.) during LC stimulation reduced the behaviors to 8.2±4.7.

The results demonstrate that diazepam, a major anxiolytic, can effectively attenuate both neural and behavioral aspects of LC activation. These results are compatible with the suggestion that anxiolytic agents can act by attenuated LC activity or net function.

Supported by MH 25642, MH 31176, MH 14276, DA 02321 and the State of Connecticut.

(1) D.E. Redmond, Jr., Y.H. Huang (1979) Life Sci. 26:2149-2162.

88.10 PARALLEL REGULATION OF BRAIN NOREPINEPHRINE (NE) NEURONS AND PERIPHERAL, SPLANCHNIC NE NERVES BY CHEMORECEPTORS BARORECEPTORS AND BLOOD VOLUME RECEPTORS. T.H. Svensson, M. Elam*, T.Yao* and P. Thorén*. Depts of Pharmacology and Physiology, University of Göteborg, S-400 33 Göteborg, Sweden.

Brain NE neurons in locus coeruleus (LC) which innervate almost the entire central nervous system, seem to participate both in behavioural reactions (alerting and alarm responses) and in modulation of cardiovascular function. Utilizing single cell recording techniques to analyze the LC function and parallel recording of splanchnic nerve activity (SNA), we have studied the effects of activation of chemoreceptors, baroreceptors and blood volume receptors.

Chemoreceptor activation by hypercapnia (3-20% CO_2 in the inspired gas mixture) with associated elevation of blood pCO_2-level (36-103 mm Hg) caused an immediate, dose-dependent and marked increase in the firing rate of central NE neurons as well as in the SNA. During hypercapnia blood pressure, heart rate and pO_2-level did not change significantly. Deafferentiation by a low cervical sympathectomy, cutting the superior laryngeal nerves and by local destruction of the carotid sinus did not change the responses of LC neurons or SNA, indicating a central chemoreceptor regulation.

Baroreceptor activation, induced by intravenous injection of NE in incremental doses (0-8 µg/kg), caused a dose-dependent, significant, immediate and parallel reduction in both the firing rate of all LC units tested and in the sympathetic discharge. Both these effects were abolished by deafferentiation (cf. above).

Blood volume load (0.5-5 ml of heparinized blood, intravenously administered) induced a volume-dependent decrease in LC neuronal firing rates and sympathetic activity, effects which both were readily reversed by withdrawal of the corresponding amount of blood: The effects seemed unaffected by deafferentiation of the baroreceptors but abolished by bilateral vagotomy in the neck.

Consequently, brain NE neurons in the LC are subject to regulation by chemoreceptors, baroreceptors and blood volume receptors entirely analogous to the peripheral, splanchnic NE nerves.

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88.11 RAPHE UNIT ACTIVITY IN FREELY MOVING CATS. I. EFFECT OF SENSORY STIMULATION. Barry L. Jacobs, Michael E. Trulson and James Heym.* Prog. in Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

Single unit recordings of serotonergic (raphe) neurons in mammals have provided a good deal of evidence regarding their spontaneous activity, and the influence of both drugs and various neurotransmitters upon this activity. By contrast, little information is available concerning the afferent control of these cells. The present study examined the effect of brief visual (flash) and auditory (click) stimuli upon the activity of serotonergic neurons in the dorsal raphe nucleus of the freely moving cat. Unit activity was recorded by means of movable 32 or 62 µ dia insulated nichrome wires (see complete methodology in Brain Res. 163: 135-150, 1979). Raphe neurons were initially identified on-line on the basis of their characteristic slow and rhythmic firing pattern. This identity was later confirmed by means of histological analysis (all cells were in the densely serotonergic dorsal raphe nucleus) and in some cells by examining their response to drugs known to depress the activity of serotonergic neurons. Click or flash stimuli were presented once every two sec. during periods of quiet or active waking. These stimuli elicited almost exclusively excitatory effects in serotonergic neurons, with a latency from approximately 30-60 msec to approximately 150 msec. This envelope of excitation was found in virtually every cell studied, and showed little variation across cells. The unit response to both stimuli typically showed some degree of habituation across trials. Clearcut inhibition was rarely seen, although some cells appeared to manifest an inhibitory phase following the initial excitation. However, upon closer examination, this was found to be the normal pause that is characteristic of the slow regular discharge of all serotonergic neurons (i.e. the spontaneous ISI). Click stimuli were generally more effective than flashes in driving raphe unit activity, however when both stimuli affected the unit, their temporal effects were largely coincident. This latter fact, plus the relatively long response latency, leads us to hypothesize that serotonergic neurons are not directly responsive to sensory stimuli, rather they appear to be under the influence of some common intermediary arousal system. Finally, these data should be contrasted with previous data on raphe unit activity in chloral hydrate anesthetized rats showing no effect of light flashes (Physiol. Behav. 13: 589-593, 1974), and showing a suppression by low frequency sciatic nerve stimulation (Brain Res. 153: 169-175, 1978). (Supported by NIMH grant MH 23433).

88.12 RAPHE UNIT ACTIVITY IN FREELY MOVING CATS. II. EFFECTS OF ADRENERGIC DRUGS. James Heym*, Michael E. Trulson, and Barry L. Jacobs (Spon: W. E. Gladfelter). Prog. in Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

Spontaneous activity of serotonin (5HT)-containing neurons of the dorsal raphe nucleus (DRN) is characterized by a slow and regular discharge rate. In freely moving cats, unit activity of 5HT cells within the DRN is positively correlated with the level of behavioral arousal, and in addition, the firing of these neurons appears to be driven by simple sensory stimuli (see preceding abstract). In anesthetized rats, recent neuropharmacological data have indicated the necessity of an excitatory adrenergic input to 5HT neurons for maintaining their spontaneous activity. This is of interest since the discharge of norepinephrine (NE)-containing neurons of the locus coeruleus has been reported to exhibit a relationship to behavioral arousal and sensory stimulation which parallels that demonstrated for 5HT neurons. When these findings are considered with anatomical evidence which indicates the existence of NE afferents to DRN neurons, it is reasonable to hypothesize that the responses of 5HT cells to arousal and sensory stimuli follow alterations in NE neuron activity, and are in fact mediated by changes in the adrenergic input to the DRN. We have explored this hypothesis by examining the effects of adrenergic manipulations upon the activity of 5HT neurons within the DRN of freely moving cats. Single unit activity of 5HT cells was recorded utilizing a methodology previously described (Brain Res.: 163, 135-150, 1979). A 30 min. period of baseline activity was recorded and included episodes of active waking, quiet waking, and sleep as judged by EEG and behavioral criteria. Following this pre-drug period, animals were administered, i.p., either WB4101 (0.5 or 1.0 mg/kg), clonidine (50-300 µg/kg), d-amphetamine (0.5-5.0 mg/kg), or saline. Although producing strong sedation, WB4101, an α adrenoceptor antagonist, did not significantly alter the activity of 5HT cells when similar pre- and post-drug behavioral states were compared. Clonidine, in doses that suppress NE cell activity through a presynaptic action, also produced strong sedation and was without effect on the activity of 5HT neurons when similar behavioral states were compared. Similarly amphetamine, a catecholamine releaser, at a dose which produced clear behavioral effects (0.5 mg/kg), failed to alter 5HT neuron activity. These data suggest that the discharge rate of 5HT neurons is not affected by alterations in adrenergic neurotransmission. Thus, arousal effects on 5HT cells may not be mediated by NE afferents. These results may be contrasted with data from chloral hydrate anesthetized rats in which WB4101 and other alpha antagonists produce complete suppression of 5HT cell firing. It seems likely that overriding influences, perhaps blocked by anesthesia, are operative in the freely moving animal.

88.13 RAPHE UNIT ACTIVITY IN FREELY MOVING CATS. III. REM SLEEP WITHOUT ATONIA. Michael E. Trulson, Barry L. Jacobs and Adrian R. Morrison. Prog. In Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544 and Dept. Animal Biol., Univ. Pennsylvania Sch. Vet. Med., Philadelphia, PA 19104.

The discharge of brain serotonin-containing neurons is almost completely suppressed during REM sleep in normal cats. Since the activity of these neurons is grossly correlated with the level of behavioral arousal, or tonic motor activity (see Brain Res. 163: 135-150, 1979), one might expect a decrease in activity during REM simply because tonic EMG activity or motoric output is at a minimum. On the other hand, raphe unit activity may be related to the state (i.e. REM sleep) of the organism. To test these competing, but not mutually exclusive, hypotheses (oneiric vs motoric), we examined raphe unit activity in cats with bilateral lesions of the pontine tegmentum (partially damaging the locus coeruleus and n. reticularis pontis oralis) which display REM sleep without atonia (C.R. Soc. Biol. 159; 895-899, 1965; Acta Neurobiol. Exp.: 34, 215-232, 1974). That is, these cats display episodes which, by all criteria, appear to be REM sleep, except that they display overt behavior, presumably because the mechanism normally responsible for producing atonia has been disrupted. The activity of raphe neurons during the waking state in lesioned cats was virtually identical to what we have previously reported for normal cats. Overall, raphe unit activity still showed a large decrease and long silent periods during REM sleep in lesioned cats. However, every cell tested frequently fired in short bursts in relation to intense gross body movements. It is interesting to note that while many raphe units are virtually silent during an entire REM epoch in normal cats, approximately one-third of the units would also occasionally discharge in short bursts in relation to eye movements and head and limb twitches. The overall level of raphe unit activity during REM sleep in lesioned cats was approximately four-fold greater than in normal cats. A few cells, however, manifested much greater increases in activity during REM sleep (10-20 times that seen in unlesioned cats). Our preliminary analysis indicates that these large increases may be related to a higher level of tonic EMG activity seen during REM sleep on these occasions. Thus it appears that the overall decrease in raphe unit activity during REM sleep is a function of the state, while motoric activity during REM is correlated with an increase in unit activity. These data indicate that increased motor output appears to "drive" raphe neurons. Whether the increased activity is directly related to the motor output, the associated proprioceptive feedback, or some other aspect of the behavior in these animals remainsto be determined. (Supported by USPHS grants MH 23433 and NS 13110).

89.1 FURTHER STUDIES ON THE ENDOGENOUS BENZODIAZEPINE RECEPTOR LIGANDS PEAK I AND PEAK II ISOLATED FROM RAT CORTEX.
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Recent studies attempting to isolate endogenous ligands for the benzodiazepine receptor have produced a growing list of ligand candidates which include the purines inosine, 2-deoxy-inosine and hypoxanthine, nicotinamide, GABA modulin, β-carboline ethyl ester and various other unidentified factors. The purines and nicotinamide have also been shown to possess "benzodiazepine-like" pharmacologic and neurophysiologic properties. Recent reports from our laboratory have described two non-purine ligand candidates extracted from bovine brain using dilute Tris-chloride buffer (Marangos, et al., Psychiat. Res., 1:121, 1979). Both factors, designated peak I and II competitively inhibit ^{3}H diazepam binding, are heat stable and not inactivated by proteolytic enzymes. Peak I is only found in brain tissue and pituitary extracts.

We now report the existence of peak I and II in rat cerebral cortical extracts and their further characterization. Several methods of tissue processing including microwave or decapitation sacrifice and buffer or dilute acetic acid extraction were tried. All procedures yielded qualitatively similar diazepam binding inhibitory profiles when chromatographed on sephadex G-10, suggesting that the factors are not artifacts of tissue preparation. The purines inosine and hypoxanthine are also obtained in all procedures with hypoxanthine being greatly reduced in the extracts prepared from microwaved animals. Rat brain peak I and II are both resistant to proteolytic enzyme degradation and incubation with ribonuclease and deoxyribonuclease suggesting that they are not simple polypeptides or polynucleotides. Molecular weight estimates for peak I range from 1,000-5,000 daltons and 500-600 daltons for peak II. Further purification of peak I using ion exchange chromatography and high pressure liquid chromatography indicate that several active factors may be present.

Kinetic characterization of peak I reveals it to be unique when compared to peak II, the purines and nicotinamide in that it specifically inhibits the GABA induced enhancement of ^{3}H diazepam binding at concentrations 10-fold below those that inhibit basal diazepam binding. This observation, coupled with the known high concentration of GABA in vivo, suggest that peak I may function by inhibiting the GABA mediated increase in diazepam binding. The physiologic relevance of peak I and II should become clearer when these factors are identified and the appropriate pharmacologic experiments are performed.

89.2 PROPERTIES OF SOLUBLE AND PARTIALLY PURIFIED BENZODIAZEPINE AND γ-AMINO BUTYRIC ACID (GABA) RECEPTORS. Moshe Gavish and Solomon H. Snyder. Dept. of Pharmacol. Johns Hopkins School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205.

Benzodiazepine and GABA receptors have been solubilized from calf brain using Triton X-100 as a detergent. Both receptors retained their drug specificity in the soluble state. GABA increases ^{3}H-flunitrazepam binding in the membrane and in the soluble states, indicating that the benzodiazepine receptor possesses GABA recognition sites. In membranes we have shown that both benzodiazepines and GABA selectively protect ^{3}H-muscimol binding from heat inactivation demonstrating that GABA receptors possess specific benzodiazepine recognition sites. The drug specificity for protection by benzodiazepines is the same as at benzodiazepine receptors. Both GABA and benzodiazepine receptors can be purified using concanavalin A-Sepharose 4B column, Sepharose 6B, DEAE-cellulose and hydroxylapatite columns, each affording 10-20 fold purification. GABA enhancement of benzodiazepine receptor binding is retained throughout these four steps of purification, the GABA recognition site appears to be intimately linked to the benzodiazepine recognition macromolecule.

89.3 COUPLING OF BARBITURATE-PICROTOXININ RECEPTORS TO BENZODIAZEPINE RECEPTORS IN RAT BRAIN. Fredrik Leeb-Lundberg*, Adele Snowman*, and Richard W. Olsen. Division of Biomedical Sciences, University of California, Riverside 92521.

CNS depressant drugs such as barbiturates and SQ 20009 (a non-benzodiazepine (BZ) anxiolytic), enhanced the binding of [^{3}H] BZ to high-affinity sites in rat cortex membranes. This effect was seen with all potent depressant drugs which bind to sites labeled by [^{3}H]α-dihydropicrotoxinin (DHP), a convulsant which blocks inhibitory GABAergic synapses at sites linked to Cl^- channels but distinct from GABA receptors. SQ 20009 (EC_{50} 8 μM) or pentobarbital (EC_{50} 100 μM) enhanced BZ binding maximally by about 100% in fresh, well-disrupted and washed membranes, assayed by filtration with 0.5 nM [^{3}H]diazepam in 10 mM $NaPO_4$, 200 mM NaCl, pH 7.0 at 0°. Background was estimated with 10 μM nonradioactive diazepam. Micromolar picrotoxinin competitively inhibited the barbiturate or SQ 20009 enhancement of BZ binding, which showed a chemical specificity (secobarbital> pentobarbital>>phenobarbital≥ barbital) correlating well with pharmacological actions of barbiturates as hypnotics or in enhancement of GABAergic postsynaptic responses. Other convulsants binding to the DHP sites (e.g. bicyclophosphates) at 10 μM also inhibited barbiturate or SQ 20009 enhancement of BZ binding. Barbiturate activation of BZ binding was stereospecific, with the active hypnotic, (+) hexobarbital much more effective than the pharmacologically less active (-) isomer. Enhancement of BZ binding by maximal concentrations of SQ 20009 (10 μM) was not further enhanced by maximal pentobarbital (0.5 mM), consistent with action at the same site (the DHP site), but GABA (10 μM) could further enhance BZ binding in an additive fashion, consistent with action at a different site. GABA enhancement of BZ binding was not Cl^--dependent but that of barbiturates and SQ 20009 required anions permeable to the GABA regulated Cl^- channels. Barbiturates increased the affinity of BZ binding with no change in the number of binding sites, and the enhancement was fully reversible. These in vitro effects of barbiturates represent a reasonable receptor site for at least part of the action of these drugs. These barbiturate receptors seem to be coupled to BZ receptors; since some BZ receptors seem to be coupled to GABA receptors, an in vitro link between DHP-barbiturate receptors and GABA receptors is now evident. The three drug receptors appear to form a complex which regulates Cl^- channels and which is the site of action for many convulsant, anticonvulsant, and sedative-hypnotic drugs.

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89.4 ^{3}H-GABA BINDING TO SYNAPTIC PLASMA MEMBRANES AND ENDOGENOUS PHOSPHOLIPIDS. G. Toffano, C. Aldinio*, M. Balzano*, A. Leon*, and G. Kirschner*. Fidia Research Laboratories, Abano Terme, Italy.

The affinity binding of ^{3}H-GABA to synaptic plasma membranes increases after freezing, thawing and Triton X-100 treatment because of the removal of soluble endogenous inhibitors (Toffano, G., et al., in "Receptors for neurotransmitters and peptide hormones" Pepeu, G., Kuhar, M.J. and Enna, S.J., eds., Raven Press, New York, 1980). Since Triton X-100 treatment removes also lipid membrane components, we investigated whether endogenous phospholipids play a role on the regulation of ^{3}H-GABA binding to its recognition sites. When single phospholipids are added to Triton X-100 treated membrane preparations, we can detect a 30% decrease of ^{3}H-GABA binding induced by phosphatidylethanolamine, with phosphatidylserine and phosphatidylcholine practically inactive. Treatment of synaptic plasma membranes with a Ca^{++}-stimulated phospholipase A_2 results in a 40% decreased binding due to the formation of lysophospholipid derivatives which solubilize ^{3}H-GABA receptors. Incubation with phospholipase C increases ^{3}H-GABA binding by about 20-30%, while phospholipase D has no effect. The increased ^{3}H-GABA binding induced by Triton X-100 treatment is 5-8 times higher than that obtained by using phospholipase C at doses that duplicate the effect of the detergent on membrane phospholipid depletion. Moreover in aged rat brains, the regional decrease of ^{3}H-GABA binding does not correlate with endogenous membrane phospholipid changes. In conclusion, the removal of endogenous membrane phospholipid, particularly phosphatidylethanolamine, only partially accounts for the increase of ^{3}H-GABA binding determined by Triton X-100 treatment.

89.5 HIGH AFFINITY GTP BINDING IN BRAIN. J.E.Rosenblatt*, R.del Carmen, R.J.Wyatt*, M.Franczak, and B. Layton. Lab. of Clin. Psychopharmacol., NIMH, St. Elizabeths Hosp., Wash., D.C. 20032

Guanine nucleotides play a role in the activation of adenylate cyclase. Although the mechanism by which GTP apparently mediates adenylate cyclase activation is not known, it is thought to involve a guanine nucleotide binding protein associated with both the cell surface hormone receptor and adenylate cyclase. We therefore studied the binding of 3H-GTP to rat brain homogenates. 3H-GTP binding was saturable; Scatchard analysis revealed a higher affinity lower capacity binding site (Kd=2.0 nM, Bmax=164 pmoles/gm tissue) and a lower affinity higher capacity site (Kd=13 nM, Bmax=690 pmoles/gm tissue). Association was rapid, reaching equilibrium within five minutes; by fifteen minutes of incubation 30-40% of 3H-GTP binding dissociated spontaneously. Scatchard analysis at this incubation time revealed only the higher affinity component of binding. By 45 minutes following addition of 100 micromolar unlabelled GTP at equilibrium 80-90% of 3H-GTP binding had dissociated. GTP was ten and one hundred times more potent in displacing 3H-GTP binding than GDP and CTP, respectively; guanosine and guanine failed to inhibit 3H-GTP binding at concentrations less than 1 mM. ATP was completely inactive in displacing 3H-GTP. 3H-GTP binding was reduced by over 90% following exposure of brain homogenate to 53 degrees Cent. for 15 minutes. Binding of 3H-GTP was relatively similar in all brain areas studied (caudate, cerebral cortex, hippocampus, cerebellum, hypothalamus, and medulla-pons). It is hoped that direct binding techniques will prove useful in studying interactions among agonists, cell surface receptors and adenylate cyclase.

89.6 PSYCHOACTIVE DRUGS MODIFY NEUROTRANSMITTER RECEPTOR CIRCADIAN RHYTHMS IN THE RAT BRAIN. M.S. Kafka, A. Wirz-Justice,* D. Naber,* P. Marangos,* I. Tobler,* A. Borbely* and T.A. Wehr*. NIMH, Bethesda, MD 20205 and University of Zürich, Zürich, Switzerland

Endogenous circadian rhythms in the number of α-adrenergic ([^{3}H]WB4101), β-adrenergic ([^{3}H]dihydroalprenolol), dopamine ([^{3}H]spiroperidol), muscarinic cholinergic ([^{3}H]QNB), opiate ([^{3}H]naloxone), and benzodiazepine ([^{3}H]diazepam) receptors have been characterized in the rat brain. Chronic treatment with the antidepressant drugs imipramine, clorgyline, and lithium, and with the neuroleptic drug, fluphenazine, alter some or all of the characterisitcs of these receptor rhythms. In contrast, 24 hours of sleep deprivation (a transient antidepressant in man) had little effect.

The daily changes in receptor number are of the same order of magnitude as those measured when rats, chronically treated with drugs, are compared at a single time point with controls. A change in the receptor number, which in most studies has been measured at only one time point, could reflect a change in the 24-hour mean receptor number, a shift in the timing of the circadian rhythm (a phase shift), or both. As changes in the timing of a number of circadian rhythms have been implicated in the pathophysiology of affective illness, the drug-mediated changes in the timing of receptor rhythms may be related to their therapeutic actions in man.

Repeated measurements throughout the year have shown that the circadian rhythms in receptor number appear to undergo seasonal changes. The rhythms, seasonal as well as circadian, may function to synchronize brain neuronal transmission to the environmental light:dark cycle.

89.7 ATP- OR GTP-INDUCED INCREASE IN ^{3}H-ADTN BINDING TO RAT STRIATAL DOPAMINE RECEPTORS IS ABOLISHED BY UV IRRADIATION. Nancy R. Zahniser, Kim A. Heidenreich* and Perry B. Molinoff. Dept. of Pharmacology, Univ. of Co. Health Sci. Ctr., Denver, CO 80262.

Dopamine receptor agonists appear to be less potent in the presence of GTP. This effect, which is not seen with antagonists, has been explored using a rigid analogue of dopamine, ^{3}H-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronapthalene (^{3}H-ADTN). In initial experiments performed at 37° C, samples were equilibrated without nucleotide and the dissociation rate of ^{3}H-ADTN was determined. There was an increase in the rate of dissociation of ^{3}H-ADTN when GTP was added along with the competing ligand. Thus, the agonist-specific decrease in affinity appeared to be due, at least partially, to an accelerated rate of dissociation of agonist from the receptor. At 37° C in the presence of either ATP or GTP, however, ^{3}H-ADTN binding to striatal membranes did not appear to reach steady-state; binding increased for approximately 10 min and then progressively declined. When ^{3}H-ADTN binding was examined at 20° C, the system reached equilibrium within 45 minutes either in the presence or absence of nucleotides. Scatchard analysis of binding isotherms performed in the absence or presence of nucleotide showed that the affinity of ^{3}H-ADTN decreased in the presence of GTP (K_D values: control = 6.8 ± 1.1 nM; experimental = 19 ± 1.7 nM). The most striking finding was that the number of binding sites increased by five-fold (Bmax: control = 3.4 ± 0.93 pmol/mg protein, experimental = 18 ± 2.9 pmol/mg). This latter effect was not specific for guanine nucleotides since ATP was equipotent with GTP (EC_{50} = 30 μM). Both compounds were maximally effective at a concentration of 300 μM. The nonhydrolyzable purine nucleotide analogs GMP-PNP and AMP-PNP did not substitute for GTP or ATP. The pharmacological specificity of ^{3}H-ADTN binding in the absence or in the presence of nucleotide was characteristic of binding to dopamine receptors. The addition of GTP or ATP did not alter the affinity, number of binding sites or dissociation rate of the antagonist ^{3}H-spiroperidol. In other experiments the tissue was exposed to UV irradiation (λmax=310 nm) prior to the initiation of the binding reaction. After 30 minutes of UV exposure, ATP no longer had any effect on ^{3}H-ADTN binding. In contrast, ^{3}H-ADTN binding in the absence of ATP and ^{3}H-spiroperidol binding in the absence or presence of ATP were stable in tissue exposed to UV irradiation. Dopamine-sensitive adenylate cyclase activity also persisted following UV irradiation. These results suggest that in the presence of purine nucleotides ^{3}H-ADTN binds to a site in the rat striatum which is labile to UV irradiation and which is not associated with ^{3}H-ADTN binding sites detected in the absence of nucleotides, with ^{3}H-spiroperidol binding sites, or with dopamine-sensitive adenylate cyclase. As expected, ^{3}H-ADTN has a lower affinity in the presence of nucleotides than in their absence; however, the amount of ^{3}H-ADTN binding increases, rather than decreases, in the presence of either GTP or ATP.

This work was supported by USPHS (NS 15756 and 09199) and by an NIH fellowship (NS 05970).

89.8 HIGH AFFINITY BINDING OF [^{3}H]DOXEPIN TO RAT BRAIN TISSUE. J. E. Taylor and E. Richelson. Depts. of Psychiatry and of Pharmacology, Mayo Foundation, Rochester, MN 55901.

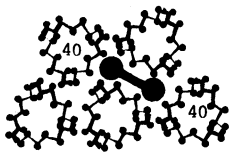
Some tricyclic antidepressants have been shown in pharmacological assays to be potent inhibitors of histamine H_1 receptors (E. Richelson, Mayo Clin. Proc., 54:669, 1979). However, the direct binding of tricyclic antidepressants to H_1 receptors in neural tissue has not been reported. In the present study we have characterized the binding of the radioactively labeled tricyclic antidepressant, [^{3}H]doxepin, to H_1 receptors in rat brain. The binding of [^{3}H]doxepin was time-dependent, and at 37°C reached equilibrium by 30 min. At 0.05 nM [^{3}H]doxepin, the association rate constant (k_1) at 37°C was $1.38 \times 10^9 M^{-1}$ min^{-1} and the dissociation rate constant (k_{-1}) was 0.038 min^{-1}. The equilibrium dissociation constant (K_B) determined from the ratio k_{-1}/k_1 was 0.027 nM. The Scatchard plot of specific [^{3}H]-doxepin binding was nonlinear indicating a multiplicity of binding sites. The K_B for the site of highest affinity was 0.043 nM ± 0.005 (mean ± S.E.M., n=6) with a maximal binding (B_{max}) of 34 ± 6 fmol/mg protein. This high affinity binding of [^{3}H]doxepin was sensitive to inhibition by pharmacologically relevant concentrations of histamine H_1 receptor antagonists such as pyrilamine (K_i=1.2 nM), d-chlorpheniramine (K_i=10 nM), and 1-chlorpheniramine (K_i=355 nM). The histamine H_2 antagonist, metiamide and the anticholinergic, atropine, were weak inhibitors with K_i's of 10,000 nM and 500 nM, respectively. The K_B for [^{3}H]doxepin binding agrees very well with its K_B value obtained from biological assays (E. Richelson, Mayo Clin. Proc., 54:669, 1979) and from the inhibition of [^{3}H]pyrilamine binding (J. E. Taylor and E. Richelson, unpublished observations). The B_{max} for [^{3}H]-doxepin binding to H_1 receptors, however, is much less than the B_{max} using [^{3}H]pyrilamine to label the H_1 receptor. These results suggest that [^{3}H]doxepin may be binding to a subclass of histamine H_1 receptors. (Supported by the Mayo Foundation and USPHS grants MH 27692 and MH 07925.)

89.9 Modification of Cholinergic Receptor Channel Properties by Sulfhydryl Reagents. A. Steinacker, Biophysics, Rockefeller University, New York, N.Y. 10021.

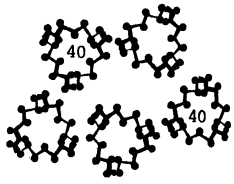
Disulfide bonds are a critical structural component of the nicotinic cholinergic receptor. It has been shown using biochemical analysis with the isolated receptor that sulfhydryl reagents show specificity for locus of action on the receptor sub-units. In addition, the locus of action of several sulfhydryl reagents has been shown by electrophysiological methods. This report concerns the modification of channel open time at the lizard neuromuscular junction (nmj) by the sulfhydryl reagents, sulfite and diamide. Sulfite has been shown to attack a disulfide bond on the α chain (Steinacker, A. Nature 278, 358-360, 1978). Using the lizard intercostal muscle and mepc decay time as an index of mean channel open time, I have shown that sulfite produces an increase in response of the receptor to ACh which is due to an increase in mean channel open time. Diamide, a sulfhydryl oxidizing agent, has been shown to act preferentially at the β chain in the isolated native receptor (Hamilton et al., Biochem. 18, 155-163, 1979). Diamide, when applied to the lizard nmj produces an increase in receptor response to ACh which is shown to be due to an increase in mean channel open time. The effects of both these reagents are extremely variable in occurrence and amplitude of response, indicating that the oxidative reductive state of these disulfide bonds vary in the in vitro nmj being predominately in the reduced state.

89.10 MODEL OF THE ACETYLCHOLINE RECEPTOR-CHANNEL COMPLEX. H. R. Guy* (SPON: D. Livengood). Armed Forces Radiobiology Research Institute, Physiology Department, Bethesda, MD 20014.

Although the acetylcholine receptor-channel complex (AChRC) has been studied extensively, its molecular structure is not known. The AChRC structure proposed here attempts to account for various data with a single model. Data suggest that the AChRC from the electric organ of Torpedo californica is a dimer in which each monomer has two 40-K, one 50-K, one 60-K, and one 65-K MW subunits (Raftery, M. et al., Adv. Cytopharmacol., 3:159-182, 1979; Lindstrom et al., Biochemistry, 18: 4465-4470, 1979). I have assumed that all of the subunits have similar backbone structures and that a channel is formed between the subunits of each monomer. The extracellular receptor portions of each model subunit have three β sheets that form triangular structures. Arrangement of the β triangles shown below is consistent with dimensions observed with electron microscopy (Klymkowsky, M. W. and Stroud, R. M., J. Mol. Biol., 128:319-334, 1979). The β backbones of the snake neurotoxins bind to the tops of the 40-K β triangles, and their invariant tails bind in the region between the two β triangles [probably corresponding to the 50-K and 65-K subunits (Raftery ref.)] that separate the 40-K subunits. When two snake neurotoxins bind to the receptor, their invariant tails meet in a manner that mimics the structure of the very potent competitive inhibitor alloferin. The binding site of the competitive inhibitors is represented below by the dumbbell. The receptor has two nonidentical agonist-binding sites: one between the 40-K and 65-K subunits and the other between the other 40-K and the 50-K subunits. The portions of the subunits that extend across the membranes are predominantly helical. Residues 5 to 20 of the 40-K subunit (Hunkapiller, M. W. et al., Biochem. Biophys. Res. Commun., 91:164-169, 1979) can form an amphiphilic α helix in which all of the side chains on one side of the helix are apolar, those in a narrow region opposite the apolar side are charged, and indifferent residues separate the apolar and charged regions. These types of amphiphilic helices probably extend through the membrane with their charged side chains extending into the channel and their hydrophobic side chains extending into the lipid phase. The model channel forms between the subunits and opens when the subunits move farther apart.



Competitive inhibitor bound to closed AChR



Open AChR

89.11 ^{3}H-2-CHLOROADENOSINE LABELS ADENOSINE RECEPTORS IN BRAIN MEMBRANES. P.H. Wu* and J.W. Phillis. Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Sask. Canada. S7N 0W0

Studies on the depressant actions of adenosine and its analogs on central neurons and biochemical investigations on the stimulation of cyclic AMP formation by adenosine and its analogs in brain slice preparations indicate the existence of adenosine receptors in CNS. We have sought evidence of binding of adenosine or its analogs to brain membranes to validate this concept of central adenosine receptors.

^{3}H-2-chloroadenosine (a ligand, 33nM) was incubated with rat cerebral cortical membranes (0.5mg protein) for a period of 60 minutes at 4°C. At the end of incubation, 5ml of the 75mM Tris-HCl buffer (pH 7.5; containing 25mM $MgCl_2$ and 25mM $CaCl_2$) was added to stop the reaction. The labelled membranes were collected on a Whatman GF/C glass fibre filter with suction. For the determination of non-specific binding of ^{3}H-2-chloroadenosine, a 2.5×10^{-4}M solution of 2-chloroadenosine was present in the incubation mixture. IC_{50} values for the inhibition of ^{3}H-2-chloroadenosine binding were obtained by adding test agents to the incubation medium at a final concentration of $10^{-8} \sim 10^{-4}$M.

The specific binding of ^{3}H-2-chloroadenosine to rat brain cortical membranes appeared to be saturable. It was linear from 0.3mg to 1.2mg of membrane protein and the amount of binding appeared to vary amongst different brain regions. A high binding capacity was found in the caudate nucleus, low binding capacity was found in hippocampus. Analysis of the binding data by the method of Scatchard gave a linear plot, suggesting the presence of a single binding site which had an apparent dissociation constant (K_D) of 23.5nM and a maximal binding capacity (B_{max}) of 476 femto mole/mg protein. Binding of ^{3}H-2-chloroadenosine to subcellular fractions indicated that the binding was mainly to the membranes prepared from the synaptosomal fraction of rat cortex. Adenosine competitively displaced ^{3}H-2-chloroadenosine binding from cortical membranes with a K_i value of 0.12μM at 10^{-5}M adenosine concentration. Other purines displaced the binding of ^{3}H-2-chloroadenosine to cortical membranes with IC_{50} values of; adenine, 0.45μM; inosine, 0.4μM; theophylline, 0.35μM; and isobutylmethylxanthine, 0.13μM. Binding was weakly inhibited by uracil, 18μM and hypoxanthine, 27μM. Guanosine and cytidine were inactive at 100μM. These findings strongly support the suggestion that there are adenosine receptors on brain cell membranes and extend previous observations that methylxanthines (caffeine and theophylline) act as adenosine receptor antagonists.

Supported by the Medical Research Council of Canada.

89.12 Are there separate Binding Sites for a Neurotransmitter and its Agonists or Antagonists. Marvin H. Winkler, (Spon: Sol Berl) Dept of Neurology, Mt. Sinai School of Medicine, New York, N.Y.

It is often difficult to find topographical similarities between the structure of a specific neurotransmitter and its physiologically active competitive binders. Frequently the chemical characteristics of the postulated topographical similarities, even when they are found, preclude the existence of a single rigid binding site. The alternatives are either configurational adaptability in a single primary site which binds both the neurotransmitter and its competitors or a primary site which binds the neurotransmitter and a secondary site which binds the competitor but can transmit information to the primary site. The large number of configurationally distinct agonists or antagonists which displace dopamine from striatal homogenates makes it difficult to conceive of even a configurationally adaptable site which could bind all of them. It is simpler to assume in analogy to serum albumen, that a component with a wide range of binding activities is to be found in neural membranes and that a configurational change induced by binding to one of a number of secondary sites is transmitted to a primary site. This configurational change must be assumed to distort the primary site in such a fashion as to render it less complementary to a bound neurotransmitter. Evidence will be discussed for the existence of communicating primary and secondary sites existing on the same molecule. However, they may exist on separate molecules which communicate through the membrane structure. According to the postulate which is to be discussed, the difference, between an agonist and antagonist is simply that while both distort the primary site upon binding, the distortion caused by an agonist alters ionic channels in the same manner as does the neurotransmitter. Antagonist binding does not cause similar alteration in ionic channels.

Supported by a grant to the Clinical Center on Parkinson's and Allied diseases USPHS Grant NS 11631.

90.1 PHARMACOLOGICAL ANALYSIS OF MULTIPLE ^{3}H-5HT BINDING SITES IN RAT BRAIN, N.W. Pedigo, H.I. Yamamura and D.L. Nelson*, Department of Pharmacology, University of Arizona Health Sciences Center and College of Pharmacy, Tucson, Arizona 85724.

A number of antipsychotic drugs can discriminate between two populations of ^{3}H-5HT binding sites in rat brain. These two distinct sites show a differential distribution in various rat brain regions. Cortical areas (frontal, parietal and occipital) and the hippocampus contain a high proportion of sites having a high affinity for neuroleptics (about 60%) while the corpus striatum contains mostly sites with a low affinity for antipsychotic drugs (about 80%). The butyrophenone neuroleptic spiperone shows the greatest selectivity for these two ^{3}H-5HT binding sites having at least a 3000-fold difference in Ki values (2-12 nM vs. 35,000 nM) for the high and low affinity sites, respectively. Drug inhibition of ^{3}H-5HT binding by spiperone in the frontal cortex, hippocampus and corpus striatum yields distinctly biphasic inhibition curves with Hill slopes significantly less than 1. Furthermore, results from saturation studies of ^{3}H-5HT binding assayed in the absence or presence of 1 μM spiperone (a concentration which completely blocks the high affinity site while exerting minimal effects at the low affinity site) reveals a parallel shift in the Scatchard plot with no change in the K_D of ^{3}H-5HT, but a significant 60% (frontal cortex) or 20% (corpus striatum) decrease in the number of specific ^{3}H-5HT binding sites.

The ^{3}H-5HT binding site with a high affinity for neuroleptics exhibits a marked degree of pharmacological specificity. Drugs which have a structure similar to spiperone, such as haloperidol, pipamperone and metoclopramide, are from 40 to 200-fold less active at this high affinity site than spiperone. The antispychotic drug (+)butaclamol is only about 3 times less active than spiperone while chlorpromazine is 50 times less potent at the high affinity site. All of these drugs show varying degrees of selectivity between the two ^{3}H-5HT binding sites, whereas the atypical neuroleptic sulpiride appears to act only at the low affinity site. While it is unlikely that the differential activities of antipsychotic drugs at these multiple ^{3}H-5HT binding sites account for differences in their theraputic efficacy, there may be a correlation to certain side effects of neuroleptics such as sedation, sleep disturbances, etc. Further characterization of these multiple ^{3}H-5HT binding sites is therefore warrented.

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90.2 ^{3}H-MIANSERIN BINDING IN CALF CAUDATE. Patricia M. Whitaker and Alan J. Cross*. Division of Psychiatry, Clinical Research Centre, Harrow, England.

Mianserin has been shown to be a serotonin receptor antagonist both in peripheral and central receptor systems. It has also been well established as a clinically effective non-tricyclic antidepressant. Recently a tritiated form of mianserin has become available with a specific activity high enough for use in direct binding assays of central neurotransmitter receptors. These assays would be of interest for two reasons: (i) they could introduce a new ligand for central serotonin receptors and (ii) the results would be interesting to compare with recent findings in ^{3}H-tricyclic antidepressant direct binding assays in order to establish a possible common site of action shared by the tricyclics and a novel antidepressant.

The direct binding assays were done in calf caudate using 5 nM. ^{3}H-mianserin and 1000 nM. cold mianserin to define the specifically bound radioligand. The unbound ligand was removed by filtration. The K_i values of a variety of drugs were determined and are given in the following table:

Drug	K_i (nM)
Tryptamines:	
Serotonin	390
Tryptamine	780
N,N Dimethyltryptamine	780
5-Methoxy-N,N-DMT	620
Ergots:	1030
LSD	
Metergoline	1.7
Dihydroergocryptine	1.7
Dihydroergotamine	30
Antidepressants:	52
Mianserin	
Imipramine	1.2
Chlorimipramine	5
Trazodone	7.2
Amitriptyline	16.7
Nortriptyline	26
Maprotiline	28
Nomifensine	38.6
	200

The sites labelled were of one type with a K_D of 0.7 nM and a B_{max} of 139 fmoles/mg. protein.

The results will be discussed in terms of antidepressant site of action.

(Supported by the MRC of Canada).

90.3 PROTEIN NEUROTOXIN FROM RUSSELL'S VIPER SELECTIVELY INHIBITS ^{3}H-SPIROPERIDOL BINDING TO DOPAMINE AND SEROTONIN RECEPTORS. Jonathan E. Freedman and Solomon H. Snyder. Dept. Pharmacol. and Exptl. Ther., Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, Maryland 21205

A neurotoxin has been purified to apparent homogeneity from the venom of Vipera russelli russelli, a snake whose bite does not cause neuromuscular paralysis, and whose venom is thought to block dopamine synapses. The toxin is a protein of molecular weight 10,000 and isoelectric point 8.9. Its activity is stable to 5 min. of boiling.

The toxin inhibits in a dose-dependent manner the specific binding of ^{3}H-spiroperidol to dopamine receptors in rat caudate nucleus and serotonin receptors in rat frontal cortex, with Ki values around 20-100 nM. Scatchard analysis reveals that the toxin lowers Bmax without raising K_D values, indicating either that the inhibition is noncompetitive or, if competitive, is of slow dissociation. The inhibition is fairly selective, in that toxin concentrations up to 1 μM have no effect on ligand binding to alpha-adrenergic, beta-adrenergic, muscarinic cholinergic, GABA-ergic, or benzodiazepine receptors, and only partially inhibit ^{3}H-doxepin binding to histamine receptors.

The molecule also possesses phospholipase A_2 activity, which appears not to account for the inhibition of binding. Binding data are obtained in the presence of 1 mM $CoSO_4$, which selectively and completely abolishes the phospholipase activity. Other phospholipase A_2 enzymes present in the same venom are substantially less potent than the toxin at inhibiting spiroperidol binding in the absence of cobalt.

The ^{125}I-toxin may be of use for receptor labeling, while toxin affinity columns may facilitate receptor purification.

90.4 CHARACTERIZATION OF ^{3}H-APOMORPHINE BINDING IN MAMMALIAN BRAIN. G. Arana,* R. J. Baldessarini. (SPON.: A. Pope) Mailman Research Center, Harvard Medical School, McLean/Massachusetts General Hospital, Belmont, MA 02178.

^{3}H-Apomorphine (APO) was employed as a radioligand to study binding to putative dopaminergic receptors in subsynaptosomal preparations of mammalian brain. With unlabeled APO, dopamine (DA), ADTN, or (+)butaclamol to displace non-specific binding, specific binding ranged from 80 to over 90% with calf caudate preparations. Optimal conditions for incubation occurred at pH 7.0-7.5 at 25°C. Binding was nearly complete within 60 min; $t_{1/2}$ for association = 14 min and $t_{1/2}$ for dissociation = 18.5 min. Analysis of kinetic data yielded K_d = 2.1 to 3.4 nM and B_{max} = 681 fmol/mg protein for a high affinity component and K_d = 212 nM and B_{max} = 1293 fmol/mg for a low affinity, high-capacity component of binding of ^{3}H-APO. Caudate nucleus or corpus striatum had the greatest density of binding sites among brain regions of several species, including man. Most monovalent or divalent cations had no significant effects on binding, but Ca^{++} and Mn^{++} decreased binding (EC_{50} = 10 to 20 mM). Several nucleotides showed no significant effect, but both GTP and ATP reduced binding of ^{3}H-APO.

Of the many aporphines tested in this system, those with a free catechol moiety yielded EC_{50} values in the low nM range; non-catechol analogs showed 10 to 1000-fold less affinity. Of a large series of phenethylamines tested, non-catechols showed 10 to 1000-fold less affinity than DA; N-alkylated analogs of DA inhibited binding of ^{3}H-APO at nM concentrations, while norepinephrine and other catechols had little effect. Other DA agonists such as ADTN and lergotrile were potent inhibitors, while a series of dopaminergic antagonists or various putative neurotransmitters, enzyme inhibitors, and other neuropharmacologically active agents were weak or inactive as displacers of ^{3}H-APO.

90.5 INTERACTIONS OF MOLINDONE WITH SEROTONIN AND DOPAMINE RECEPTORS AND WITH ADENYLATE CYCLASE IN RABBIT BRAIN. M.R.Rosenfeld* and M. H.Makman*. (SPON:R.Ginzberg). Depts. Molecular Pharmacology and Biochemistry, Albert Einstein Coll. Med., Bronx, New York 10461.

Molindone (MOBAN), a tetrahydroindole derivative is a clinically used antipsychotic agent that has been shown to be a specific antagonist of serotonin (5-HT) stimulated adenylate cyclase (AC). (Ahn and Makman, Life Sci. 23:507, 1978). We have studied the ability of molindone to bind to aminergic receptors and its effect on hormone-stimulated AC in homogenates of rabbit frontal cortex (FC) and caudate nucleus (CN).

In both regions, molindone is a weak displacer of all [3H] ligands tested. In FC IC_{50}'s for molindone are 7μM for [3H] LSD, 8.4μM for [3H] 5-HT and 6.9μM for [3H] spiroperidol. In CN IC_{50}'s are 6.5μM for [3H] LSD, 5.1μM for [3H] 5-HT and 3.6μM for [3H] ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene). In FC the guanine nucleotide analog Gpp(NH)p failed to alter the IC_{50} for displacement of [3H] spiroperidol by molindone indicating that the interaction of molindone at these sites is as an antagonist. In FC molindone is able to antagonize 5-HT and lisuride stimulation of AC but is inactive in antagonizing dopamine (DA) stimulated activity, except at high concentrations (0.1mM).

In CN we find that 66% of the [3H] LSD binding sites are serotonergic and 34% are dopaminergic. At 1μM molindone interacts with approximately 21% of the total sites of each class. At 10μM molindone interacts with 46% of the 5-HT sites and 90% of the DA sites. We also studied the interaction of molindone with the guanine nucleotide sensitive (SEN) and insensitive (INS) components of [3H] ADTN and [3H] 5-HT binding in CN. Molindone displaces both SEN and INS classes of [3H] ADTN binding sites and displays a small preference for the SEN component of [3H] 5-HT binding sites. At 1μM and 10μM molindone binds to 25% and 42% of the total INS sites respectively, and 44% and 67% of the total SEN sites, respectively.

The data show molindone to have relatively weak interactions with 5-HT and DA receptor sites in FC and CN of rabbit brain. If the guanine nucleotide sensitive component of 5-HT binding represents receptors that are coupled to AC, this, together with the ability of molindone to antagonize 5-HT-stimulated AC, raises the possibility that an important site of action of molindone is at 5-HT receptors coupled to AC. Molindone appears not to be selective for AC-coupled DA receptors but rather binds weakly to both AC-coupled and non-coupled DA receptors. The relative binding potency of molindone in these studies at either 5-HT or DA receptors is less than predicted based upon its antipsychotic potency. Molindone presents itself as an atypical neuroleptic with stronger interactions at 5-HT receptors coupled to AC than at coupled or non-coupled DA receptors. Supported by NIH Grant MH 31773.

90.7 SOLUBILIZATION OF THE NEUROLEPTIC (DOPAMINE, D-2) RECEPTOR FROM DOG STRIATUM. B.K. Madras, A. Davis and P. Seeman. Psychopharmacology Section, Clarke Institute of Psychiatry, Toronto, Ontario, M5T 1R8 and Department of Pharmacology, University of Toronto, Toronto, Ontario, M5S 1A8.

Neuroleptic drugs bind to the post-synaptic dopamine receptor (D-2) in an order of potency that parallels clinical dosage (Seeman et al., Nature 261, 717, 1976). Purification of the receptor would help clarify the molecular mechanisms involved in neuroleptic action.

We have solubilized the neuroleptic receptor from dog striatum (Gorissen and Laduron, Nature 279, 72, 1979) membrane preparations as a preliminary to purification. The receptor is solubilized with digitonin and assayed on Sephadex G-50 columns, using 3H-spiperone to label it. The soluble receptor retains the binding characteristics of the membrane-bound receptor.

	IC_{50} (nM)	
	Membrane	Soluble
Antagonists		
Spiperone	1.8	3.8
Fluphenazine	14	4
Haloperidol	19	42
(+)-Butaclamol	23	4
(-)-Butaclamol	5,000	3,900
Chlorpromazine	37	150
Agonists		
ADTN	630	950
Apomorphine	1,100	1,700
Dopamine	4,000	5,100
Serotonin	95,000	200,000
Norepinephrine	100,000	100,000
Characteristics		
K_D	0.4±0.04 nM (3)	0.8±0.1 nM (3)
Specific binding	410 fmoles/mg	193 fmoles/mg
Recovery	20.6 pmoles/g	4.2 pmoles/g

Evidence that soluble striatum receptor is dopaminergic stems from: 1) The failure of solubilized binding sites from a non-dopaminergic region, cerebellum, to bind either stereoselectively or in a saturable manner. 2) Soluble receptors from the frontal cortex have different binding profiles than the soluble receptor of the striatum. 3H-Spiperone binding in the frontal cortex is primarily serotonergic. 3) The higher affinity of dopamine for the receptor compared with other neurotransmitters is retained after solubilization.

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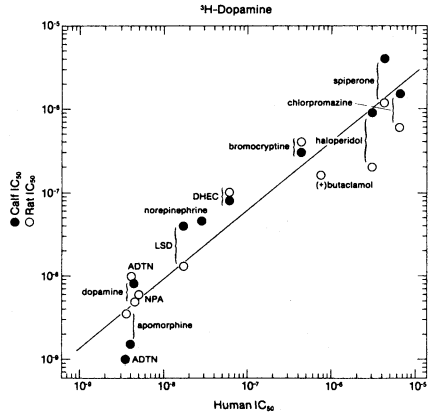
90.6 HIGH-AFFINITY 3H-DOPAMINE RECEPTORS IN THE HUMAN BRAIN. S.J. List* and P. Seeman. Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada.

Although a high-affinity binding site for 3H-dopamine has been extensively studied in the calf brain, some workers have been unable to detect this site in the rat brain (Creese et al., Eur. J. Pharmacol. 60, 55, 1979). Under our binding assay conditions, we have recently found a high-affinity 3H-dopamine binding site in the rat, similar to that found in the calf (List et al., Neurosci. Abst. 5, 654, 1979). We now report the existence of this same high-affinity binding site in post-mortem human brain tissue.

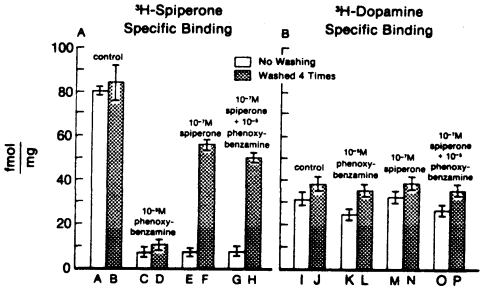
Scatchard analyses of specific 3H-dopamine binding (as defined by 10^{-6}M apomorphine) in tissue homogenates of five separate human brains revealed a high-affinity 3H-dopamine binding site in the caudate (B_{max} = 63 ± 10 fmoles/mg protein; K_D = 2.2 ± 0.4 nM) and the putamen (B_{max} = 57 ± 11 fmoles/mg protein; K_D = 2.8 ± 0.3 nM). No such sites were detectable in dopamine-poor regions in the human brain.

The high-affinity 3H-dopamine binding site in the human brain appeared to be similar to the sites previously characterized in the rat and calf, having high affinity for dopaminergic catecholamines, intermediate affinity for dopaminergic ergot agonists, and low affinity for a number of neuroleptics (as shown in the figure below). (Supported by the Medical Research Council of Canada and the Ontario Mental Health Foundation.)



90.8 IRREVERSIBLE BLOCKADE OF DOPAMINE RECEPTORS. M. Titeler. Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8.

In order to investigate the binding sites of dopamine receptors, a specific irreversible ligand is desirable. In order to determine whether there is a functional moiety at the dopamine receptor binding site capable of irreversible interactions, the effect of phenoxybenzamine on the specific binding of 3H-spiperone and 3H-dopamine to calf caudates was investigated. Phenoxybenzamine has been shown to block α-adrenergic receptors specifically and irreversibly (1), using direct binding assays to monitor the blockade of the receptors. 3H-Spiperone has been shown to label primarily a dopamine receptor in the caudate with properties associated with classical dopamine receptors (2). 3H-Dopamine labels a dopamine receptor with extremely high affinity for dopamine and low affinity for neuroleptics (2). Calf crude homogenates were prepared as previously described with minor modifications (2). The specific binding of 3H-spiperone and 3H-dopamine were determined as previoulsy described with minor modifications (2). The results in fig. 1A demonstrate that phenoxybenzamine irreversibly inhibited 3H-spiperone specific binding, while spiperone itself did not. Figure 1B demonstrates that phenoxybenzamine had no effect on 3H-dopamine specific binding. These results indicate that there is a functional moiety at the dopamine receptor capable of irreversible interaction with phenoxybenzamine, and that the receptor labelled by 3H-spiperone is molecularly distinct from that labelled by 3H-dopamine.



1. L.T. Williams and R.J. Lefkowitz, Mol. Pharmacol. 13, 304 (1977).
2. M. Titeler, J. Tedesco and P. Seeman, Life Sci. 23, 587-592 (1978).

90.9 EQUILIBRIUM DIALYSIS -- A NEW AND MORE SENSITIVE METHOD FOR MEASURING 3H-DOPAMINE.

A.Hitri*, H.L.Klawans*, W.J.Weiner, P.A.Nausieda, and C.G.Goetz* Department of Neurological Sciences, Rush-Presbyterian, St.Luke's Medical Center, Chicago, IL 60612

The ultrafiltration technique using naturally occurring dopamine in its radioactive form have yielded controversial results in the study of CNS dopamine receptors. In our recent studies, we compared equilibriaum dialysis with the ultrafiltration method using a multiple semimicro equilibrium dialyser. We performed simultaneous kinetic and saturation studies of 3H-dopamine binding to striatal membranes of guinea pigs. The studies were carried out at various temperatures. The results indicate that the amount of stereospecifically bound dopamine is greatly enhanced by the equilibrium dialysis method. Moreover, the simultaneous kinetic and saturation studies allow estimation of the intrinsic affinity constants and a direct comparison with the equilibrium constant. The equilibrium dialysis method provides a more specific means for studying dopamine receptors using the naturally occurring agonist 3H-dopamine. The equilibrium dialysis apparatus and methods will be discussed as well as the comparative results between equilibrium dialysis and ultrafiltration.

90.10 The Effects of Chronic Lithium Administration on Serotonin Receptors in Rat Brain.

Susan L. Treiser*, Nguyen B. Thoa,* Tom L. O'Donahue, David M. Jacobowitz, Caren S. Cascio* and Kenneth J. Kellar. Department of Pharmacology, Schools of Medicine & Dentistry, Georgetown University, Washington, D.C. 20007, Lab. Clin. Sci., NIMH, Bethesda, Md. 20205.

The mechanisms of action by which lithium ion exerts its therapeutic and prophylactic effects in manic-depressive illnesses remain unknown. Several recent studies have shown that chronic administration of Li alters neurotransmitter receptor processes in rat brain. Since alterations in serotonin neurotransmission are thought to be involved in affective disorders, we have examined the effects of chronic Li administration on serotonin receptors in rat brain. Two types of serotonin receptors have been described. One type binds ^{3}H-5-HT and the other binds ^{3}H-spiperone. These binding sites have been referred to as serotonin$_1$ and serotonin$_2$ receptors, respectively (Peroutka and Snyder Mol. Pharm. 16, 1979). Chronic Li administration affects both types of receptors in the rat hippocampus, but neither type appears altered in the rat cerebral cortex.

Male Sprague-Dawley rats (200-250 g) were fed a diet containing 0.25% lithium carbonate mixed in powdered rat chow. Control rats received the identical powdered rat chow without the added Li. After 4-6 weeks on the diet, rats were sacrificed by decapitation and blood was collected for measurement of Li and sodium levels by flame photometry. Blood levels of Li were approximately 1-1.2 meq/liter.

The serotonin receptor binding sites were measured using both ^{3}H-5-HT and ^{3}H-spiperone in the cortex and hippocampus.

We found no difference in either ^{3}H-5-HT or ^{3}H-spiperone binding in rat brain cortex following Li treatment. However, in the hippocampus there was a significant decrease, approximately 20%-30% in the number of binding sites measured by both ligands.

To determine whether Li exerts differential actions on presynaptic serotonin processes in cortical and hippocampal neurons which could account for the differential effects on receptor numbers in these two areas, we examined the effects of chronic Li administration on the uptake and release of 5-HT. Chronic Li administration did not alter 5-HT uptake in the hippocampus or cortex. However, our preliminary results indicate that chronic Li decreases serotonin release in the cortex, but increases serotonin release in the hippocampus. Thus, chronic Li administration appears to exert brain area specific effects on both pre- and post-synaptic neurotransmitter processes.

Supported by USPHS DA02540

91.1 EFFECTS OF M-ENKEPHALIN (ME) AND SUBSTANCE P (SP) ON THE SENSORY DISCHARGE AND RESPONSE OF CAROTID BODY CHEMORECEPTORS TO ACH AND DOPAMINE. L. Monti-Bloch* and C. Eyzaguirre. Dept. Physiol., Univ. of Utah Col. Med., Salt Lake City, UT 84108.

The carotid body and its nerve, removed from anesthetized cats, were placed in a superfusion chamber through which flowed modified Tyrode's solution equilibrated with 50% O_2 in N_2 at 36° and pH 7.43. Drugs were applied in volumes of 1-10 µl and sensory discharges were recorded from the nerve. Low concentrations (3.3 µM) of SP did not change the basal discharge or the stimulatory effect of ACh 100 µg. They did, however, reverse the normal inhibitory effect of dopamine (DA) 100 µg which induced excitation after SP. Larger concentrations of SP (33 µM) depressed the sensory discharge and potentiated the normal excitatory effect of ACh 100 µg. Low concentrations of ME (10 µM) increased the basal discharge, depressed the excitatory effect of ACh 100 µg and blocked the inhibitory effect of DA 100 µg. Larger concentrations of ME (100 µM) increased the basal discharge, reversed the excitatory effect of ACh, which after ME induced discharge depression, and also blocked the inhibitory effect of DA. Naloxone 10^{-6} g/ml (an enkephalin blocker) blocked the discharge increase induced by ME 10 µM and restored the inhibitory effect of DA on the sensory discharge. Preliminary experiments have shown that both SP and ME tend to depress the excitatory effects of anoxia (100% N_2), hypercapnia (6% CO_2 in 50% O_2 and 44% N_2) and NaCN 5 µg on the sensory discharge.

These experiments show potent effects of neuropeptides on the basal and evoked chemosensory discharge. The physiological meaning of these observations is still uncertain although peptides may have an important role in chemoreception. SP occurs in the carotid body (presumably afferent) nerves while ME has been found in the carotid body (presumably the glomus or type I) cells. If peptides are released at rest and/or during stimulation, they may interact with the better known putative neurotransmitters (ACh and DA) which are released under those circumstances. Supported by grants NS 05666 and NS 07938 from the U.S. Public Health Service.

91.2 TASTE RESPONSES TO ELECTROLYTES IN ORDINARY WATER AND DEUTERIUM OXIDE. Inglis J. Miller, Jr., Dept of Anatomy Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

Solutions of deuterium oxide (D_2O) elicit larger taste responses from rat chorda tympani nerves than corresponding solutions of ordinary water (H_2O). The study of this effect has been extended to the hamster and to a wider variety of cations and anions than in a prior report. Stimuli consisted of cations Na, Li, K, Mg and Ca with anions Cl, acetate (Ac) and benzoate (Bz) in concentrations from .03 m to 1.0 m. Summated responses from the entire chorda tympani nerves were compared during the tonic phase of a 20 sec stimulation period followed for 40 sec by an inter-stimulus rinse period. Animals were male Sprague-Dawley rats weighing 250 gm and golden hamsters weighing 125 gm. Responses to pure D_2O applied to an H_2O-adapted tongue ranged from .15 to .3 (re: 0.1 m NaCl in H_2O) in both rats and hamsters. Responses to all salts were greater for solutions in D_2O at concentrations below 0.1 m than corresponding solutions in H_2O, while at higher concentrations the responses to H_2O were greater than D_2O. The disparity between D_2O and H_2O responses was greater for potassium salts and divalent cations, Mg and Ca, than for Na and Li salts in the hamster. Suppression of the background activity produced by lower concentrations of KCl and KBz was diminished or absent in the hamster. In the rat, low concentrations of K, Ca and Mg salts in D_2O elicited larger responses than in H_2O. Rinsing KBz from the tongue with H_2O elicited responses with H_2O and D_2O as the stimulus solvent. No adequate explanation for the disparate effects of H_2O and D_2O can be demonstrated currently. It is speculated that D_2O may be sequestered differently than H_2O in the receptor phase of the taste cell membrane. An altered interaction between electrolyte stimuli and the receptor membrane may be sufficiently different in D_2O than in H_2O to enhance the response to low concentrations of stimuli and diminish the response to higher concentrations. (Supported in part by NIH Grant NS 10389).

91.3 MODIFICATION OF STIMULUS-RECEPTOR INTERACTIONS IN OLFACTORY RECEPTOR NEURONS BY ASCORBATE AND GLUTAMATE. N.S. Nadi* and T.V. Getchell, Morin Memorial Lab., Dept. Anatomy, Wayne State Univ. School of Medicine, Detroit, MI 48201.

The bipolar olfactory receptor neuron is readily accessible to electrophysiological and biochemical probes. The properties of molecular receptor and axonal membranes were investigated in the olfactory epithelium of the tiger salamander. The characteristics of the receptor membrane were studied by recording unitary spike potentials in response to orthodromic stimulation with odors in the vapour phase. The mechanisms of impulse initiation and transmission were studied by recording unitary spike and compound olfactory nerve (CONP) potentials in response to electrical stimulation. Several compounds were chosen to characterize the functional properties of each type of membrane. Their effects were studied by applying µl quantities in solution to the epithelial surface. The peak to peak amplitude of the CONP was reduced 40% by ethanol (ET, 0.5M), n-butanol (B, 0.5M), ethyl-n-butyrate (ENB, 0.5M), N-ethylmaleimide (NEM, 0.05M). Ascorbate (ASC, 0.5M), glutamate (GLU, 5mM) and carnosine (CAR, 5mM) had no effect on the CONP or the antidromically driven single units (su). At concentrations higher than 10mM ASC blocked su responses evoked by phenyl ethyl alcohol (PEA), benzaldehyde (BAL) and benzyl acetate (BAC). This was presumably due to an interaction of ASC with molecular receptor sites. At concentrations greater than 0.1mM GLU caused facilitation of the response to odor stimulation. Facilitation consisted of a decreased response latency, increased firing frequency and a prolonged response duration. In contrast, treatment with ET (0.5M) and NEM (0.05M) resulted in a decrease in the excitatory response parameters and an increased response latency. The observations with NEM suggest that both the molecular receptor and the axonal membranes may rely on sulfhydryl groups for proper function. B and ENB in solution as well as CAR had no effect on su responses elicited by PEA, BAL and BAC. The results indicate that B, ENB, NEM, and ET effect both the receptor and axonal membranes. CAR, a dipeptide found in the olfactory receptor neurons had no effect on the CONP or the odor-evoked responses. GLU caused facilitation of response to odors which may be due to a nonspecific depolarization of the neuronal membrane resulting in a decrease of its threshold to odor stimulation. Alternatively, the GLU effect could be due to its interaction with a receptor site. These two hypotheses are currently being investigated. Previously reported experiments have shown that ASC reduces the binding of neurotransmitters to membrane preparations from the brain. In agreement with these results we have shown that ASC modifies the molecular interaction of odors with receptor sites on olfactory receptor neurons. Supported by NSF-BNS-7912601.

91.4 OLFACTORY RECEPTORS RESPOND TO ODORANTS INJECTED INTO THE BLOODSTREAM. J.A. Maruniak*, W.L. Silver, and D.G. Moulton. V.A. Medical Center and Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104.

When human subjects receive odorant injections intravascularly they report an odorous sensation. How these odorants reach the receptors (either exhaled from the lungs or more directly) has long been a matter of controversy. To clarify this question and investigate the phenomenon further, we recorded pigeon olfactory nerve twig responses to both intravascularly (IV) and intranasally (IN) delivered odorants. IN odorants were generated in an air dilution olfactometer and delivered by a teflon tube inserted into the bird's external nares. Odorous solutions were injected into the arterial blood supply through cannulas in either the internal or external carotids. To stimulate IV, we injected 0.3ml of odorant in saline solution or a saline control over a 3 sec period.

IV injection of an odorant, but not the saline control elicited olfactory nerve twig responses. These responses were similar to those obtained from vapor phase stimulation, exhibiting an initial phasic component followed by a decline to a steady state tonic level. The response to IV stimulation did not arise from odor diffusing from the lungs into exhaled air since tracheotomized birds also exhibited the response. The IV response magnitude was concentration dependent, increasing with increasing odorant concentration. The highest concentrations of odorants used were determined by the solubilities of the compounds in saline. Odorants delivered IV for prolonged periods in high concentrations did not completely adapt the receptors since concurrently delivered IN odorants always produced strong responses. When the olfactory receptors were adapted to an IN delivered saturated odorant, no response to the same odor could be obtained by concurrent IV stimulation. However, IV stimulation with a dissimilar odorant did produce a response. This suggests that odors delivered IV can be discriminated and that the response is odorant specific. It is also established that the odorants do not reach receptors in exhaled air. Otherwise, it is not clear how IV delivered odorants reach the receptor sites or by what mechanisms they stimulate. What does emerge, however, is that the magnitude of the IV response approaches that of IN responses to odorants. This indicates the need for further research to determine whether such effects can lead to alterations in olfactory sensitivity following food intake or in certain disease conditions. (Supported by NIH grants HD 05547 and NS 10617.)

91.5 TRANSDUCTION PROCESSES IN REGENERATING OLFACTORY EPITHELIUM. Gloria D. Adamek*, Robert G. Mair* and Robert C. Gesteland, Northwestern University. Evanston, IL 60201.

Frog olfactory receptors bear different types of cilia which appear to be related to the age of the cell. Wigglers are 10 to 20 µm long, move rapidly and can bend in more than one plane. Strokers are 15 to 50 µm long, move more slowly than wigglers and move only in one plane. Streamers are 30 to 180 µm long and are immotile. Treatment with 0.5% Triton X-100 removes all cilia. At 8 hours after treatment, the olfactory epithelium is covered with short, immotile cilia, 3 to 16 µm long. All three cilia types can be distinguished at 16 hours. Strokers (10 to 30 µm) and wigglers (8 to 12 µm) approximate their normal lengths. Streamers continue growing from 14 to 34 µm at 16 hours post-treatment to 50 to 180 µm by 9 days post-treatment. Perfusion of frog olfactory sac with 3% $ZnSO_4 \cdot 7H_2O$ causes degeneration of the entire olfactory epithelium. Sparsely occurring motile cilia are first apparent 12 days post-treatment. By 16 days post-treatment, the epithelium is more densely covered with wigglers (8 to 16 µm) and strokers (16 to 18 µm). Sparse streamers (30 to 40 µm) are visible 27 days post-treatment. Streamers become more numerous and their length increases to 60 to 140 µm by day 45. The orderly regrowth of cilia following these treatments provides a means to isolate cilia types for electrphysiological study. EOG studies show that electrical excitability returns to near normal levels by 16 hours after detergent treatment. Activity of single units immediately after deciliation is abnormal, a brief burst of regular, 0.2 second interval action potentials interspersed between long (ca 1 minute) silent periods, or single, constant-interval continuous firing. Normal unit activity is also restored withing 16 hours. We have previously shown that receptor selectivity is different at different stages of development. We conclude that the proximal portions of the cilia are the site of olfactory reception and transduction and that the receptor neurons elaborate ciliary membranes with different properties at various stages of development.

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91.6 ODORANT SPECIFIC MAPS OF RELATIVE SENSITIVITY INHERENT IN THE SALAMANDER OLFACTORY EPITHELIUM. Alan Mackay-Sim* and David G. Moulton. Dept. of Physiology, Univ. of Penn. and V.A. Med. Ctr. Philadelphia, PA, 19104.

When patterning due to sorptive effects is eliminated the anterior and posterior regions of the salamander olfactory epithelium still show differential sensitivity to some odorants (Kubie and Moulton, Soc. Neurosci. Abstr., Vol. 5, p. 129, 1979). We have undertaken a more detailed mapping to elucidate the resolving power of inherent spatial patterning. Electro-olfactograms (EOGs) were recorded at different points on the epithelium after punctate odorant stimulation at the electrode tip. We directly compared relative sensitivity to different odorants, by measuring EOGs at 30 points across the ventral olfactory mucosa. At each of these points amplitudes of at least 3 EOGs were measured in response to 1s pulses of each of two odorants. Maps for each odorant pair were made of epithelia from at least 8 animals. These maps reveal differential sensitivity across the epithelium that is more complicated than simple anterior/posterior differences. For example, the maps for pinene and limonene, both generally posterior stimulators, also reveal smaller areas ($\frac{1}{2}$-$1mm^2$) with extremely high sensitivity. At present though, no differences in the distribution of sensitivity to pinene and limonene can be detected. In contrast, the distributions of sensitivity to eugenol and isoeugenol can be clearly distinguished. In addition to a distinct separation of the points of maximal sensitivity to each of these odorants (often 2mm apart), the epithelium can be divided into a postero-lateral segment (1/4-1/3 of the ventral olfactory epithelium) which is absolutely more sensitive to eugenol, and the rest, which is absolutely more sensitive to isoeugenol. Sensitivity maps for other odorants (propanol, butanol, decanol, heptaldehyde) reveal areas on each epithelium that are highly sensitive to these compounds. The locations of these areas are more variable, but distinct from the area most responsive to pinene and limonene. The map of sensitivity to amyl acetate reveals a distinct ridge of high sensitivity commonly stretching anteriorly for about 2mm from the posteriorly located pinene or limonene sensitive area. We conclude that there exist regions of enhanced sensitivity specific for different odorants. The variations in the shapes and positions of these regions indicate that the olfactory epithelium may have a well developed capacity to discriminate among odorants on a topographic basis.

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91.7 POSTNATAL DEVELOPMENT OF RAT OLFACTORY BULB NEURONS. Robert G. Mair* and Randy L. Gellman* (Spon: C. Enroth-Cugell). Dept. Biol. Sci. Northwestern University, Evanston, IL 60201.

Mammalian olfactory bulbs contain several distinct classes of interneurons, at least two of which (periglomerular and granule cells) make direct synapic contacts onto mitral and tufted cells. The complexity of synaptic connections within mature olfactory bulbs makes if difficult to determine the effects of interneurons on the response properties of mitral and tufted cells. Autoradiographic studies of histrogenesis in the bulb reveal that whereas the full complement of mitral and tufted cells are present at birth, most interneurons develop postnatally. Likewise, electronmicroscopic studies have suggested that synapses between receptor axons and second order neurons are more precocious than those formed by interneurons. This study describes the development of different types of interneurons and compares some response properties of olfactory bulb neurons in adult & neonatal rats. Material for anatomical study was obtained from two day postnatal (P2), P2, P15, P29 and adult rats and processed for cresyl and golgi staining. Examinationof cresyl stained material shows that the mitral cell body layer occupies a relatively large proportion of the olfactory bulb in P2 rats. The relative volumes of the external plexiform layer, the internal plexiform layer, and the granule cell layer increase dramatically by P15. With golgi impregnation we see all cell types apparent in the adult bulb by P15. In accordance with the previously cited evidence, at P2 few cell types are present, the population consisting mainly of mature and immature mitral cells and a few immature interneurons. Single unit electrophysiological studies demonstrate that the P2 rat is a useful preparation for describing response properties of mitral and tufted cells in isolation of many interneural influences.

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91.8 DISTRIBUTION OF LABEL IN THE HAMSTER MAIN AND ACCESSORY OLFACTORY BULB AFTER INTRANASAL ADMINISTRATION OF [3H]ß-ALANINE. Gail D. Burd, Barry J. Davis, Foteos Macrides, and Frank L. Margolis. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545 and Roche Institute of Molecular Biology, Nutley, NJ 07110.

Biochemical studies have shown that labeled ß-alanine, when administered to the nasal cavity, is specifically incorporated into carnosine, and this labeled dipeptide is transported to the olfactory bulb. The purpose of the present investigation was to visualize the distribution of radioactive label in the olfactory (OE) and vomeronasal (VE) epithelia and main (MOB) and accessory (AOB) olfactory bulbs after intranasal administration of [3H]ß-alanine.

Hamsters were anesthetized with Nembutal and also given 0.2 cc epinephrine I.P. to facilitate movement of fluids into the vomeronasal organ. Anesthetized hamsters received unilateral intranasal administration of purified [3H]ß-alanine (83 µCi in 50 µl). After survivals of 6 hrs, 24 hrs, or 4 days, the animals were perfused with 1% paraformaldehyde and 1% glutaraldehyde. The olfactory bulbs and decalcified nasal cavities were embedded in paraffin and processed for autoradiography.

On the side of intranasal administration, both the OE and VE were intensely labeled at 6 hrs. Label was associated with sensory and nonsensory epithelia, bundles of axons, and the lamina propria. The overall labeling of these tissues was reduced at 24 hrs and 4 days with most of the remaining labeling confined to bundles of axons. The primary nerve layer and glomeruli of the MOB and AOB were labeled at the three survival times examined. However, unlike the periphery, the most intense labeling in the MOB and AOB was observed at 24 hrs after intranasal [3H] ß-alanine administration. Less intense labeling of these areas was present after 6 hrs and 4 days. Medial portions of the contralateral nasal cavity and olfactory bulb were also labeled, but with less intensity than the ipsilateral side. The contralateral labeling was due to movement of fluids from the side of administration through the septal window of the nasal cavity.

The present results are consistent with the interpretation that carnosine is transported to the olfactory bulb in the primary afferent fibers. The similar pattern and time course of labeling in the main and accessory olfactory systems suggest that these two systems have similar transport mechanisms for carnosine.

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91.9 DISTRIBUTION OF MET-ENKEPHALIN, SUBSTANCE P AND SOMATOSTATIN IMMUNOREACTIVE NEURONS IN THE MAIN OLFACTORY BULB IN THE HAMSTER. Barry J. Davis, Gail D. Burd and Foteos Macrides. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

The unlabelled antibody peroxidase method (PAP) was used to study the locations of met-enkephalin (MET), substance P (SP) and somatostatin (SS) immunoreactive neurons in the laminae of the main olfactory bulb (MOB) in the hamster. The animals were anesthetized with Nembutal and perfused with a 2% paraformaldehyde - saturated picric acid fixative. The brains were cut on a Vibratome and sections were incubated freely floating in the primary antiserum (1:200) for 24 - 48 hrs. The size and distributions of immunoreactive neurons were compared to those of neurons in Golgi-impregnated material.

MET immunoreactive somata and dendrites were observed in all layers of the MOB. These MET somata were mostly ovoid in shape and had minor and major diameters which averaged 7 and 10 microns, respectively. Intense staining of MET somata and dendrites was observed in the periglomerular region (PGR) of the MOB. These somata outlined the glomeruli, and dendritic arborizations extended into the individual glomeruli. The location and size of these MET somata correspond with those of periglomerular cells in the Golgi material. Less numerous and more lightly stained MET somata and dendrites of the same size as the cells in the PGR were observed in the granule cell (GCL), internal plexiform, and mitral body layers. These MET somata are comparable in distribution and size to granule cells. Isolated MET somata were also observed in the external plexiform layer (EPL).

SP immunoreactive somata and dendrites were also present in the PGR. SP somata were both fusiform and ovoid in shape and had minor and major diameters which averaged 11 and 13 microns, respectively. SP somata were restricted to the PGR and these somata appeared more numerous in the deep aspect of the PGR near the EPL. The location and size of these SP somata are similar to those of superficial short axon and external tufted cells in the Golgi material.

Isolated SS immunoreactive somata were observed in the deep aspect of the GCL near the ventricle. These SS somata were well-defined, primarily ovoid in shape and had minor and major diameters which averaged 11 and 13 microns, respectively. The SS somata are larger than the somata of granule cells and correspond in size with the somata of deep short axon cells in the Golgi material.

These results suggest that the three neuropeptides are associated with different classes of neurons in the MOB.

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91.10 CHEMICAL SENSES INVOLVED IN GARTER SNAKE AGGREGATION AND SHELTER SELECTION. Steven B. Heller* and Mimi Halpern, Prog. Biol. Psychol. and Dept. Anat. Cell Biol. Downstate Medical Center, S.U.N.Y., Brooklyn, N.Y. 11203.

Recent studies have demonstrated the importance of the garter snake's vomeronasal (VN) system in prey extract trailing, prey attack and male courtship behavior. The present study investigates the relative contribution of the VN and main olfactory systems in aggregation and shelter selection by garter snakes.

Initial studies demonstrated that when groups of garter snakes are tested repeatedly in aquaria that are left uncleaned between trials, the snakes predictably return to certain (preferred) shelters and not to others. Following group testing, snakes tested individually in the same aquaria return to previously preferred shelters. Cues used by snakes in returning to preferred shelters appear to be chemicals deposited by the snakes on the substrate underlying the preferred shelter.

Snakes with VN nerve lesions or VN duct sutures (which prevent chemical access to the VN organ) do not return to previously preferred shelters when tested individually. However, when tested with a group that includes "control" animals, they return to the previously preferred shelters. The behavior of animals with olfactory nerve lesions, sham lesions or sham duct sutures is indistinguishable from preoperative levels when these animals are tested individually or in groups. Animals that fail to go to previously preferred shelters when duct sutures are in place, return to preoperative levels of performance when their sutures are removed.

Normal snakes tested in aquaria in which the substrate and other contents are cleaned between trials make shelter choices that are random with respect to position, but they select shelters occupied by a number of other snakes more often than would be expected by chance. This tendency for group aggregation is unaffected by blindfolding, olfactory nerve cuts or combined visual and olfactory deprivation. When the snakes' VN ducts are sutured closed, however, they fail to aggregate; following removal of the sutures they resume aggregating.

These studies strongly suggest that use of chemical signals by garter snakes in shelter selection and aggregation is mediated by the VN system and that neither the olfactory nor the visual system is critical for these behaviors.

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91.11 BLOOD SUGAR LEVELS AFFECT TASTE RESPONSES IN THE RAT NUCLEUS TRACTUS SOLITARIUS. Barbara K. Giza* and Thomas R. Scott. Dep't. Psychol. and Inst. for Neurosci. and Behav., U. Delaware, Newark, DE. 19711.

Subjective experience tells us that taste perceptions change with levels of satiety. Glenn and Erickson reported a neural counterpart to this experience: stomach distension caused a decrease in afferent activity in the rat's NTS. We studied the effect of blood sugar levels on NTS responses to the four basic taste qualities. Subjects were 32 undeprived female albino rats, divided into equal sized experimental and control groups. We intubated each subject in both the jugular and femoral veins for the injection of solutions and retrieval of blood samples, respectively. We inserted etched tungsten semi-micro electrodes (tip length = 20-30 mμ) into the NTS until we encountered robust gustatory activity evoked by chemical stimulation of the tongue. Stimuli were 1.0 M glucose, 0.1 M NaCl, 0.03 M HCl and 0.01 M QHCl. In each case we flowed 18 ml of solution over the tongue in 4 sec and followed that with a 30 sec DH_2O rinse and a rest period of 50 sec. We applied each stimulus four times over a 25-min period which served to monitor the stability of our recording and to establish a pre-injection response level. We regularly took small blood samples throughout this and the succeeding periods. At time T=0 we injected either 500 mg/kg glucose (1.25 ml/kg of a 40% glucose solution) or an equivalent volume of isotonic saline into the jugular vein and continued to monitor blood sugar levels and taste activity for the next 60 min. Blood glucose concentration increased in experimental animals from approximately 90 mg% pre-injection to a peak of 220 mg% at 12 min, after which it declined to almost pre-injection levels over 60 min. Controls showed no major changes in blood sugar. Integrated multiunit activity evoked by glucose declined in experimentals relative to pre-injection and control levels ($t = 2.41$; $p < .05$), reaching a nadir at 12-15 min and recovering gradually over 60 min to form a mirror image of the blood glucose curve. NaCl and HCl responses in experimentals each showed only insigificant decreases relative to their controls ($t = 0.65$; $p > 0.2$) and QHCl showed no change. Additional control animals from whom we recorded touch-evoked responses from adjacent trigeminal nuclei showed no changes with blood glucose levels. The mechanism which causes taste activity to be sensitive to blood sugar levels could be peripheral (an adaptation effect) or central. Hypothesized central mechanisms include direct inhibition of NTS, or descending inhibition imposed by forebrain feeding areas.

91.12 PREFERENCES FOR NONSEXUAL ODORS ARE INFLUENCED BY TESTOSTERONE IN HAMSTERS. Catherine A. Cornwell-Jones and Kathryn Kovanic*, Dept. of Psychology, Princeton University, Princeton, NJ 08544.

Aversion to novel odors declines with age in male hamsters (Cornwell-Jones, J. Comp. Physiol., Psych., 93). The present experiments examined the possibility that increases with age in testosterone might mediate the decrease in olfactory neophobia.

A first experiment compared the effects on olfactory neophobia of castrating juvenile vs. adult male hamsters to test the prediction that castration effects on preferences increase with age as testosterone levels rise in intact males. Male hamsters reared in pine shavings were castrated either as juveniles or adults. Sham operated males served as controls at both developmental stages. After a postsurgical recovery period of 7-10 days, animals were tested in a two-choice situation allowing them to smell but not taste or touch shavings in two compartments below a screen floor.

Adult castrates preferred the odor of familiar pine shavings to unfamiliar cedar odor, while adult sham operates showed no preference. Juveniles in both treatment groups tended to prefer pine to cedar odor. The results were consistent with the hypothesis that age-dependent increases in testosterone reduce olfactory neophobia.

A second experiment examined the effects of testosterone replacement on olfactory neophobia in castrated adult male hamsters. Pine-reared males were either sham operated or castrated. Castrates were then implanted with constant timed-release capsules of silastic tubing containing either no testosterone (T) a dose estimated to produce subnormal plasma T levels, or a dose estimated to produce physiologically normal T levels. These animals were tested on fresh cedar vs. fresh pine, and on the odor of cedar-nest in which a female had been housed vs. pine.

Castration reduced the time spent investigating both fresh cedar and cedar-nest, while the larger but not the smaller dose of testosterone restored investigation of both odors to the level of sham operates. Sham-operated animals and those treated with the higher testosterone dose, showed no preference between the fresh odors, and showed statistically similar preference for female cedar-nest odor vs. pine. In contrast, castrates receiving either no testosterone or the smaller dose, preferred pine to either cedar odor.

These data imply that testosterone dampens neophobic responses to nonsexual odors by male hamsters. Hamsters do not appear to live in social groups in the wild. High endogenous testosterone levels may help insure that novel aversive odors do not interfer with a male hamster as he searches for a female mate in unfamiliar olfactory territory.

92.1 UNMYELINATED AXONS IN THE DORSAL WHITE COLUMN. Lauren A. Langford and Richard E. Coggeshall. Depts. of Anatomy and of Physiology and Biophysics and the Marine Biomedical Institute. The University of Texas Medical Branch, Galveston, Texas 77550.

According to classical studies, the dorsal white column in the spinal cord consists of myelinated primary afferent fibers, many of which pass cranially to end in nuclei in the medulla oblongata. These fibers give rise to collaterals which enter the gray matter. Descending primary afferent fibers have also been described. The classical studies were done with light microscopy, however, and thus these conclusions are restricted to myelinated fibers since unmyelinated axons are beyond the resolution of the light microscope.

In the study we used the electron microscope to count both myelinated and unmyelinated axons in the normal S3-S4 rat dorsal white columns. Fixation was improved by the addition of potassium ferricyanide to the osmic acid postfixation. The axonal counts are listed in the table below. Before proceeding, however, it should be noted that the corticospinal tract can be distinguished from the dorsal column proper because it lies in the ventro-medial area of the dorsal white column and its myelinated axons are much smaller than in other parts of the dorsal white column. These 2 areas are:

		DORSAL COLUMN PROPER		CORTICOSPINAL TRACT		DORSAL COLUMN TOTAL	
RAT	SEG	MY	UN	MY	UN	MY	UN
1	S3	3654	4338	1695	2880	5299	7218
2	S3	3818	5214	1568	2794	5386	8008
3	S4	2162	4702	1035	3451	3197	8153
Avg.		3211	4751	1433	3042	4627	7793

In conclusion we have found that the ratio of unmyelinated to myelinated axons in the dorsal white column is approximately 1.5 to 1. Selective surgical procedures will be done to determine the origins and destinations of these unmyelinated axons in the posterior white columns. Supported by grants NS 10161, NS 11255 and NS 07377.

92.2 PAIN AND DORSAL RAPHE INPUTS TO THE PARAFASCICULARIS NUCLEUS OF THE RAT E. Andersen, B.M. Rigor and N. Dafny Depts. of Neurobiology and Anatomy and Anesthesiology, Univ. of Texas Health Science Center, Houston TX 77025.

The raphe nuclei have been implicated in an endogenous descending mechanism of pain control which suppresses pain perception at the spinal cord level. The dorsal raphe nucleus (DR) also has extensive projections to forebrain structures, including the parafascicularis nucleus of the thalamus (PF), an area which has been shown to be responsive to noxious stimuli. The present study was initiated to test the hypothesis that the DR is involved in an ascending pain modulation system.

Male Sprague Dawley rats were anesthetised with urethane and placed in a stereotaxic instrument. Bipolar concentric stimulation electrodes were lowered into the DR according to the atlas of Konig and Klippel (AP=0 L=0 H=-1). The DR was stimulated with monophasic square wave pulses (.2msec width, .1-1mA) at various frequencies. Glass micropipettes were filled with 4M NaCl saturated with fast green and lowered into the PF (AP=3.3mm L=1.0 H=0) for single unit recordings. A period of baseline firing of spontaneously active cells was recorded for 10 min. Each cell was then given four stimulation treatments: noxious tail pinch, DR electrical stimulation at 1 cycle/sec (c/s), DR stimulation at 20c/s, and both DR at 20c/s and tail pinch together. Each stimulation period lasted 2 minutes and was followed by a 2-4 min recovery period. At the end of the experiment, the animals were sacrificed, perfused with 10% formalin, and electrode sites were histologically verified. Fifty cells were analysed.

Tail pinch was found to cause an increase in the firing rate of 83% of the PF cells, a decrease in 9% and no change in 8%. DR stimulation at 1c/s caused a change in firing patterns in only 26% of the PF cells. Total number of spikes increased in 8%, decreased in another 8% and a period of inhibition lasting 20-30 msec after each stimulus pulse was seen in 10% of the cells. In contrast DR stimulation at 20c/s caused changes in 85% of the PF cells tested: 50% decreased and 35% increased firing rates. The changes usually outlasted the period of stimulation for several seconds. When DR stimulation at 20 c/s was combined with tail pinch, tail pinch did not cause the increase in firing rates as it would without the DR stimulation.

In conclusion, tail pinch causes an increase in the firing rates of PF cells in the rat, DR stimulation at 1 c/s causes only minor changes, and DR stimulation at 20c/s causes significant changes in PF firing rates. DR stimulation at 20c/s also inhibits the response of PF cells to tail pinch. It is suggested that the DR causes pain modulation in the PF.

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92.3 MEDIAL THALAMIC RESPONSE TO NOXIOUS INPUTS: INHIBITORY INTERACTIONS WITH LATERAL THALAMIC STIMULATION. A.L. Benabid*, S.J. Henriksen, J.F. McGinty, F.E. Bloom (SPON: E. Battenberg). A.V. Davis Ctr. for Behav. Neurobiology, The Salk Institute, La Jolla, 92037.

Neuronal interactions in the thalamus between sensory (specific) and noxious (non specific) inputs were investigated by analyzing the response in nucleus parafascicularis (PF) to subcutaneous electrical noxious stimulation (NS) of the limbs, with and without ventro-postero-lateral nucleus (VPL) thalamic stimulation. Extracellular recordings in chloral hydrate anesthetized rats were accomplished with either glass micropipettes (in PF), or 25 μ stainless steel electrodes (in VPL; also used for stimulation). The latter method provided information on VPL multiple unit activity and simultaneously directed the stimulating electrode placement. Computer generated post-stimulus time histograms demonstrated that, in the absence of VPL stimulation, PF cells respond to NS with peaks corresponding to A, Aδ and C fiber inputs or by inhibition of spontaneous activity. The multiunits recorded in VPL following NS consistently exhibited a response to A, Aδ and C inputs; in addition, a "M-like" histogram pattern, (as defined by Emmers (1) in the S-2 area of VPL) was also observed in S-1. Single or triple pulse stimulation of VPL without NS frequently elicited a response in PF cells with a latency from 50 to 250 msec. For the majority of PF cells, train stimulation of VPL induced a strong alteration in the post stimulus time histogram elicited by NS, ranging from the disappearance of one peak (A, A or C fibers response) to, more often, a complete and long-lasting (>500 msec) inhibition of the PF cells activity. This inhibition was positively correlated with the VPL stimulation parameters. Medial thalamic cells responsive to NS but not in PF were differentially influenced by VPL stimulation, depending on their anatomical location. The long lasting inhibition of PF cells by VPL train stimulation suggests the existence of a lateral to medial thalamic mechanism modulating pain. The intrathalamic connection between medial and lateral thalamus is supported by the time-locked response of PF cells to VPL single shocks. The possible chemical and anatomical basis underlying this VPL stimulation-induced PF inhibition are under investigation. 1) EMMERS, R.: J. Comp. Neur., 1965, 124, 215-228. Supported by DA 01785 and a NATO fellowship to A.L.B.

92.4 RESPONSE OF PRIMATE SI CORTICAL NEURONS TO NOXIOUS STIMULI. D. R. Kenshalo, Jr. and O. Isensee*. Division of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

Previous results indicated that many neurons in VPLc responsive to noxious stimuli project to SI cortex. To study the possibility that SI neurons receive nociceptive information, we recorded from single cortical neurons in the Java Monkey (Macaca fascicularis). The animals were anesthetized with α-chlorolose and maintained with supplements of sodium pentobarbital. Single units were isolated using tungsten microelectrodes and in 5 cases the position of the electrode was marked with an electrolytic lesion. The cutaneous receptive field was tested with innocuous and noxious stimuli. Innocuous stimuli included hair movement, light tough and joint rotation. Noxious stimuli applied to the skin included pinching and noxious heat pulses (from 35° adapting temperature to 43, 45, 47, 50, 53 and 55°C)

To date 20 cortical neurons have been sampled that responded to noxious stimuli. Of these 13 were classified as having a wide dynamic range receptive field (responded to both innocuous and noxious stimuli) and 7 were classified as having a narrow dynamic range high threshold receptive field (responded to noxious stimuli). Cortical cells responsive to noxious stimuli exhibited a progressive increase in the discharge rate as the intensity of a mechanical stimulus increased. The threshold for initiation of activity during an ascending series of noxious heat pulses was near a mean of 50°C. The threshold for cortical neurons is higher than that reported for either spinothalamic tract or thalamic neurons. The increase in the thermal threshold may be due to anesthesia. Reconstruction from electrolytic lesions for 5 cells indicated that the neurons responsive to noxious stimuli were located in area 1.

From these data, we speculate that neurons in SI receive nociceptive information and thereby play a role in the processing of this information.

92.5 CORRELATIONAL ANALYSIS OF HYPERALGESIA IN HUMANS WITH RESPONSES OF NOCICEPTIVE PRIMARY AFFERENTS IN THE MONKEY. J.N. Campbell, R.A. Meyer*, and S.M. Lancellotta*, Dept. Neurosurgery, Appl. Physics Lab., The Johns Hopkins Univ., Baltimore, Md. 21205

Thermal injury to the skin induces hyperalgesia in man and increases the response of certain nociceptive primary afferents in monkey. Previous investigators have described an increased response (i.e., sensitization) in C-fiber polymodal nociceptive afferents (CPNs) that innervate the hairy skin of the monkey following a thermal injury. We demonstrate here that in the glabrous skin of the hand hyperalgesia resulting from a 53°C thermal injury correlates better with activity in A-fiber nociceptive afferents, sensitive to mechanical and heat stimuli (AMHs), than with activity in CPNs. The responses of AMHs and CPNs to noxious radiant heat before and after thermal injury to their receptive fields were compared with estimates of the magnitude of pain by human observers given the same stimuli. An infrared laser under radiometer feedback control provided a step increase in skin temperature over a 7.5 mm diameter spot with rise rates greater than 30°C/s. Using standard methods of single fiber recording in monkeys, we studied the changes in the response properties of 18 CPNs and 41 AMHs after injury to the skin caused by a 53°C stimulus of 3 to 30s duration. The changes in response were similar in all cases though more pronounced after the 30s, 53°C stimulus. In 7 CPNs, 8 AMHs, and 5 experiments with 3 human subjects, we measured the response to thermal stimuli before and at varying times after an injury induced by a 53°C, 30s stimulus using a random sequence of stimuli (3s duration, delivered every 30s) ranging from 41° to 49°C. Stimuli below 45°C failed to elicit pain prior to the thermal injury. However, 10 minutes after the injury, even the initial 38°C base temperature evoked pain. Also, all stimuli from 41 to 49°C were quite painful, and the 42°C stimulus was judged more painful than the 49°C stimulus prior to the injury. The 49°C stimulus after injury induced 5.5 times more pain than it did before injury. Before the injury, 63% of the AMHs failed to respond to 49°C and none responded to stimuli below 44°C. After injury, 63% responded to the initial 38°C base. The mean response to 49°C was 110 impulses compared to 12 impulses prior to injury. The mean threshold of CPNs was 44.1°C prior to the injury and 45.2°C after injury. The response to 49°C decreased from a mean of 15.3 (±2.7 S.E.M.) impulses before injury to 6.6 (±1.8) impulses after injury. These results suggest that activity in the AMHs and not the CPNs is responsible for the hyperalgesia that occurs after a 53°C, 30s thermal injury to the glabrous skin of the hand.

92.6 DETECTION THRESHOLD AS AN OBJECTIVE INDICATOR OF SENSITIVITY TO THERMAL PAIN IN NORMAL AND HYPERALGESIC SKIN IN HUMANS. C.J. Robinson, R.H. LaMotte, J.G. Thalhammer* and H.E. Torebjörk*. Dept. of Anesthesiology, Yale University Sch. of Med., New Haven, CT 06510.

We have found that the capacity of human observers to discriminate differences in painful heat stimuli is preserved at the expense of the capacity to make judgments of the absolute magnitude of a painful stimulus temperature. As a possible consequence of this, human observers appear to have a remarkably precise and accurate capacity to detect very small increments in temperature superimposed upon base temperatures that are continuously rated as painful.

Five human subjects, each of whom gave informed consent to an approved protocol, made continuous ratings of the magnitude of pain sensations evoked by brief temperature increments superimposed on a base temperature maintained either at 38°C or 47°C. Stimulus increments were also delivered on a 38°C base to skin made hypo- or hyperalgesic. Subjects additionally made forced choices as to which of two paired intervals of time contained each increment (regardless of whether or not it was painful). Detection threshold (DT) was the minimum increment in temperature detected on 75% of the trials in the forced choice task. Pain threshold (PT) was the minimum increment rated as painful, or as an increase in pain sensation on 50% of the trials.

For a 38°C base, perceived as warm but never painful in normal skin, DTs averaged 0.4° and PTs 5.0°C. In contrast, on a base of 47°, which was continuously rated as painful, DT and PT were each equal to 0.1°C or lower. Following a conditioning stimulus (CS) of 50°C and 20 sec duration the skin became hypoalgesic and thresholds elevated to 0.8° (DT) and 6-8° (PT). Following a longer 50° CS (100 sec.), the skin was hypoalgesic for up to 4 min., with DT and PT elevated to 1.2°C and 9°C, respectively; but by 10 min after this CS, the skin became hyperalgesic, and DT was lowered to 0.25° and PT to 0.5°C. A similar lowering of DTs and PTs were observed in skin made hyperalgesic by a topical application of 1% capsaicin (Sigma Chem. Corp.). Thus, in normal skin, the DT is lower for painful than for nonpainful base temperatures and may serve as an objective test of thermal pain sensitivity. Furthermore, following injury to the skin, a lowered DT is an objective indicator of the presence of hyperalgesia.

(Supported by NIH Grant NS14624)

92.7 THE EFFECTS OF MORPHINE ON EXPERIMENTAL PAIN RESPONSE IN CHRONIC PAIN PATIENTS. Patricia J. Wolskee* and Richard H. Gracely* (SPON: Catherine Bushnell) Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205 USA.

Noxious thermal stimuli were delivered to 5 patients suffering from chronic low back pain to assess the effects of morphine on experimental pain responsivity. Forty-two noxious thermal stimuli ranging from 46-51°C were delivered via a contact thermode to the volar surface of the forearm of 5 chronic pain patients before and after the administration of morphine or saline placebo. Subjects assessed sensory intensity and unpleasantness of the stimuli by cross modality matching to handgrip force and by choosing verbal descriptors from randomized lists. The dose of morphine (mean = 12.2 mg) was determined individually to produce clinical pain relief without significant respiratory or cardiovascular depression. In comparison to placebo, morphine reduced verbal descriptor responses of sensory intensity ($t = 4.10$, $p < .01$) and unpleasantness ($t = 2.47$, $p < .05$). In contrast, handgrip measures of sensory intensity and unpleasantness were not significantly reduced by morphine in comparison to placebo.

Spatial variability of cutaneous thermal sensitivity was assessed in a preliminary study by randomly presenting 6 stimuli ranging in intensities from 46-51°C to each of 8 sites on the volar surface of the forearm. Three-sec duration stimuli were presented at 30 sec intervals to successive sites; each site received a stimulus at 240 sec intervals. Five subjects rated the sensory intensity of the stimuli by choosing verbal descriptors quantified previously. Statistical analysis (ANOVA) showed that there were no significant differences in thermal sensitivity between sites.

These results support a previous study with normal subjects showing that in comparison to placebo, fentanyl, a narcotic analgesic, significantly reduced sensory intensity responses to painful electrical toothpulp stimulation. Unlike the finding in the previous study with pain-free subjects, morphine also reduced unpleasantness. This result supports the hypothesis that the effect of a drug on perceived unpleasantness is dependent, in part, on the side effects experienced. After narcotic administration, pain-free subjects usually experience dysphoria and nausea, while chronic pain patients, in contrast, experience euphoria, warmth, and relaxation. These results further support the utility of the verbal descriptor method for assessment of different dimensions of the pain experience. Our findings also suggest that the verbal descriptor scaling of contact heat may provide an experimental model for the evaluation of pharmacological and non-pharmacological pain control methods.

92.8 DOES NALOXONE ALTER EXPERIMENTAL PAIN PERCEPTION? Richard H. Gracely*, William R. Deeter*, Patricia J. Wolskee* and Ronald Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

The influence of the narcotic naloxone on the perception of pain sensations evoked by electrical tooth pulp stimulation was assessed by pain-free volunteers experienced with cross-modality matching techniques. Forty-six subjects matched handgrip force to the intensity or unpleasantness of sensations produced by 42 stimuli before and after the random, double-blind, intravenous administration of either 10 mg naloxone or placebo (naloxone vehicle). The stimuli were 1-sec trains of 1-msec, monophasic, cathodal pulses delivered monopolarly at 100 Hz. Stimulus intensity varied in 7 equal-log steps between pain threshold and pain tolerance.

Mean handgrip responses to sensory intensity or unpleasantness were not significantly altered after either naloxone or placebo. However, a non-probabilistic index of discriminability based on analysis of variance and independent of mean response ($r = .06$) showed that handgrip responses matched to the intensity of the sensations were significantly more variable after naloxone in comparison to placebo ($t = 4.08$, $df = 20$, $p < .001$). The variability of unpleasantness responses also was increased by naloxone in comparison to placebo, but this effect was not statistically significant. The unpleasantness effect was significantly less than that observed for sensory intensity responses ($t = 3.27$, $df = 22$, $p < .01$).

These results show that naloxone did not change mean pain perception but that it did increase response variability. The negative effect with mean response is consistent with previous evidence that naloxone does not alter mean pain response to electrical, ischemic, or cold-pressor stimulation. The significant difference found between naloxone's effect on the variability of sensory intensity and unpleasantness responses suggests that verbal instructions to attend to either sensory intensity or unpleasantness can influence the outcome of cross-modality matches to painful experimental stimuli. The significant increase in the variability of sensory intensity responses produced by naloxone suggests that naloxone degrades psychophysical performance. This hypothesis is supported by observations that subjects receiving 10 mg of naloxone become restless, agitated and irritable. Thus, studies that use response measures based on sensory discriminability may show a naloxone-produced change in response that reflects reduced psychophysical performance and not a change in the magnitude of pain perception.

92.9 SELECTIVE DEPLETION OF SPINAL CORD MONOAMINES FAILS TO ALTER STIMULATION PRODUCED ANALGESIA (SPA). J.N. Johannessen*, L.R. Watkins*, & D.J. Mayer, Dept. of Physiology, Medical College of Va., Richmond, Va., 23298.

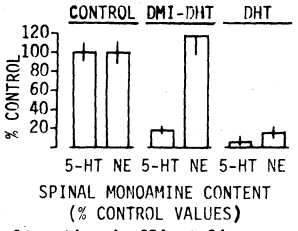
Striking parallels between SPA and morphine induced analgesia (MIA) have recently emerged. Both seem largely dependent on the integrity of central serotonergic (5-HT) systems and a descending inhibitory pathway within the spinal dorsolateral funiculus (DLF). Evidence suggests that monoamines may mediate this descending inhibitory control. Neurotoxins which destroy 5-HT terminals attenuate MIA. In addition, pCPA (a 5-HT synthesis inhibitor) reduces both SPA and MIA. To date, assessments of SPA or MIA after monoamine depletion have relied on systemic or ventricular drug injections - procedures which must necessarily affect supraspinal and spinal sites. Intrathecal (i.t.) administration of 5,7-dihydroxytryptamine (5,7-DHT) was therefore chosen to test the effect of spinal monoamine depletion on SPA.

Rats, implanted with stimulating electrodes in the dorsal raphe (DR) and with lumbosacral spinal catheters, were tested for SPA at one week intervals using the tailflick test. Results are given as % MPE (L. Grumbach, 1966). After two testing periods, rats received either i.t. saline (CONTROL), i.t. 5,7-DHT preceded by desipramine (DMI-DHT) or i.t. 5,7-DHT (DHT). In experiment 1, rats were tested 2 and 3 weeks post treatment. A second experiment tested rats at 24 hrs. to look for a transitory alteration in SPA. Spinal cords were assayed for 5-HT and norepinephrine (NE).

(Table 1)

	% MPE	CONTROL	DMI-DHT	DHT
EXP 1	PRE-	63±10	45±11	69±10
	POST-	64±11	65±11	44±12
EXP 2	PRE-	77±9	72±8	69±9
	POST-	76±9	52±17	69±15

(Figure 1)



Our results show no significant alteration in SPA at 24 hrs. or 2-3 weeks after treatment (table 1.). Chemical data (fig. 1) shows selective depletion of 5-HT or 5-HT and NE. These results suggest that the role of spinal monoamines in mediating SPA may be overemphasized. They are certainly not necessary for SPA. Supported by PHS Grant DA-00576.

92.10 ANALGESIC TOLERANCE TO CLONIDINE AND CROSS-TOLERANCE TO AUTOANALGESIA. William T. Chance. Department of Surgery, University of Cincinnati Medical Center, Cincinnati, Ohio 45267.

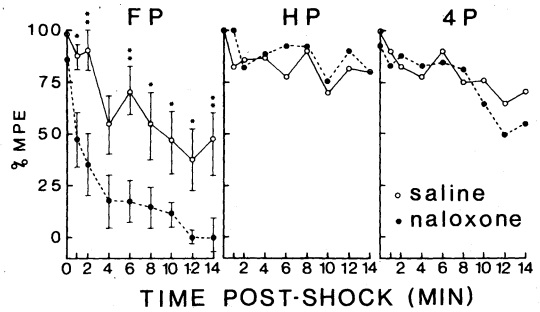
The antihypertensive drug, clonidine, has been reported to elicit analgesia across a wide variety of tests. Although this analgesic response appears to result from activation of descending inhibitory systems, it is not opioid in nature. In the present experiments, tolerance to clonidine-induced analgesia and cross-tolerance to autoanalgesia were investigated in adult, male, Sprague-Dawley rats. Analgesia was assessed using a radiant-heat tail-flick procedure with response latencies of approximately 3 sec in control rats and an 8 sec latency cut-off criterion. Initially, tail-flick latencies were determined prior to and 15, 30, 60, 90, 120 and 180 min following the injection (ip.) of 0.5 mg/kg (n = 8), 1.0 mg/kg (n = 8) clonidine HCl (free base, from Boehringer Ingelheim) or normal saline (n = 7). Each dose of clonidine elicited significant analgesia 15 min after injection, with peak analgesia occurring at 30 min (0.5 mg/kg = 6.59 sec, 1.0 mg/kg = 6.78 sec). Across the next 7 days, these treatments continued to be administered with tail-flick latencies being assessed prior to and 30 min after the injections. By the 8th drug administration, tail-flick latencies had significantly declined in both drug groups (0.5 mg/kg = 4.57, 1.0 mg/kg = 4.12 sec). To assess tolerance, on the following day, 2 mg/kg clonidine was administered to all rats. The reduced analgesic response revealed that tolerance had developed in both clonidine-treated groups (saline gp. = 7.04, 0.5 mg/kg = 5.00, 1.0 mg/kg = 4.17 sec). On the next day, the acute analgesic response to footshock (1.0 ma/15 sec) was assessed in each of these groups as well as in another experimentally-naive group (n = 6). An additional group (n = 6) served as non-shocked controls. Although significant analgesia was observed in the saline, 0.5 mg/kg and no-drug groups, the 1.0 mg/kg group exhibited a much attentuated response. Across the next 4 days, autoanalgesia was assessed in these groups by obtaining tail-flick latencies prior to the administration of footshock. Clonidine tolerance was maintained throughout this phase of the experiment. Within this paradigm, both clonidine-tolerant groups exhibited attenuated analgesia. To allow dissipation of clonidine tolerance, no further treatments were administered. Eight days after the last tail-flick-shock pairing, the rats were again placed on the grid and tail-flick latencies were determined with no shocks being administered. Although the clonidine-tolerant groups had previously exhibited very attenuated analgesia in this situation, the tail-flick latencies of all groups were over 7 sec. Thus, when tolerance to clonidine had been allowed to dissipate, the autoanalgesic response to conditioned fear was observed. These data suggest that clonidine and autoanalgesia may elicit analgesia through similar descending inhibitory mechanisms.

92.11 DISSOCIATION OF OPIATE AND NON-OPIATE FOOT-SHOCK PRODUCED ANALGESIA (FSA). D.A. Cobelli*, L.R. Watkins* & D.J. Mayer (SPON: R.L. Hayes) Dept. Physiol., Med. Coll. Va., Richmond, Va. 23298.

Aversive footshock has been shown to produce analgesia (Hayes et al., 1978). In this experiment we examined the effects of footshock applied to the front paws (FP), hind paws (HP) or four paws (4P) of rats. The effect of naloxone was examined to determine the involvement of endogenous opiates. The measure of analgesia was the tail flick (TF) test.

We studied 3 groups (20/group): FP, HP & 4P shock. Within each group, ½ received 2 i.p. injections of 10 mg/kg naloxone and ½ received equivolume saline. The first injection was given just prior to baseline (BL) TF testing & the second 5 min later, immediately post BL. Rats were then shocked for 90 sec (1.5 mA, rms).

Immediately post shock, TF latencies of virtually all rats increased to 100% MPE. TF latencies remained significantly elevated throughout the 14 min test for all groups except FP naloxone. At 1 min post-shock the FP naloxone rats were reliably less analgesic than FP saline rats ($p<0.02$). By 4 min, TF latencies of FP naloxone rats were not reliably above preshock values ($p>0.07$).



At all times tested, FP FSA was significantly attenuated by naloxone, indicating the involvement of an endogenous opiate-like mechanism. These observations suggest that noxious stimuli entering the cord at levels contiguous with that of the test area (ie. tail) result in potent non-opiate FSA. In contrast, input to cord levels distant from the test area elicit opiate FSA. We conclude that both opiate and non-opiate systems are involved in FSA. We propose that opiate FSA is more generalized both in production & effect. In contrast, non-opiate FSA may be highly specific in that zones of analgesia are confined to the shocked region. These results may have important implications for TENS & acupuncture theory & practice. Supported by PHS Grant DA-00576.

92.12 BEHAVIORAL RESPONSES TO DENTAL PULP STIMULATION IN PRIMATE-EFFECT OF TRIGEMINAL TRACTOTOMY. Ronald F. Young and Terrance D. Oleson, Division of Neurosurgery and Department of Anesthesiology, UCLA School of Medicine, Harbor/UCLA Medical Center, Torrance, CA 90509.

Macaque monkies were trained to press a bar to terminate a brief period of electrical stimulation of either the dental pulp, delivered via electrodes chronically implanted into the maxillary canine teeth, or facial skin delivered via intradermal electrodes. Unilateral trigeminal tractotomy was subsequently carried out and escape thresholds for dental and cutaneous stimulation were redetermined. Responses to facial pin-scratch were also observed following tractotomy. In two animals rhizotomy of the C 2-4 posterior cervical nerve roots was also accomplished, six weeks after tractotomy. Escape thresholds and responses to facial pin-scratch were again determined. Completeness of all lesions was histologically verified.

No animal was rendered analgesic to dental pulp stimulation after tractotomy. Slight, (mean 22%) but statistically insignificant elevations in dental escape thresholds were observed ipsilateral to tractotomy. On the other hand, marked (mean 124%) and statistically significant elevations were observed in escape thresholds for cutaneous stimulation ipsilateral to tractotomy. Responses to pin-scratch were absent in the peripheral portion of the face ipsilateral to tractotomy, however near the midline of the face and including the oral and buccal mucosa and tongue, aversive responses were either normal or slightly reduced when compared to the contralateral side. The addition of cervical rhizotomy had no effect on dental escape thresholds but further increases (mean 50%) were seen in escape thresholds for cutaneous electrical stimulation. In addition the zone of reduced or absent aversive responses to pin-scratch spread to include the side of the neck and shoulder.

The results suggest that trigeminal nucleus caudalis is not the exclusive brainstem relay for pain related information transfer from orofacial structures. Nuclear zones rostral to the obex, acting free of the influence of nucleus caudalis may process pain related information, particularly from the oral cavity and teeth. Results of cervical rhizotomy suggest that facial skin (exclusive of the paramedian area) is innervated by both the trigeminal and cervical nerve roots. Integrated processing of afferent input from these two pathways apparently occurs in trigeminal nucleus caudalis.

93.1 PRENATAL DEVELOPMENT OF CORTICOSTRIATAL PROJECTIONS IN THE RHESUS MONKEY. P. S. Goldman-Rakic, Sec. of Neuroanatomy, Yale Univ. School of Med., New Haven, Ct. 06510

In the adult rhesus monkey, prefrontal corticostriatal fibers are not uniformly distributed but form segregated clusters that surround round or elliptically shaped territories that are free of prefrontal afferents (Goldman & Nauta, J. Comp. Neurol. 1977 171: 369). To examine the time and mode of development of this pathway, a mixture of ^{3}H-leucine and ^{3}H-proline was injected into the prospective dorsolateral prefrontal cortex of eight embryonic (E) rhesus monkeys that had been temporarily removed from the uterus and then subsequently replaced in utero for 24-48 hours. Following delivery by Cesarean section, the fetuses were perfused intracardially and their brains prepared for autoradiography.

In the youngest fetus of this series, injected at E69, a light even spray of radioactivity was present over the appropriate area of the ipsilateral neostriatum, indicating that a small number of corticostriatal afferents have reached their subcortical target by this age. In a fetus injected at E85 and in two slightly older fetuses injected at E95 and E105, respectively, labeling over the ipsilateral neostriatum increases in density but continues to be uniformly distributed. The first indication of segregation of corticostriatal afferents appears in a fetus injected at E123. In this specimen, areas of higher and lower grain density begin to emerge and, indeed, ten days later at E133, ingrowing corticostriatal fibers assume the adult pattern of cortical fibers surrounding core territories devoid of cortical input. The configuration of the corticostriatal afferents at this stage are not very different from that observed in older fetuses (E151; E153) or postnatal monkeys (e.g. P1; P4; P60).

Thus, corticostriatal projections in the rhesus monkey are established within the first half of the 165 day gestation period in this species, expand throughout the middle of gestation and achieve the organizational features of the mature pathway a full month before birth. Throughout this period of fetal development, the prefrontal neostriatal pathway is well delineated ipsilaterally and there is no comparable contralateral component. The cortical innervation of the neostriatum displays a biphasic mode of developement proceeding from an overlapping, diffuse phase to a segregated and patterned phase similar to the transformation previously reported for the thalamocortical pathways of the primary visual system in the same species (Rakic, Nature, 1976 261; 467). This mode of development may therefore reflect a general rule governing the formation of long tract connections - one that applies to descending efferent fiber systems of association cortex as well as to ascending afferents of sensory systems. Supported in part by NS 16666.

93.2 THE ASSEMBLY OF THE RADIALLY STRIATED PATTERN OF THE NEOCORTEX: A GOLGI-EM ANALYSIS IN NORMAL MOUSE EMBRYOS. M.C. Pinto Lord*, P. Evrard*, V.S. Caviness, Jr. Department of Neurology, Massachusetts General Hospital, Boston, MA 02114 and Southard Laboratory, Eunice Kennedy Shriver Center for Mental Retardation, Inc., Waltham, MA 02154.

The cortical plate of the developing mammalian neocortex has a radially striated appearance in general cell stains. This reflects the assembly of neuronal somata into radially aligned groups, separated by intervals free of somata. The means of assembly of the somata and dendritic components of postmigratory cells into these general patterns has been examined in the cortical plate of normal E17 mouse embryos. The configurations of cells has been reconstructed from skip serial thin sections of tissue wherein radial glialfibers (RGF) are identified by the Golgi-gold toning method of Fairen et al ('77). The somata of neurons appear to be in contact with a single RGF. A radial assembly corresponds to somata associated with 3-4 adjacent RGF. The apical dendrites of cells are directed externally with respect to the somatic assembly. The dendrite of a cell contacts the cell soma immediately above its soma of origin but is progressively removed tangentially from successively more superficial somata. The dendritic ensheathments of adjacent somatic assemblies abut upon each other so that there is a continuous dendritic field throughout the cortical volume. The radial somatic assemblies are embedded as mosaic elements.separated from each other within the continuous dendritic field.

Supported in part by NIH grant 1-R01-NS12005-02

93.3 INTRACORTICAL MIGRATION OF NEURONS ALONG RADIAL GLIAL FIBERS. A GOLGI-EM ANALYSIS IN NORMAL MOUSE EMBRYOS. P. Evrard*, M.C. Pinto Lord*, V.S.Caviness, Jr. Department of Neurology, Massachusetts General Hospital, Boston, MA 02114 and Southard Laboratory, Eunice Kennedy Shriver Center for Mental Retardation, Inc., Waltham, MA 02154.

Neurons which form the mammalian neocortex migrate from the ventricular zone to the cortical plate along radial glial fibers (RGF) (Rakic, '72). They complete their migrations with their perikarya at the interface of the molecular layer and cortical plate. The ascent of migrating neurons through the cortical plate of N1 E17 mouse embryos has been examined in electronmicrographs of tissue in which RGF are impregnated by the Golgi-gold toning method of Fairen et al ('77). The configuration of migrating cells is reconstructed from skip serial thin sections. The migrating neuron is readily identified because its nuclear and cytoplasmic size and degree of differentiation are much less than those of adjacent postmigratory elements. The tapered leading process does not extend fully to the molecular layers. The intracortical migrating cell is invariably closely applied to a RGF throughout its length. It is coiled about the RGF with increasing tightness of coil at outer levels of the cortical plate. It is invariably interposed between RGF and postmigratory elements that it encounters in its ascent. These observations suggest that the surface of RGF is an obligatory corridor of ascent for migrating cells through the cortical plate and that their binding affinity for this surface is greater than that of postmigratory cells.

Supported in part by NIH grant 1-R01-NS12005-02

93.4 IMMUNOCYTOCHEMICAL STUDY OF RADIAL GLIAL CELLS AND ASTROCYTES IN THE DEVELOPING RHESUS MONKEY BRAIN. P. Levitt and P. Rakic. Sec. of Neuroanatomy, Yale Univ. School of Med., New Haven, Ct. 06510.

Gliogenesis in the central nervous system (CNS) of the rhesus monkey was examined at various embryonic (E) and postnatal (P) ages utilizing a specific antibody to the glial fibrillary acidic protein (GFA) and peroxidase-antiperoxidase immunohistochemical staining. Positive staining was noted by the presence of dark brown horseradish peroxidase (HRP) reaction product in the cytoplasm and processes of radial glial cells, Bergmann glial cells and astrocytes. The HRP product was conspicuously absent from all neuronal elements examined at the light microscopic level.

Radial glial cells are the first glial cells to appear, being present within the first quarter of the 165 day gestational period in the spinal cord and brainstem (E41), and the first third of gestation in the diencephalon (E45) and telencephalon (E47). In each of these areas, radial glial fibers fan out from the ventricular and subventricular zones, where their cell bodies reside, and course approximately 3-8 um apart through the developing mantle zone or cortical plate to terminate at the pial surface with conical endfeet. The Bergmann glial cell is the next glial cell type to appear. These cells, which are positively stained for GFA by E54, are located below the Purkinje cell layer. Evenly spaced parallel Bergmann fibers (3-6 um apart) run through the molecular layer to end along the pial surface. At specific times within each major subdivision of the CNS, there is an increase in the number of positively stained radial glial fibers. As tectogenetic changes occur, the massive number of elongated glial fibers alter their scaffolding patterns, but maintain constant ventricular-pial relationships. In all areas of the CNS, columns of unlabeled migrating neurons are seen juxtaposed to GFA-positive radial and Bergmann glial fibers in Nissl counterstained material. Radial glia undergo a variety of changes to assume transitional cell forms during the process of transformation into fibrillary and protoplasmic astrocytes. This transformation occurs at specific embryonic ages within each structure, usually commencing after neuronal migration has begun to subside.

The immunocytochemical localization of glial cells during embryonic development in the primate brain indicates that 1) a transient glial cell class, the radial glial cell, is present concominently with neurons during periods of neurogenesis and 2) two distinct cell precursor populations may compose the ventricular and subventricular proliferative zones during development. The close structural relationship between large numbers of migrating neurons and immunohistochemically identified radial glial fibers support the concept of a role for radial glial cells in migration guidance and compartmentalization of the brain during development. (Supported by NS 14841 and EY 02503).

93.5 MIGRATION OF EXTERNAL GERMINAL CELLS IN THE CEREBELLAR CORTEX OF NEONATAL RATS. I. S. Zagon and W. E. Rogers*. Dept. of Anatomy, The M.S. Hershey Medical Center, Hershey, PA 17033 and Dept. of Biology, Shippensburg State College, Shippensburg, PA 17257.

A close interrelationship between migrating external germinal cells and the Bergmann fibers of Golgi epithelial cells has been postulated by Rakic (1971) and others, with vertically oriented fibers thought to be imposing radial constraints and providing guidelines to facilitate the migration of young neurons. Evidence for this concept has been based largely on examination of animals at later stages of cerebellar development when a myriad of cells and processes are encountered in the molecular layer. The present study was undertaken in order to evaluate whether "glial guidance" is necessary at early developmental stages when the migration route is less densely packed with cell processes. Newborn Sprague-Dawley rats were fixed by intracardiac perfusion with 1% paraformaldehyde and 2% glutaraldehyde in 0.07 M phosphate buffer with 2% sucrose. Cerebellar were post-fixed in a 1% OsO_4 solution and embedded in Epon. A series of thin (700Å) sections were prepared in the sagittal (perpendicular to the long axis of the folia) and transverse (parallel to the long axis of the folia) planes and observed by electron microscopy; serial sections of at least 4 migrating cells/plane were examined. Migrating cells were often elliptical in shape, with both the perikaryon and the migrating cell process elongated in a plane parallel to the folium (i.e., transverse plane). Adjacent to migrating cells was a loosely fashioned network of processes from other germinal cells, as well as numerous areas of extracellular space; in some cases, these areas of extracellular space were quite extensive. Bergmann glial fibers were not observed in association with migrating cells, however, Bergmann glial fibers were occassionally noted in other areas of the cortex. These results suggest that, at least in the early stages of cerebellar development, germinal cells can navigate through the molecular layer without being dependent on glial fibers for guidance.

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93.6 SENSORY INFLUENCE ON THE POSTNATAL RESHAPING OF THE CORPUS CALLOSUM - TEMPORAL FACTORS. G. M. Innocenti and D. O. Frost. Inst. of Anat., University of Lausanne, Switzerland.

Callosally projecting neurons, retrogradely labeled by horseradish peroxidase (HRP) were counted in areas 17 and 18 of 9 kittens deprived of vision by binocular eyelid suture performed on postnatal days 7-8 (prior to natural eye opening). Of these kittens, 7 were sacrificed on postnatal days 42-143 while 2 had their eyes reopened at the beginning of the second postnatal month and were allowed, respectively, 109 and 114 days of normal vision. In addition, a normal adult cat received 91 days of visual deprivation and two kittens were allowed normal vision for their first 29 and 32 postnatal days, prior to sacrifice.

In all but one of these animals 10 to 12, 0.5 µl injections of a 40 % HRP solution were injected unilaterally into areas 17 and 18 two days before perfusion with 3 % paraformaldehyde; in one animal deprived continuously from birth the solution was directly applied to cut callosal axons. The HRP was visualized with multiple substrates (diaminobenzidine, with or without cobalt intensification; tetramethylbenzidine) for each brain, according to standardized procedures. Charts of single coronal sections and reconstructions from serial sections, showing the distribution of labeled neurons, were made using automated procedures.

Binocular deprivation during the first two postnatal months or longer causes more than a 50 % decrease in the number of labeled neurons normally found in areas 17 and 18; it also narrows their mediolateral tangential distribution.

Deprivation during adulthood or restricted to the first postnatal month does not appear to have such an effect. Actually, the two kittens whose eyes were reopened after one month's deprivation showed a widening in the tangential distribution of callosally projecting neurons in area 17 similar to that previously observed in strabismic kittens (Innocenti and Frost, Nature, 280, 1979, 231-234). The widening is probably due to the persistence of juvenile callosally projecting neurons which at birth are distributed over the entire extent of areas 17 and 18 and which at the end of the first postnatal month, in both normally reared and binocularly deprived kittens, still occupy wider portions of both areas than in adults.

These results suggest the existence of a period during which juvenile callosal connections are sensitive to the stabilizing effects of visual experience. This sensitive period probably extends at least until the end of the second postnatal month.

93.7 EFFECTS OF AXOTOMY ON DEVELOPMENT OF PYRAMIDAL TRACT NEURONS. L. Ramirez*, K. Kalil and T. Reh (SPON: M. Javid). Dept. of Surgery, Dept. of Anatomy, and Neurosciences Training Program, Univ. of Wis., Madison, WI 53706.

Previous experiments have shown that when the pyramidal tract is severed in the adult hamster there is severe cell shrinkage but not cell death in the neurons of origin in layer 5 of the sensorimotor cortex (Kalil and Schneider, 1975, Brain Res. 89). In the present experiments, unilateral lesions of the medullary pyramids were carried out on infant hamsters to determine the effect of axotomy on developing cortical neurons.

Animals ranging from 2 days of age to adulthood received a unilateral lesion of the medullary pyramid and were allowed to survive for periods ranging from 4 days to several months. The fixed brains were embedded in celloidin and 20µ coronal sections were Nissl stained. Histograms of cell sizes were based on drawings made under 100X oil immersion and traced with an electronic planimeter of 100 cells in layer 5 of the sensorimotor cortex of each operated animal. The corresponding region of the contralateral cortex served as the normal control in all cases.

The results showed that, as previously reported in the adult lesioned animals, there is no significant cell loss after infant lesions. Rather, infant lesioned animals show a severe cell shrinkage such that the size of axotomized cortical neurons is reduced to 65% of normal controls. Moreover, an examination of pyramidal tract neurons one week after the lesion indicates that the neurons react quite rapidly to axotomy by a temporary shrinkage or, alternatively, by simply lagging behind in development.

These results suggest that the cortical neurons do not have an independent program of development, at least with respect to the size of the cell body, but one that can be severely disrupted by damage to their efferent fibers.

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93.8 REGENERATIVE CHANGES IN COUPLING OF AMBYSTOMA BLASTOMERES MEDIATED BY VOLTAGE DEPENDENT JUNCTIONS, M.V.L. Bennett, A.L. Harris and D.C. Spray, Div. Cellular Neurobiology, Dept. Neuroscience. A. Einstein Col. Med., Bronx, New York 10461.

The junctional conductance between coupled Ambystoma blastomeres is strongly voltage dependent as characterized under voltage clamp (Spray et al., Science 204: 432,'79). This analysis was used to calculate steady state and transient potentials during current injection into one cell of a coupled pair. The predicted coupling shows hysteresis for slowly increasing and decreasing ramp currents. As applied current increases a voltage is reached where a further increase in voltage decreases junctional conductance and thereby increases voltage in the injected cell and decreases voltage in the coupled cell. The transjunctional voltage is increased and junctional conductance is further decreased. Thus, the transition is regenerative and a state of reduced coupling and higher input resistance is reached. As current is decreased again, the cells remain "uncoupled" to a lower current level because the transjunctional voltage is greater for a given applied current when the junctional conductance is low. Eventually a lower transition voltage is reached where a further decrease in voltage decreases junctional conductance and thereby decreases the voltage in the injected cell and increases voltage in the coupled cell. The transjunctional voltage is decreased and the junctional conductance is further increased. Again, the transition is regenerative. The calculations also predict that over a range of constant currents applied in one cell superimposed pulses of appropriate sign will cause transitions between stable states of more and less close coupling with lower and higher input resistances respectively. These predictions are readily verified experimentally. Furthermore, calculations predict that if the resting potentials of the cells are different, currents of one polarity will uncouple the cells at a lower level than currents of the opposite polarity; the junctions rectify under these conditions. Large differences in resting potentials (including those produced by a non-specific leak in one cell) can allow pulses to produce transitions between coupled and uncoupled states without maintained current application. Still larger differences can cause transition to the uncoupled condition without application of pulses. Bistability due to differences in resting potentials is readily demonstrated experimentally and can last for many minutes. This mechanism may cause rapid and sustained changes in intercellular communication during development, although longer term changes must involve growth and dissolution of junctions. In any case an unexpected plasticity of coupling can arise from voltage dependent junctions between amphibian blastomeres.

93.9 QUANTITATIVE ANALYSIS OF ELECTRICALLY COUPLED CELLS. N.G. Publicover* (SPON: D.G. Lawrence). Microsurgical Laboratories, McGill University, Montreal, P.Q. Canada, H3A 1A1

The transient response of an electrically coupled network of cells can be approximated with passive electrical components. A capacitance in parallel with a resistance may be used to represent the cell body. Communication with other equivalent cells via electrical synapses can be treated as purely resistive. The responses of this system can be described mathematically in terms of a pair of simultaneous differential equations and can be solved numerically by computer simulation.

The validity of the model has been assessed using standard intracellular recording techniques in identified pairs of electrically coupled neurons (L4 and R4 of the buccal ganglion) in the freshwater snail Helisoma trivolvis. A microprocessor-based computing system has been developed to 1) inject hyperpolarizing steps of current, 2) monitor the cells' voltage responses, and 3) perform all of the subsequent analysis using non-linear curve-fitting algorithms.

Two exponential decay modes are found in both the injected neuron and in the coupled cell responses. As seen in the model, the amplitude of one mode of the coupled response may be inverted, depending on the relative magnitudes of the membrane and coupling resistances. The presence of these two decay modes in the coupled response changes our interpretation of coupled cell behavior. For example, the prolonged latency of the appearance in a coupled neuron of an injected pulse of current is explicitly a function of the coupling circuit and not due to an excessive propagation along an axon. This accounts for the observation that action potential latency from one coupled cell to the next is related to the degree of coupling.

The two time constants of the injected cell response and the magnitude of the response in the injected neuron and one coupled cell provide enough information to calculate the values of each of the components in the equivalent circuit. This can be used as a mathematical foundation to describe neuronal responses mediated by electrical synapses.

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93.10 POSSIBLE ELECTROTONIC EPSPs IN DYE-COUPLED NEOCORTICAL NEURONS. M.J. Gutnick* and D.A. Prince. Dept. of Neurology, Stanford University Medical School, Stanford, CA 94305.

Recent demonstrations of electrotonic coupling in a variety of subcortical mammalian CNS structures raise the possibility that this form of intercellular communication may be a general feature of the brain's neuronal connectivity. In the present experiments we obtained anatomic and physiologic evidence for electrotonic coupling in mammalian neocortex. In several systems, electrotonic coupling is associated with "dye-coupling", the cell to cell diffusion of an intracellularly injected, low molecular weight dye, presumably through gap junctions. Intracellular iontophoretic injections of the fluorescent dye Lucifer Yellow CH were made in 17 neurons in slices from guinea pig sensory-motor cortex maintained in vitro. Each of 6 injections at a depth greater than 1.2 mm below the pial surface caused staining of only one neuron. However, 10 of 11 superficial (< 0.4 mm beneath the pial surface) injections resulted in a staining of aggregates of 2-7 neurons. In 5 slices, injection of a single Lamina III cell caused staining of 3-6 pyramidal type neurons organized in columnar-shaped clusters oriented perpendicular to the pial surface. These dye-coupled neurons had extensive overlap of their dendritic arborizations. Dye-coupling with complete filling of the dendritic trees of all stained cells was observed as soon as 5 min after the end of the 5-8 min injection period. It was not dependent on evoked chemical synaptic activity, since it was present in slices bathed in a solution containing 3 mM Mn^{++}. Injection of superficial neurons never caused staining of deeper cells.

In parallel experiments, intracellular recordings were obtained from 14 superficial neurons which had membrane potentials of 65-76 mV and input resistances of 28-42 MΩ. In bathing medium containing 3 mM Mn^{++} and 1 mM Ca^{++}, all evidence of chemical synaptic transmission disappeared, and low intensity (< 100 μA) stimuli applied deep in cortex, near the subcortical white matter, evoked only antidromic activity. In 4 of these cells, subthreshold stimulation evoked small (< 4 mV) all-or-none depolarizing potentials at a fixed latency of 1-2 msec. These rose to peak within 1 msec and decayed over 10-20 msec. Their amplitudes and latencies were not affected by intracellularly applied depolarizing or hyperpolarizing currents. They were clearly distinguished from M or IS spikes by their different waveform and by failure to collide with intracellularly evoked spikes. These Mn^{++}-resistant potentials may reflect passive propagation of antidromic spikes that actively invade other, electrotonically coupled neurons. Thus, both the morphological and the electrophysiological evidence is consistent with the hypothesis that superficial neocortical neurons are electrotonically coupled.

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93.11 CRYOPRESERVATION AND TRANSPLANTATION OF EMBRYONIC RAT NEOCORTEX: PARAMETERS INFLUENCING ITS SURVIVAL AND GROWTH. J. D. Houle* and G. D. Das* (SPON:J.A. Altman). Dept. Biol. Sci., Purdue University, W. Lafayette, IN. 47907.

It has been demonstrated that embryonic neural tissues can be successfully transplanted into the brains of neonatal and adult animals. Tissues for these studies have always been fresh and transplanted with minimal delay following dissection of the donor embryos. A recent study in our laboratory showed that neocortical tissue from 16-day-old rat embryos could be frozen and stored at -95°C, then thawed and used for transplantation. This prompted a more detailed study of various parameters related to the freezing of neural tissue, media and cryopreservative used, storage of tissues, and rates of thawing prior to transplantation.

Neocortical tissue was dissected from 17-day-old rat embryos. Individual hemispheres were placed in micro test tubes containing either Eagles' MEM, lactated Ringer's solution, or amniotic fluid obtained at the time of embryo dissection, and with DMSO added to a final concentration of 10% or 2%. Tissues were frozen and stored at -90°C for 21-35 days before thawing at either room temperature (slow) or by immersion in a 37°C water bath (fast) and washing with Ringer's solution. One entire hemisphere was transplanted into the cerebellum of a 10-day-old rat. Animals were sacrificed 30 days post transplantation and their brains processed for Golgi-Cox impregnation, cresyl violet and Bodian staining.

Qualitative and quantitative analyses were performed to define the conditions of cryopreservation which provided for optimal survival and growth of the transplanted tissue. In general it can be stated that the concentration of DMSO was most crucial to the survival of the neural tissue. Use of 10% DMSO in combination with any of the 3 media and employing either fast or slow thawing gave the most desireable results, i.e., all the transplants appeared as normal, healthy cortical tissue, occupying a volume of 1/3 to 1/2 of the host cerebellum. The transplants contained fully differentiated pyramidal and stellate neurons and normal neuropil. Anatomical integration of the transplants and host cerebellum was demonstrated by sharing of neuropil and presence of fibers crossing at the interface. In no combination was a concentration of 2% DMSO found to adequately protect the tissue during freezing and thawing as demonstrated by small transplants, if present at all. The use of amniotic fluid resulted in fragmented tissue after thawing which adversely affected the transplantability though apparently not the survival of the tissue.

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93.12 ORNITHINE DECARBOXYLASE ACTIVITY IN THE DEVELOPING RAT CEREBELLUM: BIOCHEMICAL AND CYTOCHEMICAL STUDIES. G.M. Gilad and V.H. Gilad* Dept. Isotope Research, Weizmann Institute of Science, Rehovot, Israel.

We have recently characterized the developmental pattern of ornithine decarboxylase (ODC) activity, the enzyme catalyzing the first step in polyamine (PA) biosynthesis, in the rat cerebellum (Gilad & Kopin, J. Neurochem., 33:1195-1204, 1979). A transient increase with a sharp peak at 7d after birth occurs in ODC activity over the first two weeks after birth. Thereafter, the activity declines to very low levels which persist throughout adult life. This transient elevation in ODC activity coincides with the period of major cell proliferation, migration and beginning of differentiation in the cerebellar cortex. In the present study, therefore, we sought to determine if ODC activity is associated with a specific cell population. In order to do this, we had developed a new technique for cytochemical localization of ODC, using labelled α-difluoromethylornithine, an enzyme activated irreversible inhibitor (i.e. suicidal inhibitor) as a marker. Both rhodamine for fluorescence cytochemistry and biotin labelled inhibitor for biotin-avidin-horseradish peroxidase light microscopical studies were used. Using this technique, we have localized ODC, both in proliferating cells of the external granular layer of the developing cerebellar cortex at 6-10d after birth, and in the developing internal granular layer. No staining was observed in the molecular layer or deeper structures of the developing cerebellum. Adult cerebellum was also devoid of staining. We conclude that ODC activity and therefore, most probably, PA themselves, are associated both with neuronal proliferation and early stages of differentiation in the cerebellar cortex. Further studies are in progress to determine the subcellular localization of ODC.

93.13 AXOLOTL PROSPECTIVE FOREBRAIN-MIDBRAIN CAN BE REPROGRAMMED TO DIFFERENTIATE AS MAUTHNER CELL-CONTAINING MEDULLA. Pat G. Model. Depts. Neuroscience and Anatomy, Albert Einstein Coll. Med., Bronx, N.Y. 10461.

In premetamorphic amphibians, the Mauthner cells (M-cells), a single pair of large neurons, are present in the medulla at ear level. M-cells develop early and are easily recognized morphologically.

That the major axes of the CNS in the axolotl (Ambystoma mexicanum) are labile through mid-neural plate stages (Model, 1978, Brain Res. 153:135-143) suggests that regionalization of the CNS does not occur prior to that time. Prospective forebrain-midbrain from early midneurulae was unilaterally substituted for prospective hindbrain in hosts of the same stages. LM examination of feeding larvae showed that the implanted tissue differentiated as hindbrain and, in addition, produced an M-cell. EM examination of the M-cell surface revealed normal localization of vestibular input. Proof that the graft itself actually differentiated as medulla was obtained through implantation of H^3-thymidine labelled tissue into unlabelled host embryos and through implantation of pigmented tissue into albinos. That prospective forebrain-midbrain taken from early midneurulae is competent to produce medullae as well as M-cells indicates that regionalization of the developing CNS has not occurred by this stage and that regulating factors from the surrounding tissues can override the original fate of the graft to bring it into accord with the system as a whole.

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94.1 STRUCTURAL ASPECTS OF THE 66,000 DALTON SUBUNIT OF THE TORPEDO MARMORATA ELECTROPLAQUE ACETYLCHOLINE RECEPTOR ANALYZED BY LIMITED PROTEOLYTIC DIGESTION. R.E. Oswald, L.P. Wennogle*, T. Saitoh* and J.P. Changeux*. Neurobiologie Moléculaire, Institut Pasteur, 75015 Paris, France.

Preparations of purified Torpedo acetylcholine receptor (AChR) contain peptides of molecular weights 40,000 (40K), 50K, 60K, and 66K. The 40K peptide can be affinity labeled by acetylcholine analogs and thus carries the acetylcholine binding site (rev. by Heidmann & Changeux, Ann. Rev. Biochem. 47, 317, 1978). Recently (Oswald, et al., FEBS Lett. 111, 29, 1980), the 66K peptide has been labeled with a photoaffinity local anesthetic analog, 5-azido ^{3}H-trimethisoquin (5AT). The labeling has all of the characteristics of noncovalent local anesthetic binding, indicating that the 66K chain may carry the local anesthetic binding site. The 66K chain also carries binding sites for lectins (Wennogle & Changeux, Eur. J. Biochem. 1980, in press), a specific site of phosphorylation by an endogenous kinase (Saitoh & Changeux, Eur. J. Biochem. 105, 51, 1980), and a disulfide which crosslinks two 66K chains (Hamilton, et al., Biochem. 18, 155, 1979). We have used trypsin digestion of the 66K chain to determine the relative positions of the specific sites mentioned above.

When 5AT is used as a specific marker for the 66K chain, SDS gel electrophoresis (in the presence of β-mercaptoethanol) of a series of samples containing increasing quantities of trypsin (15 min at 25°C) indicates that the 66K chain is degraded first to a 50K fragment, then to a 48K chain, and finally to a 47K chain. With concentrations of trypsin up to 3 mg/ml, no further degradation is observed and the 5AT and lectin binding sites are conserved. When β-mercaptoethanol is omitted from the electrophoresis sample buffer, a dimer of the 66K chain is observed. The dimer is cleaved by trypsin to 50K chains going through an 82K intermediate which corresponds to a 66K chain crosslinked to a 16K fragment. The loss of this fragment does not correlate to a conversion of the AChR complex from a 13S to a 9S species. When the 66K chain is labeled with ^{32}P by an endogenous kinase and subjected to trypsinization, the phosphate label is lost during the degradation of the 50K fragment to the 48K fragment. Finally, N-terminal analysis of the 66K chain and the 47K fragment demonstrated that both have valine as the N-terminal amino acid, suggesting that the tryptic cleavages are made closer to the C-terminal.

We conclude that the cysteine involved in the disulfide bridge resides between the C-terminal and the 50K cleavage, that the phosphorylation site is between the 50K and 48K cleavages, and that the 5AT and lectin binding sites are between the 47K cleavage and the N-terminal.

94.2 DOPAMINE RECEPTORS: TEMPERATURE EFFECTS ON ^{3}H-SPIPERONE AND ^{3}H-N-n-PROPYLNORAPOMORPHINE BINDING. D.L. Niehoff, J.M. Palacios, and M.J. Kuhar. Depts. of Pharmacology and Psychiatry, Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

^{3}H-spiperone (^{3}H-SP) has been widely used to study dopamine receptors in in vitro binding assays. Recently, ^{3}H-N-n-propylnorapomorphine (^{3}H-NPA), a novel dopamine agonist, has also been used by several investigators to characterize dopamine receptors in vitro; however, this ligand appears to label at least some receptors which differ pharmacologically from those labeled by ^{3}H-SP. Experiments in our laboratory with both homogenates of rat striatum and slide-mounted tissue sections revealed temperature-related differences in the kinetics and pharmacology of ^{3}H-NPA binding. Further investigation of both ^{3}H-SP and ^{3}H-NPA binding in tissue homogenates indicated that the binding of both ligands is temperature sensitive and that subpopulations of the receptors labeled by each are differentially affected by temperature.

The binding of ^{3}H-SP and ^{3}H-NPA was performed according to published procedures (Creese, et al., Europ. J. Pharmacol., 56, 1979; Creese, et al., Europ. J. Pharmacol., 46, 1977) at 37°, 25°, and 0° with homogenates of rat striatum. Association of both ligands at all three temperatures was complete at 45 min. Binding at equilibrium was reduced at 0°, suggesting either a K_d or B_{max} change. Kinetic experiments supported this observation. Scatchard analysis of ^{3}H-SP binding revealed a fourfold increase in K_d and 30% decrease in B_{max} at 0°. ^{3}H-NPA binding was more complex, revealing 2 components of binding at 37° and 25°, but probably only 1 at 0°, due to the apparent progressive loss of low-affinity sites with decreasing temperature. The K_d of the high-affinity site appeared to increase with decreasing temperature. Displacement experiments with (+)-butaclamol and dopamine revealed that agonists become increasingly potent or are unchanged in potency with decreasing temperature, while antagonists become less potent. All temperature-related effects have been shown to be independent of pH and tissue concentration, and occur at equilibrium. Thermodynamic analysis of ^{3}H-SP and high-affinity site ^{3}H-NPA binding according to Weiland, et al. (Nature, 281, 1979) showed that the binding of dopamine antagonists is entropy-driven, while the binding of agonists in enthalpy-driven. These results are similar to those obtained for the beta-adrenergic receptor.

Models for the binding of ^{3}H-SP and ^{3}H-NPA based on the selective effects of temperature on the kinetic parameters of the multiple sites labeled by these ligands will be presented.

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94.3 SOLUBILIZATION OF γ-AMINOBUTYRIC ACID (GABA) AND BENZODIAZEPINE (BZ) BINDING-SITES BY DETERGENTS. F. P. Bymaster* and D. T. Wong. Lilly Research Laboratories Indianpolis, IN 46285.

In order to separate the molecular entities involved in the interaction between the receptors of GABA and BZ we have compared the effects of the detergents Triton X-100 (TX), deoxycholate (DOC), and lysolecithin (LLC) on the solubilization of components responsible for the binding of GABA and BZ. As previously reported (Wong and Horng, Life Sci. 20, 445, 1977), treatment with TX at concentrations up to 0.1% increased the binding of ^{3}H-GABA to the synaptosomal membranes of rat cerebral cortex, up to 5 times the levels found in native tissue, but higher concentrations increased the binding only 1- to 2-fold as expressed in pmole/gm tissue. However, the binding of the BZ, ^{3}H-flunitrazepam (FLU), to membranes was decreased at concentrations of TX greater than 0.01%, but FLU binding and protein were recovered in the soluble fraction. The rank of inhibition of FLU binding by 3 BZ (lorazepam, diazepam, chlordiazepoxide) in the soluble fraction paralleled that in FLU binding to cortical membranes. The soluble fraction of 0.2% DOC-treated membranes exhibited monophasic saturable binding of FLU with a dissociation constant (Kd) of 1.69 ± 0.06 nM and number of binding sites of 600.7 ± 14.5 fmoles/mg protein. Similar values were obtained on saturation binding of FLU to the soluble fraction of 2% DOC-treated membranes. Characteristics of FLU binding in membrane, such as stimulation by GABA agonists and rank order of inhibition by BZ drugs, were retained in DOC-solubilized fractions. Saturable and monophasic binding of FLU was also observed in fractions solubilized by 0.4% LLC. GABA agonists stimulated FLU binding to the soluble fraction and BZ had the same rank order of potency as in membranes. GABA agonists and bicuculline, a GABA antagonist, inhibited the binding of ^{3}H-muscimol to LLC-solubilized fractions. Scatchard analysis revealed the possibility of two components with similar affinity as seen in membranes. Thus the binding of BZ and GABA in the DOC or LLC-solubilized preparation retains some characteristics of the membrane bound receptors and the differential solubilization by TX suggested the involvement of separate molecular entities in binding of GABA and BZ.

94.4 CHARACTERIZATION OF ALPHA ADRENERGIC INHIBITION OF PROSTACYCLIN-STIMULATED ADENYLATE CYCLASE IN THE HUMAN PLATELET. Robert H. Lenox, C.L. McMains* and D.A. VanRiper*. Dept. of Psychiatry, Univ. of Vermont College of Med., Burlington, VT 05405

The prostaglandins E_1 (PGE_1), D_2 (PGD_2)and prostacyclin (PGI_2) have been shown to inhibit human platelet aggregation while stimulating adenylate cyclase (AC) activity. Alpha (α) adrenergic agonists, e.g. norepinephrine (NE), are potent stimulators of platelet aggregation and inhibit PGE_1-stimulated AC. Pharmacological studies of this response indicate that it is mediated by α adrenergic receptors with characteristics similar to the α_2 subtype. Since PGI_2 is reported to be one of the most potent endogenous stimulators of human platelet AC, we have proceeded to examine more closely the characteristics of the α adrenergic inhibition of PGI_2-stimulated AC in an intact platelet preparation.

Human platelets for these studies were obtained from volunteer subjects. Blood was collected and centrifuged to separate platelet rich plasma (PRP). AC activity was determined using a modification of the pulse labelling technique with [^{3}H] adenine developed by Kuo and DeRenzo (J. Biol. Chem., 224, 1969). Following termination of the reaction, the [^{3}H] cyclic AMP (cAMP) formed was assayed using two-stage column chromatography (Salomon et al. Anal. Biochem.,58, 1974). Recovery of cAMP ranged between 70-80%.

Maximal stimulation of AC was 32-fold over basal levels for PGI_2 (1μM) but only 7-fold for PGE_1 (1μM). The conversion of [^{3}H]ATP to [^{3}H]cAMP in the platelet in the presence of PGI_2 was found to be linear with time during the reaction period, over a wide range of platelet concentrations. NE inhibition of PGI_2-stimulated AC was found to be concentration dependent with the IC_{50} for PGI_2 at 0.5μM NE compared to 10μM for PGE_1-stimulated AC. NE inhibition of PGI_2 stimulation was effectively blocked by the α antagonist phentolamine and the selective α_2 antagonist yohimbine but not by the α_1 antagonist prazosin. Clonidine inhibited the PGI_2-stimulated AC to a lesser extent than NE, and its action was also antagonized by yohimbine. Beta adrenergic agonists and antagonists were not significantly active in this AC system.

Pharmacological characterization of NE inhibition of PGI_2-stimulated AC in the human platelet was consistent with the modified α_2 subtype demonstrated for PGE_1-stimulated AC. The increased sensitivity and range of response to NE inhibition of PGI_2-stimulated AC results in a more statistically reliable detection of changes in slope and IC_{50}, indicative of an alteration in α adrenergic receptor activity. Current studies are underway to determine whether altered α adrenergic receptor activity may be related to biochemical processes underlying various neuropsychiatric disorders.

94.5 RECEPTORS FOR α-AND β-BUNGAROTOXIN IN CHICK EMBRYO RETINA. Heinrich Betz* and Hubert Rehm* (SPON: H. Holländer). Max-Planck-Institute of Psychiatry, 8033 Martinsried, Germany.

^{125}I-labelled α-Bungarotoxin (α-BTX) binds with apparent nicotinic-cholinergic specificity to membranes of chick embryo retina (Vogel and Nirenberg (1976) PNAS 73, 1806). In primary cultures of retinae from 8 day old embryos, a large portion of the neurons present (20 to > 60% depending on culture conditions) develops cell surface receptors for this toxin. Despite α-BTX is ineffective in blocking carbamylcholine-induced ^{22}Na uptake in these cultures, it may be a selective ligand for the nicotinic acetylcholine receptor (AChR) present in this tissue: i) preincubation with carbamylcholine decreases the initial rates of α-BTX binding in the presence of this agonist, suggesting that the toxin receptor is susceptible to cholinergic desensitisation; ii) 30 to 60 % of the detergent solubilised α-BTX binding sites are precipitated by antisera to Torpedo or Electrophorus AChR. The accumulation in culture of α-BTX receptors is increased by 0.1 to 1 mM 8-Bromo-cAMP and different steroid hormones, whereas dibutyryl cGMP (0.1 to 0.5 mM) and veratrine (o.Ol to O. M) inhibit receptor appearance. In the absence of external Ca^{2+}, the latter compounds are ineffective.

ß-Bungarotoxin (ß-BTX) is thought to selectively bind to and destroy the retinal ganglion cells in 18 to 20 day-old chick embryos (Hirokawa (1978)J.Comp. Neurol. 180, 449). ^{125}I-labelled ß-BTX binds in a specific and saturable manner to P2 membrane fractions isolated from retinae of 17 to 20 day old chick embryos. Binding is Na^+-sensitive and completely abolished by 0.25 mM Ca^{2+}. Saturation of specific binding sites is reached within 1 to 3 min depending on temperature; dissociation of the ß-BTX-receptor complex, however, proceeds only slowly ($t_{1/2}$ > 10 min). Scatchard analysis reveals one class of binding sites with approximately 10^3 to 10^4 receptors per retina cell. Supported by the Deutsche Forschungsgemeinschaft.

94.6 C6 GLIOMA CELLS GROWN WITHOUT SERUM HAVE INCREASED β-ADRENERGIC RECEPTOR NUMBERS. Mark D. Dibner and Paul A. Insel*
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The β-adrenergic receptor system was compared in rat C6 glioma cells grown in serum and in a serum-free defined medium. In parallel cultures, cells were grown in a DME:F-12 medium with 5% fetal bovine serum or with a defined mixture of 5 hormones in the absence of serum. These include insulin, transferrin, fibroblast growth factor, linoleic acid and serum spreading factor. (R. Wolfe, D. McClure and G. Sato, J. Cell Biol., in press) Cell growth was similar under both conditions. β-Adrenergic receptors were measured in cells dissociated from the dish by incubation with the antagonist radioligand [^{125}I]iodohydroxybenzylpindolol (IHYP). All studies were carried out in washed cells in the absence of serum or hormones. Several IHYP binding properties were similar with cells grown under both conditions such as association rates for IHYP and stereospecific affinities of the (+) and (-) isomers of isoproterenol and propranolol. In spite of these similarities of IHYP binding in cells grown under both conditions receptor numbers were increased by approximately 50% in cells grown without serum. Scatchard analysis of specific binding data (1.0 μM propranolol used to define specific binding) showed that there were approximately 9,500 receptors/cell with serum growth for 7 days and 14,000 receptors/cell with the defined medium ($p<0.01$). This increase in receptor number was even greater when expressed as receptors/μm^2 surface area. This effect took more than 3 days to occur and was not readily reversible by changing back to serum-containing medium. We also found that serum added to our in vitro IHYP binding assay led to a decreased affinity of the receptor for isoproterenol. Thus, our studies demonstrate that C6 cells grown in serum-free media have an increase in receptor number and that serum added to our IHYP assay decreases the affinity of β-adrenergic receptors for agonists.

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94.7 IDENTIFICATION AND CHARACTERIZATION OF AN INVERTEBRATE ACETYLCHOLINE RECEPTOR. W.E. Thomas* and J.G. Townsel (SPON: R. Greenberg). Dept. of Physiol. Biophys., Univ. of Illinois Medical Center, Chicago, Il. 60612

The snake venom neurotoxin α-bungarotoxin has been used to investigate an acetylcholine receptor in the central nervous system of the horseshoe crab, Limulus polyphemus. Levels of α-bungarotoxin binding of 500-700 pmoles/mg of protein were detected in the subesophageal ganglia, ventral nerve cord and cardiac ganglion. This binding was inhibited by greater than 80% by 100 μM d-tubocurarine. The level of toxin binding in nonneuronal tissues was less than 10% of that observed in central nervous system tissues. The physiological specificity of α-bungarotoxin in Limulus nervous tissue was accessed by recording spontaneous activity from the dorsal root of the ventral nerve cord. In those cases where the spontaneous activity was stimulated by μM concentrations of nicotine, α-bungarotoxin caused a reduction in spontaneous activity and a pronounced inhibition of the stimulation by nicotine. Autoradiographic studies have shown the neuropil regions and longitudinal connectives to be the major sites of localization of toxin binding in the abdominal ganglion. Subcellular fractionation of the subesophageal ganglia revealed the greatest enrichment of toxin binding in the mitochondrial fraction. This fraction also displayed the greatest enrichment of acetylcholinesterase activity. Sedimentation studies of toxin binding in solubilized extracts of the subesophageal ganglia resulted in the identification of three α-bungarotoxin binding proteins. The approximate sedimentation coefficients of these proteins were 9S, 15.4S and 17.4S. Acetylcholinesterase and choline acetyltransferase from the same tissue had approximate sedimentation coefficients of 6S and 5S, respectively. The formation of the 15.4S and 17.4S toxin binding proteins from isolated fractions of the 9S protein was observed. Therefore, the 15.4S and 17.4S proteins are suggested to be aggregates of the 9S protein. This 9S protein is proposed as an acetylcholine receptor from the central nervous system of Limulus polyphemus. (Supported by NIH Grant HL 24140)

94.8 SNAKE α-NEUROTOXINS ANTAGONIZE CHOLINERGIC AND NONCHOLINERGIC Cl^--MEDIATED RESPONSES IN APLYSIA CNS. P.M. Salvaterra, J.K. Ono and R.E. McCaman. Div. of Neurosciences, City of Hope Research Institute, Duarte, CA 91010

Neurons in the CNS of the marine mollusc, Aplysia californica, may respond to acetylcholine (ACh) and other transmitters and agonists with a conductance increase (↑g) to Na^+, K^+, Cl^- or a combination of these ions. Confirming the observations of Kehoe et al. (Brain Res. 107:527, 1976), we find that α-bungarotoxin (αBTX), as well as α-naja toxin (αNTX), will block only the ↑gCl ACh response. In addition, our observations indicate that the α-neurotoxins also block ↑gCl responses mediated by noncholinergic agonists and transmitters. We have observed that responses involving an ↑gCl induced by the iontophoretic application of histamine, and in some cases glutamate, are reversibly antagonized by the perfusion of saline containing the α-neurotoxins at a level comparable to that affecting the ACh response of the same neuron. The α-neurotoxins also antagonize certain synaptically induced ↑gCl responses mediated by identified, noncholinergic, presynaptic neurons. Characterization of [^{125}I]αBTX binding to Aplysia CNS membranes indicates a single toxin binding site (Kp≈3nM) which is competitively antagonized by cholinergic ligands (nicotine, carbachol, curare). Noncholinergic ligands (glutamate, histamine) and Cl^- channel blockers (picrotoxin, penicillin) were without significant effect on toxin binding.

We believe that toxin binding in the Aplysia CNS is confined to a nicotinic cholinergic receptor mediating the ↑gCl ACh response. Despite the observation that toxin antagonizes noncholinertic ↑gCl responses, we do not believe that the α-neurotoxins are generally blocking Cl^- channels because: a) they do not antagonize all ↑gCl responses, e.g., GABA and in certain cells glutamate and histamine ↑gCl responses are not blocked by toxin; and b) the α-BTX binding is not antagonized by Cl^- channel blockers. Rather, we have formulated a working hypothesis that several neurotransmitter recognition sites may share the same ionophore. The α-neurotoxins block noncholinergic ↑gCl responses in cells where the recognition sites for these agonists and transmitters share the same Cl^- ionophore with cholinergic receptors. Experiments testing this hypothesis will be presented. [Supported by NS 13813 (PMS) and NS 15583 (REM & JKO).]

94.9 MULTIPLE AFFINITIES FOR MUSCARINIC ANTAGONISTS IN THE RAT BRAIN. J. Ellis* and W.Hoss, Center for Brain Research, University of Rochester, Rochester, NY 14642

Gallamine, a cholinergic antagonist at the (nicotinic) neuromuscular junction, possesses antimuscarinic potency in several systems. We report here that gallamine inhibits the binding of [^{3}H]-quinuclidinyl benzilate (QNB) in a competitive manner in several regions of the rat brain. The occupancy curves derived from these studies suggest that gallamine has widely varying affinities for different subpopulations of muscarinic receptors, a finding which sets gallamine apart from classical muscarinic antagonists such as atropine and QNB. The greatest difference in affinities for gallamine occurs in the brainstem, where the data can be satisfactorily fit to a two-site model, with 78% of the receptors having high affinity (K_d =20nM) and 22% low affinity (1mM) (a 50,000-fold difference). Further, these affinities display rank order correlation with those of carbachol (an agonist), although gallamine has so far displayed no agonistic (or partial agonist) activity. For example, carbachol, but not gallamine, enhances the turnover of phosphatidyl inositol in brain slices. We have also found that another antagonist, scopolamine, has a significantly higher affinity in the forebrain than in the brainstem, in contrast to the higher affinity of most muscarinic agonists for the brainstem (forebrain ID_{50} 0.2nM; brainstem ID_{50} 1 nM). The finding that certain antagonists, as well as agonists, display more than one affinity for muscarinic receptors suggests that there are fundamental differences among subpopulations of these receptors.

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94.10 CHARACTERIZATION OF THE GLUTAMATE RECEPTOR-LIKE PROTEIN RECONSTITUTION INTO LIPOSOMES. Robert D. Grubbs and Elias K. Michaelis Dept. of Human Development and Family Life, Neurobiology Section, Univ. of Kansas, Lawrence, KS. 66045.

Initial attempts at the reconstitution of a glutamate binding glycoprotein into liposomes were reported at this meeting last year (Grubbs & Michaelis, 1979). The continued effort to reconstitute this protein isolated from rat brain synaptic membranes (Michaelis, 1975) has allowed for a preliminary characterization of the effects of this procedure on the specific, high-affinity L-[^{3}H]-glutamate binding of the protein.

The reconstitution procedure utilized for these experiments is based on the method of Epstein & Racker (1978) in which the protein, liposomes, and buffer are incubated together at room temperature for 30 minutes (total volume 0.5 ml). This mixture is then transferred to a short column (0.8 x 2.0 cm) of Bio-Beads SM-2 for 4 hr. at room temperature to remove Triton X-100 from the mixture. The columns are then centrifuged in a table-top clinical centrifuge at 1000 x g for 4 minutes. The eluate recovered from each column is centrifuged at 38,000 x g for 30 minutes. The pellet, which contains the reconstituted system, is resuspended in 0.5 ml of 50 mM Tris-HCl buffer.

The Bio-Beads incubation step in this procedure was found to reduce by 80% the Triton X-100 concentration of liposomes prepared in 0.5% Triton X-100 / 50 mM Tris-HCl buffer. This removal was monitored as the absorbance decrease at 280 nm, the peak absorbance wavelength of the detergent. This step was also observed to quantitatively remove the soluble, non-reconstituted binding protein from solution. The eluate of a column incubated with 30 µg of the binding protein has consistently yielded negligble amounts of binding activity and protein.

Using this reconstitution procedure, the effect of the liposome composition on the L-[^{3}H]-glutamate binding of the protein has been examined. The addition of Triton X-100 to the reconstituted system was found to enhance glutamate binding at low concentrations (20-40 µM), while higher concentrations had no effect. Data will be presented concerning the effects of Na^+ and Ca^{++} on the kinetics of L-[^{3}H]-glutamate binding.

This research was partially supported by DHEW Research Service Award HD 7066 from NICHHD to the Kansas Center for Mental Retardation and Human Development, by a grant from NIGMS, GM 22357, and grant #DAAG29-79-C-0156 from the Army Research Office.

94.11 INTERACTION OF CARDIAC NEUROTRANSMITTER RECEPTORS WITH A GUANINE NUCLEOTIDE BINDING SITE. T. K. Harden. Dept. Pharmacology, Univ. North Carolina School of Medicine, Chapel Hill, NC 27514.

Guanine nucleotides, which are obligatory for hormone effects on adenylate cyclase, have been shown to influence the interaction of muscarinic and β-adrenergic receptor agonists with their receptors. To date it is unclear whether a single nucleotide binding site is responsible for these effects. The present series of experiments was initiated to examine the interaction of guanine nucleotides with rat heart muscarinic cholinergic receptors. The muscarinic receptor antagonists, atropine and scopolamine, inhibited the binding of ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) to a washed cardiac membrane preparation with K_I values of 0.6 and 1.3 nM, respectively. GTP exhibited no effect on the binding affinities of ^{3}H-QNB, atropine, or scopolamine. The K_I values for oxotremorine and carbachol for inhibition of ^{3}H-QNB binding were 15 nM and 42 nM, respectively. In the presence of GTP the concentration effect curves for these two agonists were shifted 1.0 to 1.5 orders of magnitude to the right. $K_{0.5}$ values for the effects of guanine nucleotides on agonist binding were generated for a series of nucleotides by incubating ^{3}H-QNB in the presence of a fixed concentration (0.1 µM) of oxotremorine and various concentrations of nucleotides. The $K_{0.5}$ values determined in this manner for GTP, Gpp(NH)p, GDP and GMP were 8, 72, 84, and 5000 µM, respectively. The sulfhydryl reagent, N-ethylmaleimide (NEM), was employed to probe potential differences in sensitivities to inactivation of components of the muscarinic receptor system. ^{3}H-QNB binding was not affected by concentrations of NEM less than 30 µM. In contrast, concentration effect curves for oxotremorine were shifted to the right by 1.0 to 1.5 orders of magnitude following preincubation of membranes with 10 µM NEM. GTP had no effect on apparent agonist affinity in NEM-treated membranes. Thus, high affinity cholinergic agonist binding and the effects of GTP appear to be lost in concert as a result of inactivation of NEM-reactive groups in cardiac membranes. The relationship between the effects of guanine nucleotides and NEM on muscarinic receptors versus β-adrenergic receptors in rat heart will be discussed. (Supported by USPHS HL22490 and the American Heart Association, North Carolina affiliate.)

94.12 RECEPTOR-MEDIATED INCREASE IN PHOSPHATIDYLINOSITOL TURNOVER IN CLONED CELL LINES. D.M. Schmidt, R.C. McGlennen, and W.L. Klein. (Biological Sciences, Northwestern University, Evanston, IL 60201).

Activation of muscarinic ACh receptors of cultured N1E-115 neuroblastoma and NG108-15 hybrid cells by 10^{-3} M carbachol causes a 60-100% increase in incorporation of $^{32}P_i$ into phosphatidylinositol (PI) during a 15 minute period. Enhancement of turnover can be observed within one minute after adding carbachol to cultures, and little or no desensitization occurs for at least 30 minutes. Stimulation of opiate, alpha$_2$, or serotonin receptors of NG108-15 cells does not increase PI turnover. Measurements of the specific activity of $^{32}P_i$ in PI have been facilitated by use of one-dimensional TLC for the rapid separation of phospholipids (butanol:acetone:acetic acid:water:: 5:5:1:1 on Whatman LK5D plates).

Because the muscarinic receptor-mediated increase in intracellular cGMP in N1E-115 cells appears dependent on calcium gating (R.E.Study, et al., Proc. Natl. Acad. Sci. 75: 6295,1978), it was of interest to examine the effect of changing the concentration of extracellular ions on the receptor-mediated stimulation of PI turnover in these cells. When Na^+ is replaced by choline or K^+, total incorporation of $^{32}P_i$ into PI is reduced by 90% and 50%, respectively. In contrast, removal of extracellular K^+ or Ca^{++} has little or no effect on $^{32}P_i$ incorporation. In all four cases, addition of carbachol increases PI turnover, although the percentage increase in the absence of K^+ or Ca^{++} is somewhat smaller than when these ions are present.

This work represents the first use of cloned cells to study receptor-mediated increases in PI turnover. In cloned neuroblastoma and neuron-like hybrid cells, muscarinic ACh receptors can mediate increases in PI turnover independently of the presence or absence of specific extracellular cations. The data are consistent with a possible role for enhanced PI turnover in the receptor-coupled calcium gating proposed to regulate intracellular cGMP. (Supported by NIH grant 5 R01 NS15299-02 to WLK.)

94.13 PURIFICATION OF THE SOLUBILIZED BENZODIAZEPINE RECEPTOR, J. Fong* and M. Goldstein (SPON: R. U. Margolis). New York Univ. Med. Cntr Dept. of Psychiatry, Neurochemistry Lab., New York, N. Y. 10016

Recently several methods for the solubilization of the benzodiazepine receptor from cerebral cortical membranes were described. We have attempted to purify the solubilized benzodiazepine receptor, using 1% Triton X 100 as a detergent. The solubilized membrane proteins were dialyzed against 0.1% Triton X 100 and 10 mM K-phosphate (KPi) buffer pH 6.3. Following dialysis and centrifugation at 100,000xg for 1 hr., the adsorption of the solubilized benzodiazepine receptor on different resins was tested. Chromatography on Concanavlin A- Sepharose 4B resulted in a 95% adsorption and 25% recovery following elution with 0.5 M α-methyl-D-mannoside. Chromatography on phosphocellulose resulted in a 75% adsorption and 25% recovery following elution with a gradient of 0.01-0.4 M KPi buffer (pH 6.3). Chromatography on hydroxylapatite(HA) resulted in a 95% adsorption and 40% recovery following elution with a gradient of 0.01-0.5M KPi (pH 7.0) + 0.1% Triton X 100. The solubilized benzodiazepine receptor was eluted from the HA column in two separate peaks. The first peak was eluted with 0.2 M Pi buffer and contained approx. 20% of the receptor, and the second peak was eluted with 0.35 M KPi buffer and contained also approx. 20% of the receptor. Scatchard plot analysis of H^3-flunitrazepam binding to the receptor isolated in the first peak revealed a Kd of 1.4 nM and that isolated from the second peak of 6.8nM. The binding of ^{3}H-flunitrazepam to the solubilized receptor obtained from both peaks was stimulated by GABA (approx. 90% stimulation at 10 μM GABA). Bicuculline at a concentration of 50 μM inhibited the stimulation elicited by 1μM GABA. The properties of the solubilized benzodiazepine receptors isolated from HA column are now being further studied.

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94.14 INTERACTIONS BETWEEN THE BENZODIAZEPINES AND ALPHA-MELANOCYTE STIMULATING HORMONE (α-MSH). E. Matthew, D.L. Engelhardt*, J.D. Laskin*, and E.A. Zimmerman. College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

High affinity binding sites for benzodiazepines (BDZ) have been demonstrated in the nervous system and in other organs, but no functional effects of these compounds have been established outside the nervous system. In earlier reports, we described melanotropic effects of benzodiazepines and high affinity binding sites (K_D=1.8±0.7nM) in the cell line B16/C_3 derived from a spontaneous melanoma in a C_{57} BL/6JAX mouse. These cells are of neural crest origin and are probably part of the APUD system. The combination of high affinity binding sites and a biological effect permits the study of the 'benzodiazepine receptor' in relation to other cell components. We now report the effects of alpha melanocyte stimulating hormone (α-MSH) which shares sequences 1-13 with ACTH. As with the benzodiazepines, α-MSH induced melanogenesis and differentiation in these cells in culture. However, the addition of α-MSH to radioreceptor assay for diazepam caused a two-fold enhancement of 3_H diazepam binding in cells at 70-90% confluency. When the cells were in stationary phase, this effect was not evident. Thus, although the benzodiazepines and α-MSH have similar melanotropic effects, they apparently do not act at the same binding sites. There are, however, significant modulatory influences between these substances which are most evident during cell growth and differentiation. This is the first demonstration of an association between the benzodiazepines and a peptidergic system. It emphasizes the value of the melanoma cell as an effective and accessible model in which to study benzodiazepine compounds.

94.15 ASCORBIC ACID INHIBITION OF DOPAMINE AGONIST BINDING IN STRIATUM T.N. Thomas, C. Koteel*, L.D. Middaugh and J.W. Zemp. Departments of Biochemistry, Psychiatry and Behavioral Sciences, Medical Univ. of S.C., Charleston, SC 29403.

Ascorbic acid (vitamin C) is a normal constituent of the brain and has long been known to be an important biological catalyst. Concentration of the vitamin in the brain of several mammalian species, including man, is higher than that of any other organ except the adrenal cortex, but the significance of ascorbic acid in brain function is yet to be realized. Recent findings in this laboratory indicate a role for ascorbic acid in regulating dopaminergic transmission. We have previously reported that ascorbic acid, like other known dopamine receptor blockers inhibited the dopamine-sensitive adenylate cyclase in rat striatum at physiological concentration. Subsequent studies revealed that elevation of ascorbic acid in rat and mouse brain specifically altered a variety of neurochemical, behavioral and physiological systems mediated by dopamine. This report is a further attempt to characterize the action of ascorbic acid on postsynaptic dopamine receptors in rat and bovine striatum.

Rat and bovine striata were removed and synaptic membranes were isolated using standard procedures. The membranes were washed thoroughly and the binding of the dopamine agonist, 8 nM ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) and the antagonist, 0.2 nM spiroperidol were studied by published procedures, in the absence of ascorbic acid. Nonspecific binding of the ligands to membranes were estimated by parallel incubation in presence of excess agonist apomorphine or dopamine (for ADTN) and antagonist + butaclamol (for spiroperidol).

In both rat and bovine striata, ascorbic acid specifically inhibited ADTN binding, with no evident effect on spiroperidol binding. In both species, a 50% reduction in ADTN binding was observed at a concentration of less than 5 μM ascorbic acid. The antioxidant properties of ascorbate have resulted in extensive use of this compound in studying the binding of ligands to neurotransmitter receptors. At 1 mM concentration normally used in dopamine receptor binding studies, there was 80% reduction in ADTN binding. Scatchard analysis of binding data in presence of varying concentrations of ADTN (0.1 to 8 nM) indicated that ascorbic acid caused a significant reduction in ADTN binding sites.

These results support our previous reports showing the ability of ascorbic acid to block the dopamine receptor and the effect seems to be on the agonist state of the dopamine receptor. Ascorbic acid might be thus useful in probing the agonist binding site of the dopamine receptor. This report questions the validity of previous dopamine receptor studies in presence of high concentrations of ascorbic acid.

94.16 GUANINE NUCLEOTIDES MODULATE RECEPTOR BINDING FOR TRH IN PITUITARY BUT NOT IN CNS. D. R. Burt and R. L. Taylor*. Dept. Pharmacol. Exp. Ther., U. Md. Sch. Med., Baltimore, MD 21201.

Guanosine-5'-triphosphate (GTP) and related nucleotides have been shown in vitro to lower the affinity of agonist binding for a variety of receptor types. This phenomenon is thought to reflect the existence of receptor-linked GTP-regulatory proteins as intermediates in the coupling of agonist binding to an adenylate cyclase or other response mechanism (Rodbell, 1980, Nature 284: 17). We report the detection of guanine nucleotide effects on receptor binding for thyrotropin releasing hormone (TRH) in membranes of sheep anterior pituitary gland and the inability to detect such effects in membranes from sheep retina or nucleus accumbens. Tissue resuspensions (100-200 mg wet weight/ml) in 20 mM $Na\text{-}PO_4$ buffer, pH 7.4, were incubated with 1-1000 μM GTP, guanyl-5'-yl imidodiphosphate (Gpp(NH)p, a nonmetabolizable analog), and other nucleotides for 5-20 min at 37°C, chilled, and diluted with an equal volume of 0.5-20 nM ^{3}H-TRH or ^{3}H-[3-Me-His^2]TRH with or without added nonradioactive TRH or [3-Me-His^2]TRH. The mixture was incubated for an additional hour on ice and filtered as previously described (Burt, 1979, Exp. Eye Res. 29:353). Gpp(NH)p, 100 μM, added to pituitary membranes during the 37° preincubation, increased the apparent K_D of ^{3}H-TRH binding from 27 ± 1 nM (6) to 47 ± 6 nM (4) with no effect on the B_{max} (13.1 ± 1.6 and 14.2 ± 1.7 pmol/g wet weight, respectively). Similar effects were observed with lower concentrations of Gpp(NH)p, with GTP and GDP (but not GMP, ATP, etc.), and with the new ligand for TRH receptors, ^{3}H-[3-Me-His^2]TRH (Taylor and Burt, 1980, Proc. Endocrine Soc., 62nd meet., in press). However, with neither ligand were we able to demonstrate nucleotide effects on TRH receptor binding in the retina and nucleus accumbens. This lack of effect was shown not to be due to soluble factors present in the central nervous system incubations by the ability of supernatant solutions from such incubations still to produce an effect when incubated with pituitary membranes. The apparent difference between brain and pituitary receptors for TRH in their response to guanine nucleotides is surprising in view of the close similarity of receptors in the 2 tissues in their kinetic properties and specificity for TRH analogs (Burt and Taylor, 1980, Endocrinology 106, in press), but is not unprecedented, e.g., for muscarinic (Ehlert et al., 1980, Life Sci. 26:245) or β (Hegstrand et al., 1979, J. Pharmacol. Exp. Ther. 210:215) receptors in certain rat brain regions as opposed to other tissues. It is not yet known whether such differences in nucleotide response among receptor binding sites in different tissues reflect actual differences in the binding proteins or rather differences in other components involved in coupling to a response. (Supported by grant MH-29671)

94.17 SOME STEREOCHEMICAL PROPERTIES OF THE 1-17 N-TERMINAL SEGMENT OF THE α-SUBUNIT OF THE ACh PROTEIN IN RELATION TO THE ACTUAL ACh BINDING SITE. J.R. Smythies. Neurosciences Program, UAB Medical Center, Birmingham, Alabama 35294.

The amino acid sequence of the 1-25 N-terminal segment of the α-subunit of the AChR protein is H - ser - glu - his - glu - thr - arg - leu - val - ala - asn - leu - leu - glu - asn - tyr - asn - lys - val - ile - arg - pro - val - glu - his - his - A Chou and Fasman analysis indicates that the segment from ser(1) to tyr(15) will probably be in the α-helical conformation and the segment from lys(17) to his(25) in the β-sheet conformation. Such an α-helix will be amphoteric with the array of hydrophilic amino acids down one side: glu(2), arg(6), asn(10), glu(13). This can be completed by lys(17) to form a regular array with the charge distribution - + ± - + . There are 4!/2 = 8 possible combinations of (+ or -) x 4 of which two are self-complementary. This sequence is one of these two. The α-subunit of the AChR exists as a dimer and is known to bear the actual ACh binding site. Thus two such (1-17) segments laid anti-parallel can form a four-runged grid made up of ionically linked complementary amino acids as follows: glu(2) to lys(17'); arg(6) to glu(13'); asn(10) and asn(10') (broken rung); glu(13) to arg(6') and lys(17) to glu(2'). This forms a symmetrical complex of two pairs of ionically bound rungs on either side of a central hole left by the short residues asn(10) and asn(10'). Experiments with CPK molecular models show that such a grid is highly complementary to a wide range of ACh agonists and antagonists. There are two pairs of potential ACh binding sites as follows. Site 1 (x2): lys(17) h bond to CO; asn(14) h bond to ether O; onium head in pocket between asn(10) and glu(13). Site 2 (x2): arg(6) to CO: his(3) to ether O: onium head to glu(2). The hypothesis is that ACh molecular binding in this manner would disrupt these ionic links and allow the two segments to separate, thus opening an ionic channel between them. Molecular model building experiments show that the separated segments (R1) are now complementary to antagonists such as curare, decamethonium, histrionicotoxin and snake neurotoxins. The closed conformation (R) is complementary to β-erythroidine. The hypothesis may be experimentally verified since it predicts that each α-subunit may be cross-linked by a covalent bond (using an agent such as carbodiimide) to its partner α-subunit via lys(17) to glu(2) (x2). Molecular models demonstrating these relationships will be presented.

94.18 DETERGENT SOLUBILIZATION OF AGONIST AND ANTAGONIST DOPAMINERGIC BINDING. E. Stefanini*, E. Suen* and Y. Clement-Cormier. Department of Pharmacology, The University of Texas Medical School, Houston, Texas 77025.

The binding of a radiolabelled agonist and antagonist have been used to characterize brain receptors solubilized with selected detergents. An extract, which binds (^{3}H) Spiroperidol exhibits stereoselectivity for (+) and (-) Butaclamol but does not bind the agonist (^{3}H)N-propyl-norapomorphine, was isolated from the dog striatal microsomes using the detergent digitonin. Conversely, the detergents deoxycholate and octyl-B-glucosylpyranoside solubilized receptor sites with a high affinity for (^{3}H)N-propylnorapomorphine but with a low affinity for (^{3}H) Spiroperidol. The extract that was solubilized with octyl-B-glucosylpyranoside was found to have essentially the same biochemical and pharmacological properties for (^{3}H)N-propylnorapomorphine as the native membrane bound receptor. Moreover, the site labelled with (^{3}H)N-propylnorapomorphine was heat stable up to 60 C whereas that for (^{3}H) Spiroperidol was heat labile. The results demonstrating both the differential solubilization of agonist and antagonist dopaminergic binding sites and the selective thermolability of the antagonist binding site, suggest that multiple binding sites may be involved in dopamine receptor responses in the brain. (This work was supported by grants from the Pharmaceutical Manufacturer's Association and the National Science Foundation).

94.19 SOLUBILIZATION AND PURIFICATION OF THE DOPAMINE RECEPTOR FROM CALF STRIATAL MICROSOMES: MULTIPLE BINDING SITES. Yvonne Clement-Cormier and Andrew McIsaac*. (SPON: B. T. Ho) Department of Pharmacology, University of Texas Medical School, Houston, Texas 77025.

Specific dopamine binding sites were solubilized from calf striatum using potassium chloride. The solubilized salt extract was found to have the same properties as the native membrane preparation including binding affinity and stereoselectivity. Gel filtration chromatography of the salt extract on Sephadex G-100 Superfine revealed the presence of multiple binding sites for the dopamine antagonist (^{3}H) Spiroperidol and the dopamine agonist (^{3}H) N-propyl-norapomorphine (NPNA). Some components bind only (^{3}H) Spiroperidol indicating a distinct and exclusive antagonist binding site and some components can bind both ligands indicating that a conformational change may occur to accomodate both antagonist and agonist drugs. The predominant site labelled by both (^{3}H) Spiroperidol and (^{3}H) NPNA had a calculated molecular weight of about 50,000 daltons as calibrated for globular proteins. Hill coefficients for selected agonists and antagonists of the dopamine receptor confirmed the presence of more than one population of agonist binding sites. Furthermore, the presence of high salt concentrations such as sodium chloride or potassium chloride can increase the affinity of (^{3}H) NPNA for the agonist binding sites but not the antagonist sites. These data support the existence of multiple binding sites of the dopamine receptor in the central nervous system. (This work was supported by grants from the Pharmaceutical Manufacturer's Association and the National Science Foundation).

94.20 LIGAND BINDING TO ACETYLCHOLINE RECEPTORS IN WILD TYPE AND ACETYLCHOLINESTERASE-DEFICIENT STRAINS OF THE NEMATODE C. ELEGANS W.M. James, C. DeCrease*, J.G. Culotti* and W.L. Klein. (Biological Sciences, Northwestern University, Evanston IL 60201)

Crude homogenates of the nematode worm C. elegans bind the cholinergic antagonists (^{3}H)QNB and (^{125}I)alpha bungarotoxin with high affinity. Specific binding sites are saturated by 10^{-9} M (^{3}H)QNB and by 2.5×10^{-9} M (^{125}I)toxin. In wild type strains, the maximum number of specific binding sites for (^{3}H)QNB is 15 fmols per mg total worm protein and for (^{125}I)toxin is 7 fmols per mg total worm protein. Competition experiments using unlabeled atropine, oxotremorine, carbachol, nicotine, and d-tubocurarine indicate that (^{3}H)QNB binding is to muscarinic ACh receptors and that (^{125}I)toxin binding is to nicotinic ACh receptors. The presence of acetylcholine receptors in C. elegans is in harmony with previous experiments showing locomotor deficits due to mutations in genes for acetylcholinesterase (J.G. Culott, et al., submitted to Genetics). Locomotor deficient ace-1 ace-2 double mutants have the same levels of acetylcholine receptors as wild type, although the locomotor normal ace-1 single mutant has only half the specific binding of the wild type when assayed using 10^{-9} M (^{3}H)QNB.

This is the first report of identification and quantification of any neuroreceptor in the nematode worm, an organism with a simple nervous system particularly amenable to genetic and developmental studies. The ability to measure acetylcholine receptors in this system suggests that it is possible to use the genetic advantages of C. elegans to assess the role and mechanism of action of specific neuroreceptors in neural development and also in simple behavioral traits. (Supported by NIH grant 5 R01 NS15299-02 to WLK.)

94.21 REGULATION OF β-ADRENERGIC RECEPTOR SUBTYPES IN RAT CEREBELLUM, CEREBRAL CORTEX, AND CAUDATE. B.B. Wolfe, K.P. Minneman*, M.D. Dibner and P.B. Molinoff. Dept. of Pharmacology, Univ. Colo. Health Sci. Ctr., Denver, CO 80262.

The effects of chronic alterations in β-adrenergic receptor stimulation on the densities of β_1 and β_2-receptors in regions of the rat CNS have been investigated (see Table). Noradrenergic stimulation was decreased by the chronic administration of the β-adrenergic antagonist propranolol or the neurotoxin 6-hydroxydopamine (6-OHDA). Both treatments led to increases in the density of β_1-receptors in the cerebellum and cerebral cortex without affecting β_2-receptors. To study the effects of increased norepinephrine availability, desmethylimipramine (DMI) or pargyline was administered. In the cerebral cortex these drugs decreased the density of β_1-receptors without changing β_2 receptors. In the cerebellum, on the other hand, administration of these drugs led to decreases in the the density of β_2-receptors. Decreases in the density of β_1-receptors were not observed, but since this receptor subtype comprises only 2-3% of total cerebellar β-adrenergic receptors, small decreases would have been difficult to detect. The administration of either propranolol or pargyline led to changes in the density of β_1-receptors in rat caudate.

	CORTEX		CEREBELLUM		CAUDATE	
	β_1	β_2	β_1	β_2	β_1	β_2
6-OHDA	↑59%	NS	↑250%	NS	NS	NS
PROPRANOLOL	↑51%	NS	↑150%	NS	↑11%	NS
DMI	↓42%	NS	NS	↓20%	NYD	NYD
PARGYLINE	↓23%	NS	NS	↓12%	↓22%	NS

Data are presented as % change from control values. All changes are significant ($p<0.0\%$). NS = $p>0.05$. NYD= Not yet determined

The data suggest that β_1-receptors in the cerebral cortex normally have a neuronal input. Interruption or facilitation of neurotransmission leads to reciprocal, compensatory changes in the density of these receptors. β_2-Receptors, on the other hand, are not regulated by these manipulations and may not receive a neuronal input. The density of cerebellar β_1-receptors increased as a consequence of manipulations that cause decreases in noradrenergic input, suggesting that these receptors also receive a tonic innervation. The decrease in the density of β_2 receptors in the cerebellum following DMI or pargyline suggests that drug induced increases in catecholamines released from the adrenal medulla or from nerve terminals have access to these receptors. Since chronic propranolol administration had no effect on these receptors they are unlikely to have a neuronal input under normal conditions. Surprisingly, some of these manipulations altered the density of β_1-adrenergic receptors in the caudate. These findings were unexpected since there does not appear to be a noradrenergic input to this brain region. These receptors may be on vascular elements affected by catecholamines released from the adrenal medulla. Supported by HL 24353, NS 13289.

94.22 BARBITURATES, BENZODIAZEPINES AND PURINES INHIBIT THE BINDING OF [^{3}H] α-DIHYDROPICROTOXININ. M.K. Ticku, T.P. Burch* and W. Davis*. Department of Pharmacology, University of Texas Health Science Center at San Antonio, Texas 78284.

[^{3}H] α-Dihydropicrotoxinin (DHP), a GABA synaptic antagonist having biological activity similar to picrotoxin, binds to rat brain membranes with an apparent K_D of ~2μM and a B_{max} of 5 pmol/mg protein (Ticku et al, Mol. Pharmacol. 14:319, 1978). The DHP binding sites are distinct from the GABA receptor sites and appear to be associated with the GABA receptor-ionophore system. Besides picrotoxin analogues, DHP binding is inhibited potently by depressant and convulsant barbiturates, benzodiazepines and purines. Depressant barbiturates inhibit DHP binding with IC_{50} values of 5-50μM. Stereoisomers of barbiturates which differ in pharmacological activity also inhibit DHP binding potently. In the case of depressant barbiturates like pentobarbital, the (-) isomer was three to four-fold more potent than its (+) isomer. Displacement curves with some barbiturates deviated from the law of Mass Action. Displacement curves with the (+) and (-) isomers of 1-methyl-5-phenyl-5-propyl barbituric acid, which show opposite pharmacological activity, were biphasic with a distinct plateau. These data suggest heterogenity of DHP binding sites. DHP binding was also inhibited by various benzodiazepines, including RO5-3553, with IC_{50} values ranging from 0.1-20μM. DHP binding was also inhibited by purines like inosine, hypoxanthine and adenosine, albeit with low potencies. It is interesting that purines inhibit the binding of DHP, GABA and diazepam to three distinct sites; nonetheless, all of these sites appear to be associated in the synaptic membranes. DHP binding was also inhibited by some of the fractions obtained by gel filtration of the supernatant of 100,000g rat brain pellet. Our preliminary studies indicate that deoxycholate extract of rat brain membranes binds [^{3}H] DHP with the expected properties. The DHP binding to deoxycholate extract was inhibited by picrotoxinin, DHP and pentobarbital.

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94.23 DIFFERENTIAL SENSITIVITY OF MOUSE BRAIN SEROTONIN RECEPTORS TO ETHANOL IN VITRO. James D. Hirsch. Dept. Bio. Res., G.D. Searle & Co., Chicago, IL 60680.

During in vitro screening of drugs dissolved in ethanol it was observed that serotonin (5HT) receptors in whole mouse brain membranes labeled with [^{3}H]LSD were more sensitive to the alcohol than those labeled with [^{3}H]5HT. Further work revealed that versus [^{3}H]LSD in the presence of 1μM pimozide and 10μM dopamine (DA) to block [^{3}H]LSD binding to DA receptors, ethanol in the range of .054 to 2.17M had an IC_{50} of .63M. Versus [^{3}H]5HT, ethanol had an IC_{50} of 1.8M. These IC_{50}'s represent 2.7% (v/v) and 8.1% (v/v) ethanol respectively. With both ligands, non-specific binding was determined ± 10μM unlabeled 5HT. In the presence of 1μM pimozide and 10μM DA, [^{3}H]LSD labeled a single site noncooperatively (n_H=.95) and binding was saturable with a K_D of 13.8nM and a B_{max} of 86.3 fmol/mg protein. When assayed under identical conditions in the presence of .543M ethanol, binding was still saturable and noncooperative (n_H=.91), but had a K_D of 7.8nM and a B_{max} of 46.3 fmol/mg protein. Thus, ethanol caused a 46% decrease in the number of binding sites and a 43% increase in affinity. Double reciprocal and Scatchard plot analysis revealed that ethanol acts as an uncompetitive inhibitor of [^{3}H]LSD binding to 5HT receptors. In the presence of 10μM unlabeled 5HT, [^{3}H]LSD also labels DA receptors. In these experiments, non-specific binding was determined ± 100μM DA. Under these conditions, ethanol had an IC_{50} of .96M. Benzodiazepine, GABA, opiate and muscarinic receptors were not sensitive to the ethanol concentrations used here. These results lend support to the hypothesis that [^{3}H]LSD and [^{3}H]5HT label different populations of brain 5HT receptors and suggest that 5HT receptors labeled by [^{3}H]LSD are particularly sensitive to membrane perturbation by ethanol.

94.24 ADENOSINE RECEPTORS: ELECTROPHYSIOLOGICAL ACTIONS AT PRE- AND POSTSYNAPTIC SITES ON MAMMALIAN NEURONS. Barbara K. Henon, Diane K. Turner*, and Donald A. McAfee, Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

Our studies suggest that adenosine specifically antagonizes Ca^{++}-dependent processes in the rat superior cervical sympathetic ganglion in a receptor-mediated fashion. Postsynaptic effects were determined by intracellular potential measurements from single postganglionic neurons. These neurons show three Ca^{++}-dependent potentials: the Ca^{++} spike, the shoulder on the falling phase of the Na^+ spike, and the hyperpolarizing afterpotential (HAP). Presynaptic effects were determined by measuring synaptic depression. Repetitive preganglionic stimulation results in compound action potentials whose amplitudes depress as a function of time and stimulus frequency. This synaptic depression is decreased in lowered external [Ca^{++}], presumably by reducing depletion of readily releasable ACh.

Adenosine antagonizes these pre- and postsynaptic processes in a dose-dependent fashion. The EC_{50} for its effect on the HAP was 10 μM with maximal inhibition of 35% at 100 μM adenosine. Adenosine was less efficacious at 1 mM than at 100 μM. Other evidence for specific receptor-mediated action of adenosine is the observation that the enzyme, adenosine deaminase (10 μg/ml), blocks the effect of adenosine (250 μM) on the HAP and on synaptic depression. Cyclic AMP (250 μM) also decreased the pre- and postsynaptic signals, but this action was blocked by adenosine deaminase. It is possible that cyclic AMP must be metabolized to adenosine in order to antagonize the Ca^{++}-dependent responses. Theophylline (1 mM), an adenosine receptor antagonist, reduced by 85% the inhibitory effect of adenosine (100 μM) on the Ca^{++} spike. Theophylline alone had no effect on the Ca^{++} spike or on the shoulder of the Na^+ spike, two signals thought to be a direct result of the inward Ca^{++} current. Dipyridamole, which specifically blocks the uptake of adenosine in other cells, doubled the inhibitory effect of 10 μM adenosine in 3 of 4 cells tested. The response to 100 μM adenosine was unaffected by dipyridamole. This observation is consistent with the idea that adenosine acts on extracellular adenosine receptors.

The effects of adenosine are similar to those observed with α-adrenergic agonists. However, the effects of adenosine on synaptic depression were not altered by the α-antagonist, phentolamine. In addition, the postsynaptic effects of adenosine (10 μM) were additive with noradrenaline (1 μM). These results suggest that discrete pre- and postsynaptic receptors exist for both adenosine and catecholamines to control Ca^{++} influx and thus neuronal excitability. Supported by NSF Grant BNS 79-12394.

94.25 BINDING AND INTERNALIZATION OF ^{125}I-TETANUS TOXIN IN CEREBRAL CELL CULTURES. E. Yavin*, Z. Yavin*, W. H. Habig*, M. C. Hardegree* and L. D. Kohn* (SPON: J. W. Daly). Section on Biochem. of Cell Regulation, Lab. of Biochem. Pharmacology, and Bacterial Toxin Branch, Division of Bacterial Products, Food and Drug Administration, NIH, Bethesda, MD 20205

Tetanus toxin is a potent neurotoxin which is presumed to affect transmitter release at the nerve terminals and cause a syndrome of dysinhibition. The putative receptor for toxin binding has been postulated to be a polysialoganglioside. Studies with cultured cells of neural origin have established that tetanus toxin can be used as a highly specific neuronal marker because of its binding properties and its ability to alter the electrophysiology of these cells. There is currently no evidence whether and how the toxin is taken up by nerve cells, and whether uptake is important in its pathologic action. This concern is relevant given the ability of the toxin to undergo retrograde axonal transport. In the present report we document receptor mediated internalization of tetanus toxin by neuronal cell preparations grown for long terms in the absence of serum.

Dissociated rat embryo cerebral cells maintained in monolayer cultures in the presence of a mixture of 6 hormones to permit expression of neuronal characteristics and prevent glia proliferation, incorporate ^{125}I-tetanus in a time and ligand concentration-dependent fashion and with properties of a receptor mediated process. Uptake occurs equally effectively at 0° as at 37°C. Uptake can be partially prevented by the presence of 3×10^{-7}M unlabeled toxin or by a goat antitoxin. It is also inhibited (50%) by 5 mM EDTA. Pulse-chase studies in which radiolabeled toxin is exposed to cells and then chased with unlabeled tetanus toxin (25 µg/ml) tetanus toxoid (50 µg/ml) or tetanus antitoxin indicate that after 2 h radioactivity released in the medium approaches similar steady state levels irrespective of the compound added. This finding suggests a receptor-mediated process for uptake. The nonchased radioactivity associated with the cells can be completely extracted by sequential treatment with 5 M acetic acid or 0.5 N NaOH. About 25 to 35% of the radioactivity associated with the cells after chase can be extracted by either 0.1% Nonidet P-40 or 0.1% Triton X100. The bioactivity of this fraction is entirely preserved. The localization of the detergent-resistant toxin has been monitored by indirect immunofluorescence technique. Preliminary evidence suggests that it is associated with the cytoskeleton proteins.

95.1 PRELIMINARY OBSERVATIONS ON THE RELEASE OF LHRH FROM ISOLATED STORAGE GRANULES UNDER IN VITRO CONDITIONS. G.H. Burrows, J.C. Porter, and A. Barnea. Depts. Ob-Gyn and Physiology, Univ. of Texas Southwestern Med. Sch., Dallas, TX 75235.

Luteinizing hormone releasing hormone (LHRH), released from the hypothalamus, is involved in the regulation of the secretion of gonadotropins from cells in the pituitary gland. Although it is known that LHRH is stored in granule-like particles in hypothalamic neurons, the mechanism of release of LHRH from these granules is not known. In order to elucidate this mechanism, we have investigated under in vitro conditions the release of LHRH from LHRH-containing granules. Granules containing LHRH were isolated from homogenates of hypothalami obtained from adult male rats, employing differential centrifugation. After incubation, LHRH released into the medium was separated from the LHRH-containing granules by means of affinity chromatography. The LHRH that was released and the LHRH in the granules were quantified by radioimmunoassay. The granules were incubated for 1 hr at 22°C in a phosphate-buffered (10 mM, pH 7.8) solution of 320 mM sucrose. Of the total LHRH recovered, 7% was present as free LHRH in the medium and 93% was present in the storage granules. In the study of the effect of KCl on the release of LHRH, storage granules were incubated in a buffered (pH 7.8) solution of KCl (40, 80, 120, or 160 mM) for 1 hr at 22°C. The osmotic strength of the medium was adjusted to approximately 330 mOsm with sucrose. Twice as much LHRH was released in the presence of 160 mM KCl as in the absence of KCl. This small KCl-induced release of LHRH was found to be linear within the range of 40 to 160 mM KCl. The effect of H^+ concentration on the release of LHRH in the presence of 160 mM KCl was investigated. Incubation of granules at pH 7.8 resulted in a release of 14% of the stored LHRH, whereas incubation at pH 6.2 resulted in a release of 30% of the stored LHRH. This increased release of LHRH appears to be a saturable function of the H^+ concentration with maximal release occurring near pH 6.2. The effect of Mg^{2+} on the release of LHRH from LHRH-containing storage granules was also investigated. The presence of Mg^{2+} in buffered KCl (150 mM) solution at pH 7.8 enhanced the release of LHRH in a dose-dependent manner; at 10 mM $MgCl_2$, 25% of the stored LHRH was released. It is concluded that KCl, H^+, or Mg^{2+} induced the release of LHRH from its isolated storage granules under in vitro conditions. Based on these results, we speculate that these ions participate in the release of LHRH in the intact hypothalamus.

95.2 MECHANISM OF MONOVALENT IONOPHORE INHIBITION OF ACETYLCHOLINESTERASE SECRETION. Henry Smilowitz, Dept. of Pharmacology, U. Conn. Health Center, Farmington, CT 06032.

The monovalent ionophores nigericin and monensin inhibit acetylcholinesterase (ACHE) secretion from cultured chick embryo myotubes at 10^{-8}M to 10^{-7}M concentrations (Smilowitz, Molec. Pharm. 16:202-214, 1979). Our recent evidence suggests that K^+ transport is principally involved in the inhibition. Valinomycin inhibits ACHE secretion; half maximal inhibition is obtained at 10^{-8}M. Hence valinomycin is more potent (>) than nigericin > monesin. Concentrations of ionophore which lead to maximal inhibition of ACHE secretion do not result in net potassium loss from the cells or cell membrane depolarization. Nigericin at 10^{-8}-10^{-7}M does not alter intracellular K^+ and Na^+ after three hours; yet μM nigericin decreases intracellular K^+ and increases intracellular Na^+ 40%. Substitution of culture medium NaCl by KCl inhibits ACHE secretion while substitution of culture medium NaCl by potassium isethionate does not. The use of H^3 TPP (tetraphenylphosphonium) supports the conclusion that membrane depolarization is not involved as does direct electrophysiologic measurement of the resting potential in the presence and absence of nigericin. Additionally: (1) A23187 cannot overcome the effects of the monovalent ionophores, and (2) both monensin and nigericin can inhibit ACHE secretion in the absence of external sodium.

Ouabain mimics the effects of the monovalent ionophores. ACHE secretion and Rb^{86} uptake are both inhibited by ouabain; half maximal inhibition is obtained at 2 x 10^{-6}M ouabain at 4.5 mM external potassium, 10^{-7}M ouabain at 1.5 mM external K^+ and 10^{-4}M ouabain at 40 mM external K^+. ACHE secretion is inhibited by low external KCl and NaCl as is the Na^+, K^+ ATPase (Rb^{86} uptake). ACHE also accumulates on perinuclear intracellular membrane vesicles after ouabain or monovalent ionophore treatment as viewed by histochemical staining. Hence the Na^+, K^+ ATPase is involved in ACHE secretion. Since vanadate has no effect on ACHE secretion at concentrations which inhibit Rb^{86} uptake, these two processes are not obligatorily coupled.

We propose that potassium transport by the monovalent ionophores directly affects a putative regulatory site on the Na^+, K^+ ATPase which affects ACHE secretion. The monovalent ionophores inhibit Na^+, K^+ ATPase activity (hydrolysis of γ-P^{32} ATP) of aged red cell ghosts by 50% at .05-1.0μM levels. Rb^{86} uptake of cultured chick myotubes is twofold more sensitive to ouabain in the presence of 0.1μM nigericin. Hence we propose that ouabain affects both the ATP hydrolysis and the putative regulatory component of the Na^+, K^+ ATPase; monovalent ionophores primarily affect the regulatory component and vanadate inhibits ATP hydrolysis without an effect on the regulatory component. (NIH NS13860)

95.3 CONTRACTILE PROTEINS IN CHROMAFFIN CELLS IN CULTURE. D. AUNIS* (SPON : J. BABITCH). Ctr. Neurochem., U-44 Unit, F-67085 Strasbourg Cedex (France).

Isolated bovine chromaffin cells were plated on collagen-coated glass slides. After 2-4 hr, chromaffin cells started to attach and 12 hr later 50 % of the attached cells adopted an elongated bipolar shape. 24 hr later, up to five neuritic-like expansions could be observed on each individual cell. Cells cultured for more than 72 hr formed fine, colchicine-sensitive, neuritic extensions with numerous varicosities along their length and terminal growth cones. Anti-α-actinin, anti-actin and anti-myosin antibodies, together with anti-dopamine-β-hydroxylase (DBH) antibody, were used to localize homologous antigens by indirect immunofluorescence in parallel cultured of chromaffin cells. Staining with antiDBH antiserum showed a bright fluorescence, with a punctate distribution. Neurite-like elongations, varicosities and terminals were fluorescent. Labelling with anti-myosin antiserum was diffuse in cytoplasm and no fibrillar arrangement could be observed ; myosin was detectable in neurite-like elongations. Labelling with anti-actin displayed a punctate distribution : a fluorescent, granular pattern was visible in the cytoplasm and in neurite-like elongations. Faint, thin fibrillar structures, particularly in neurite-like expansions were also visible. Staining with anti-α-actinin was found in a punctate, fluorescent pattern ; fluorescence was detectable in neurite-like outgrowths and terminals where it was found to accumulate.

Chromaffin granules, the catecholamine-storing organelles, were isolated from intact bovine adrenal glands and the limiting membrane was isolated and solubilized with 2 % sodium docecylsulphate. Solubilized polypeptides were submitted to 4-30 % polyacrylamide gel electrophoresis under limiting pore size conditions. 37 bands with M.W. > 27,000 could be identified ; an actin-like and an α-actinin-like components (comprising, respectively, 0.5-1.5 % and 4 % of recovered proteins) were found to comigrate with actin and α-actinin. The α-actinin-like component was extracted from the granule membrane with low ionic strength buffers used to solubilize α-actinin from myofibril Z-lines and partially purified by chromatography on DEAE-cellulose. This component was enriched 3-fold and found to cross-react with anti-α-actinin antibody. External lactoperoxidase-coupled iodination of intact granules resulted in incorporation of radioactivity into the α-actinin-like component, an indication that this polypeptide is facing the cytoplasmic side of the granule.

These data show that the contractile machinery is present in chromaffin cells and its close relation with secretory vesicles suggests the contractile apparatus may be involved in granule transport and/or the release of stored material.

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95.4 ADAPTIVE REGULATION OF PROLINE TRANSPORT IN MUSCLE CELLS. W.J. Logan, A. Klip*, E. Gagalang*. Neuromuscular Research Laboratory, The Hospital for Sick Children, University of Toronto, Toronto, Ontario. M5G 1X8.

L-proline (Pro) is accumulated by L-6 rat muscle cells in culture by a sodium dependent process. In other cell lines the transport of Pro and several other amino acids which utilize the A system adapt to substrate deprivation by increasing transport activity. In the present study we demonstrate the adaptive regulation of Pro uptake by L-6 muscle cells and further investigate its mechanism.

L-6 cells were incubated in a buffered solution with or without Pro (10 mM). Initial rates of pro uptake were then determined. Cells deprived of Pro for different periods of time showed a steady increase in initial velocity of uptake. After 6 to 7 h the maximum stimulation (4 to 6 fold) was attained. Under these conditions Vmax was 1.38 fmoles/cell/min and Km was 0.38 mM. This represents a 2 to 3 fold increase in Vmax and a 2 fold decrease in Km relative to basal uptake. Uptake of tryptophan was not enhanced by either tryptophan or Pro deprivation.

The stimulation of Pro uptake observed after 6 h of Pro deprivation could be reversed by a further 30 min incubation in 10 mM pro prior to uptake determination. This decrease in transport coincided with the build-up of free Pro concentration inside the cells. The stimulation was also reversed by loading the cells with other amino acids transported by the A system but not by those which are preferentially transported by other transport systems.

The decreased uptake rate in Pro loaded cells cannot be explained solely by the possible efflux of the accumulated amino acid, which could in turn dilute the external radiolabeled Pro during the uptake assay. This event would result in an apparent increase in Km, but Vmax should not be affected. The observed change in Vmax induced by Pro can be explained by the following mechanisms: (a) Decreased translocation rate (e.g. as a result of transinhibition). (b) A change in the number of transport sites. These possibilities are being tested by means of chemical and kinetic approaches. Differences in sensitivity to inhibition by SH-group reagents and in pH dependence suggest that the basal and stimulated transport may be mediated by two different populations of transport entities.

(supported by Muscular Dystrophy Association of Canada)

95.5 Na^+,K^+-STIMULATED ADENOSINETRIPHOSPHATASE IN THE CNS OF THE HAWK MOTH, MANDUCA SEXTA. A. L. Rubin*, A. F. Clark*, and W. L. Stahl. Depts. of Physiology, Biophysics, and Medicine, Univ. of Washington Sch. of Med., and the Neurochemistry Laboratory, VA Med. Ctr., Seattle, WA 98108.

Hemolymph from certain phytophagous insects has a low Na^+/K^+ ratio, and it has been postulated that for the nervous system to be functional, hemolymph Na^+ must be concentrated five- to ten-fold. To better understand the role of the Na^+,K^+-ATPase in insect nervous system homeostasis of Na^+ and K^+, we have studied the nerve cord and brain of M. sexta. Ventral nerve cords from immediately pre-emergent pupae were homogenized, briefly sonicated, and centrifuged to yield microsomes. Beef brain microsomes were used for comparative studies. The mammalian and insect Na^+,K^+-ATPases had similar pH optima and K_m values for Mg^{2+} and ATP. However, in M. sexta much higher levels of K^+ are required for full enzymatic activity. In the moth, a Na^+/K^+ ratio of 0.6 to 1.7 supported optimum enzymatic activity, whereas in beef brain optimum activity occurred with ratios between 1.3 and 10.8. Scatchard plots of ^{3}H-ouabain binding to nervous tissue in both species also yielded markedly biphasic curves under certain conditions. This different ion sensitivity of the Na^+,K^+-ATPase in M. sexta may reflect an adaptation for the high K^+ content in this insect's hemolymph. Additional studies demonstrated that inhibition of the M. sexta and mammalian Na^+,K^+-ATPase by cardiotonic steroids is complex and apparently biphasic (each prep. had I_{50} values of 10^{-6}M and 10^{-4}M with strophanthidin). Significant differences in sensitivity to K^+ and ^{3}H-ouabain binding to the enzyme were also noted. Finally, the enzyme was labelled with ^{32}P from AT^{32}P and was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Two phosphorylated large subunits of the enzyme were observed in both the M. sexta and mammalian Na^+,K^+-ATPase. These results are consistent with the presence of two distinct Na^+,K^+-ATPases in the two preparations studied. The relative simplicity of the moth CNS compared to the mammalian CNS may provide a good model for direct study of the localization and role of these different Na^+,K^+-ATPases.

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95.6 EFFECT OF DIETHYLDITHIOCARBAMATE ON BRAIN UPTAKE OF CADMIUM. L. Cantilena*, G. Irwin,* C. Klaassen* and S. Preskorn (SPON: E. Othmer). Depts. of Pharmacology and Psychiatry, University of Kansas Medical Center, Kansas City, KS 66103.

The element, cadmium (Cd) is largely excluded from the central nervous system by the blood:brain barrier (BBB). In a study of chelating agents to treat Cd poisoning, Cd (1 mg/kg) was administered intravenously 24 hours prior to measuring tissue concentration; the chelators were given intraperitoneally (i.p.) within a few minutes of the Cd. DDC, the metabolic product of disulfiram, produced a twenty-fold increase in the brain concentration of Cd while all other chelating agents tested decreased or had no effect on brain Cd concentrations. The effect of DDC on brain uptake of Cd following a single cerebral transit was then tested. DDC (10 mg/kg i.p.) given thirty minutes prior to intracarotid Cd administration caused a 6-fold increase in the single pass extraction of Cd into the brain.

Two mechanisms could explain this effect. First, DDC blocks norepinephrine synthesis by inhibiting dopamine-beta-hydroxylase. Since the central adrenergic system may act to modulate BBB (Preskorn et al., JPET, 1980), DDC might increase brain uptake of Cd by altering cerebral capillary permeability (CCP) or cerebral blood flow (CBF). Second, DDC might chelate with Cd to form a product which is more permeable across BBB.

To distinguish between these two mechanisms, a series of studies have been undertaken. The effect of this i.p. dose of DDC on CCP, ^{14}C and CBF was assessed. CBF was determined from the ratio of ^{14}C-butanol taken up by the brain to the ^{14}C-butanol in an arterial blood sample withdrawn at a constant rate following intravenous injection of a tracer bolus. Cerebral capillary permeability was determined as E_w (the extraction fraction of water) by the double diffusion tracer technique (Preskorn and Hartman, Biol. Psychiat., 1979). DDC did not alter either parameter, suggesting that the second mechanism--the formation of a more permeable product--accounts for the increase in brain uptake of Cd. Supported by USPHS grants GM 15956, ES 01142 and MH 27201.

96.1

Withdrawn by Author

96.2 QUANTITATIVE MEASUREMENT OF LOCAL METABOLIC RATE FOR GLUCOSE UTILIZING TRITIATED 2-DEOXYGLUCOSE. G. M. Alexander*.R. J. Schwartzman, R. D. Bell*,J. Yu and A. Renthal*. Lab. of Neurology, The University of Texas Health Science Center, San Antonio, Texas 78284.

The ^{14}C-deoxyglucose (2DG) technique has been widely utilized for the quantitative measurement of cerebral metabolism (ℓCMRG) in animals. This technique as presently used is limited by the energy of the ^{14}C B-particles, which can travel relatively great distances in tissue. This results in limited autoradiographic resolution and in computed ^{14}C concentrations which are a function of tissue section thickness. ^{3}H-2DG has less energetic B-particles; hence, the autoradiographs have better resolution and the optical densities are independent of tissue thickness for thicknesses greater than 5μ. We have recently developed a method for the quantitation of ℓCMRG in the rat using ^{3}H-2DG and a newly-developed ultrasensitive X-ray film. Autoradiographic tissue standards were prepared by injecting 8 rats with ^{3}H-antipyrine so that brain tissue concentration varied between 900 and 15,000 nci/g.

The antipyrine brains were cut in half, along the sagital plane. One half was cut into 20μ sections and used as tritium tissue standards; the other half was assayed for its tritium concentration in nci per gram of brain.

Four male wistar rats 250-350 g were used in this experiment. They were fasted for 16 hours; the femoral vein and artery were cannulated under light barbiturate anesthesia. The rats were allowed 24 hours to recover from the anesthesia, while the catheters were kept patent by slow continuous infusion of heparinized saline (1 ml/24 hr).

The animals were injected with a bolus of ^{3}H-2DG 200μci/g. Arterial blood samples were obtained, the animals sacrificed, and the brains frozen, sectioned and plated, along with the antipyrine tissue standards, for 20 days on LKB Ultrofilm. Tissue ^{3}H concentration was obtained autoradiographically; computation of the ℓCMRG gave values for the rat which were similar to those reported by Sokoloff et al, (J. Neurochem. 28:897-917, 1977) and Hawkins et al. (Stroke 10:690-703, 1979). The ℓCMRG from brain structures of adjacent sections showed no variation due to inadequate microtome precision. The resolution of discrete nuclei and particularly of fiber tracts is greatly enhanced, as compared with ^{14}C-2DG or ^{14}C glucose.

96.3 An autoradiographic analysis of ^{3}H-2-D-deoxyglucose uptake in the hippocampal region of the rat. J.P. Lund, J. Courville and J.J. Miller. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec, H3C 3J7.

Two series of experiments were carried out to examine a) the effects of various drug treatments on the uptake of deoxyglucose in the hippocampal region and b) to compare the relative distribution of radioactivity over cell bodies and neuropile.
a) 5 male rats (180-200 gm) were divided into 5 groups, one control and 4 others which received:
(1) chlorpromazine (12.5 mg I.M.); (2) ketamine (35.0 mg I.M.); (3) urethane (1.8 gm I.P.); (4) eserine (0.35 mg I.P.).
Ten minutes after injection of the drug, 1 mCi of ^{3}H-2-D-deoxyglucose was given I.P. and animals were killed by decapitation 30 min later. The whole brain was quickly dissected free and frozen in 2-methylbutane kept at -40°C in liquid nitrogen. Sections were cut at -18°C, dried and placed in contact with tritium-sensitive film for 3 weeks. Ketamine was found to increase deoxyglucose uptake above control levels in the molecular layer of the dorsal and ventral CA1 regions of the hippocampus and the ventral anterior nucleus of the thalamus (VA). Urethane reduced it markedly, while chlorpromazine and eserine had no effect. In the hippocampus, the labeling was heaviest in a band which appeared to cover the distal portion of the CA1 dendritic tree. The dendritic trees of the dentate granule cells were also densely labeled.
b) Blocks of tissue including the hippocampus and VA were removed from control and ketamine treated rats and frozen at -120°C in liquid nitrogen, cut in the dark at -30°C and placed in contact for 1 week with slides previously coated with NTB2 emulsion. Grain counts were carried out at consecutive positions across sections of the hippocampus and dentate gyrus and analyses of variance were used to evaluate the distribution of grains across the section. In the dorsal hippocampus, counts were approximately equal in strata oriens, pyramidale and radiatum, but were significantly higher in stratum lacanosum-moleculare. Ventrally, the heavily labeled band was wider, covering the distal half of the dendritic trees. Within stratum lacanosum-moleculare grains were equally numerous over neuropile and scattered cell bodies. Counts were higher over the molecular layer of the dentate granule cells than over their perikarya. In VA, the regions of highest grain density were also in the neuropile.

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97.1 RESPONSE PATTERNING, T-MAZE ALTERNATION AND SEPTO-HIPPOCAMPAL CIRCUITRY. G.N.O. Brito and G. J. Thomas. Center for Brain Research, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Four experiments investigated the ability of rats to discriminate a brain-built "cognition" of spatial (T-maze alternation) and temporal ("patterning") events as affected by small septo-hippocampal lesions inflicted before (acquisition) and after (retention) learning. In the T-maze hungry rats received 13T/day (12 opportunities to alternate) with a brief ITI under conditions of contingent reinforcement for alternating. Reinforcement consisted of about 10-sec access to wet mash introduced into the goal boxes after the rat had entered. In the runway rats also received 13T/day with a brief ITI, but they received similar reinforcement only on odd numbered trials. Under these conditions normal rats rapidly learn spatial alternation in the T-maze in few sessions, and in the runway normal rats learn to "pattern," i.e., to alternate running fast and slow. After behavioral testing, the lesions were evaluated histologically.

Small electrolytic lesions in posterodorsal septal area interfered significantly with acquisition of spatial alternation in a T-maze when the lesions were inflicted prior to learning. When the lesions were inflicted after learning and the animals were tested postoperatively (retention), they dropped to chance levels, but they recovered to normal levels within 9 sessions. The effects on temporal alternation in the runway were similar. Animals with lesions inflicted prior to learning acquired speedy running as quickly as controls, but they did not learn to pattern as well. When the rats had learned prior to surgery (retention) they dropped to chance levels of temporal alternation, i.e., they did not pattern, but by 15 training sessions they were patterning again, but not as well as controls.

The data suggest that interference with septo-hippocampal circuitry (some extrinsic afferent and efferent connections of the hippocampus) temporarily reduces the ability of rats to discriminate a brain-built cognition of where and when they last received reinforcement, a capability presumed to depend on "working memory."

97.2 REDUCTION OF THE SPATIAL REVERSAL DEFICIT IN MICE WITH SEPTAL LESIONS BY DISTRIBUTION OF TRIALS. C. R. Goodlett*, R. G. Burright* and P. J. Donovick. Dept. of Psychology and Center for Neurobehavioral Sciences, SUNY Binghamton, Binghamton, NY 13901.

The deficits in learning and performance following destruction of the septum have been shown to depend on task-specific parameters. For example, 24 hour spacing of trials eliminates the typical passive avoidance deficit of rats with septal lesions, indicating that deficits in response inhibition may result from disruption of temporal integration processes. Since spatial reversal is also known to reveal behavioral deficits in animals with septal damage, a water maze was used to assess the effect of distribution of trials on reversal performance.

Adult, male Binghamton-Heterogeneous mice were given large, bilateral septal lesions or control surgery, and were tested for position preferences in the water maze on the ninth post-operative day. Each surgical group was then randomly sub-divided into massed practice and distributed practice groups, and spatial discrimination to their non-preferred side was begun the next day. The mice in the massed practice groups were given 5 trials per day, with a 30 second inter-trial interval, while the distributed practice groups were given only 1 trial per day. A correction procedure was employed, and after reaching a criterion of 9 correct choices over 10 consecutive trials on the original spatial discrimination, the mice were reversed under the same training conditions to the 9/10 criterion.

There was no effect of surgery or training procedure on acquisition of the original spatial discrimination. For reversal, mice with septal lesions exhibited deficits only in the massed practice procedure. Control mice had similar trials to criterion (TTC) scores on reversal learning for massed and distributed practice (medians 26.5 and 24, respectively). The lesioned mice had significantly elevated TTC scores in the massed regimen (median 42), but were similar to controls when the trials were distributed (median 23). This pattern was also seen for initial errors to criterion, with controls having equivalent scores for massed and distributed practice, and lesioned mice having elevated initial errors with massed practice but not with distributed practice. In general, the distribution of trials dramatically improved reversal performance of the brain-damaged mice for measures taken across trials, but it did not affect their tendency to make repeated errors on the early trials during reversal training.

The results support the contention that septal lesions disrupt temporal integration processes. Factors contributing to the improved performance of brain-damaged animals with temporal spacing of trials will be discussed.

97.3 HOME ENVIRONMENTAL STIMULI ATTENUATE PERFORMANCE DEFICITS RESULTING FROM SEPTAL BRAIN DAMAGE. Gregory J. Smith*and Charles R. Goodlett* (SPON: Norman E. Spear). Department of Psychology, Case Western Reserve University, Cleveland, Ohio 44106 and State University of New York at Binghamton, Binghamton, New York, 13901

Previous reports have indicated that learning and performance deficits common to immature organisms may be attenuated if training occurs within the context of stimuli associated with the home nest environment.This effect,however,appears to disappear sometime between the ages of 20-30 d. postpartum in rats. The present series of experiments examined whether performance deficits following septal damage in adult rats (60-100d) might also be attenuated by the presence of home environmental stimuli during training. This hypothesis stems from the growing body of data suggesting that infantile behavior patterns may reappear following damage to the hippocampus or hypothalamus and neural degeneration accompanying normal aging.

The first experiment examined the spontaneous alternation behavior of septal lesioned female Sprague-Dawley rats and sham-operates in the presence of 7-day vintage litter shavings removed immediately prior from their nest cage (LS) or unsoiled,clean wood shavings (CS). The shavings were spread evenly beneath the wire mesh floor of an adult-size T maze during training. To assure a blind experimenter condition, another worker handled and gentled both lesioned and control groups for 7 days prior to training.

The results of Experiment 1 indicated that adult rats given septal lesions and trained in the presence of LS alternated in the T maze reliably more than lesioned rats trained in the presence of CS. Furthermore,septal rats trained in the presence of LS did not differ from controls trained in the presence of either LS or CS treatments. The presence or absence of litter shavings had no affect on the normal alternation behavior observed in sham control rats.

A second experiment examined the effects of home environmental stimuli on two types of passive-shock-avoidance behavior in septal lesioned adult rats.

These data suggest that performance deficits accompanying septal damage may be due to the stress of the test condition which can be attenuated by providing familiar home environmental stimuli in the test environment.

97.4 STRAIN DIFFERENCES IN THE BEHAVIORAL EFFECTS OF SEPTAL LESIONS IN MICE. R. J. Fanelli, P. J. Donovick, R. G. Burright* and W. J. Engellenner*. Dept. of Psych., Center for Neurobehavioral Sciences, SUNY Binghamton, Binghamton, NY 13901.

Over the past several years we have examined the behavioral effects of septal lesions in different strains of mice. We started with the assumption that destruction of the septal region enhances acquisition of two-way active avoidance tasks and impairs performance on passive avoidance tasks. We found that both of these brain damage effects are dependent upon the genotype of the animal. In attempts to explain these interactions between genotype and lesion effects, neurochemical analyses were performed, as well as batteries of tests designed to measure motor behavior and sensory reactivity. While genotype, lesion and interaction effects were present, they were not simply related across measures. The present investigations were designed to examine whether post-surgery and motivational reactivity might help clarify our previous findings.

C57BL/6J, RF/J or Binghamton Heterogeneous (HET) mice received either control operations or bilateral electrolytic lesions of the septal region. All animals were handled daily for a seven day recovery period during which reactivity (to handling) measures were taken. Then half of the animals were placed on a 23 1/2 hr water deprivation schedule and trained for acquisition and reversal of a spatial discrimination in a dry T-maze reinforced by water. The remaining animals were trained for acquisition and reversal of a spatial discrimination in a water maze placed in cold water. Following training, all animals were examined for shock sensitivity.

The results from the dry T-maze task further indicated the limitation of utilizing a single strain when examining the behavioral effects of brain damage. While the HET lesioned mice showed a clear decrement in performance relative to HET controls on acquisition of the discrimination, there was no difference between the lesion and control mice of the C57 strain on this measure, and in the RF strain, the lesioned animals actually performed better. In contrast, the three strains of mice performed relatively alike on the water maze task. There was no major differences on the acquisition of the spatial discrimination among any of the groups in the water maze but there was a lesion decrement on reversal of the discrimination. The results of the handling reactivity and shock reactivity measurements indicate significant strain by surgery interactions and seem to provide a possible explanation for some of the above findings. Thus, the need for a multifaceted approach to the study of the behavioral effects of brain damage, using a variety of tasks and genotypes, is indicated.

97.5 PARADOXICALLY ENHANCED LEARNING IN SEPTAL RATS: EFFECT OF NUMBER OF NONREINFORCED TONE PREEXPOSURES. H.A. Burton* and A.W. Toga (SPON: C. Tamminga). Neuroscience Program, Maryland Psychiatric Research Center, P.O. Box 3235, Baltimore, MD 21228, Neurology Department, Washington University, St. Louis, MO 63110.

Discrimination performance of rats with bilateral destruction of the lateral septal nuclei was enhanced by nonreinforced tone preexposure. Nonreinforced preexposure to a stimulus normally makes more difficult subsequent use of that stimulus for learning. The effect of this preexposure on learning is termed latent inhibition and was demonstrated in sham operated animals. Prior to surgery all animals were trained by appetitive conditioning to press a bar upon illumination of a cue (S^+) but not to the house light. Neither was lit during the intertrial intervals. The criterion for entering the second phase of the experiment was 80% response to S^+. Upon reaching this level subjects were divided into 8 groups matched for response rate. Four of these groups were assigned to the brain lesion and four to the sham condition. Bilateral ablation of the lateral septum was accomplished stereotactically. The sham controls underwent similar surgery except that the electrodes were lowered to just above the septum. Each operate and each sham group was assigned to a preexposure phase in which they received 0, 90, 180, or 270 presentations of a 1000 Hz tone within 60 minutes. The operate and sham groups receiving 0 preexposures sat in an identical training box without tone for 1 hour. Immediately following preexposure all animals were subjected to the first of four daily successive discrimination training sessions. S^+ now consisted of a simultaneous 5 sec. exposure to the tone, house and cue lights, and S^- the latter two alone. Latent inhibition or a delay in the initial learning following preexposure was less pronounced in operates. After testing the animals were killed by formolsaline infusion and the brains were removed for verification. The septal operate group receiving 0 preexposures exhibited the poorest discrimination performance as was evidenced by a high rate of S^- responding. A response perseveration model seems appropriate. Sham discrimination performance decreased with greater numbers of preexposures (latent inhibition effect), however septal discrimination performance was paradoxically enhanced. The septal operate group which received 270 preexposures exhibited discrimination performance significantly superior to all other groups. One may conclude that septal lesions have a disruptive effect upon the occurrence of latent inhibition, possibly by the release of behavior from those mechanisms which normally link it to appropriate stimuli, and that the lesion-preexposure compound appears to result in enhanced stimulus saliency such that successive discrimination performance can be controlled, and even made superior to that of controls.

97.6 EFFECT OF EARLY BRAIN DAMAGE TO HIPPOCAMPAL CONNECTIONS ON ACQUISITION OF A SPATIAL WORKING MEMORY TASK IN THE RAT. John A. Walker, Lydia E. Skinner*, and Eva S. Hersh*. Department of Psychology, Reed College, Portland, OR 97202.

In the adult rat, damage to the hippocampus or its extrinsic connections results in a severe and apparently permanent loss of ability to solve tasks requiring working memory, such as spatial alternation tasks. Animals with damage induced in adulthood perform at levels expected by chance, with no improvement with extensive testing for periods up to at least several months after surgery. However, the hippocampal circuitry is capable of remarkable plasticity. Moreover, in humans early brain damage results in little loss of certain kinds of abilities, while equivalent damage in the adult results in a profound and permanent loss. The present experiment investigated whether brain damage in young rats resulted in a deficit in working memory equivalent to that seen after adult brain damage, or whether there was a sparing of function as a consequence of the age at which the damage was induced.

The task was a spatial alternation test conducted on an elevated two-arm maze. At the beginning of each test, both arms contained a single 190 mg Noyes pellet in an opaque food cup at the end of the arm. After the rat had chosen one arm, only the choice of the other arm was rewarded (a correct choice). If the rat instead returned to the previously chosen arm (an incorrect choice), the test continued until the rat either chose the remaining arm, or had rechosen the first arm on six consecutive choices.

Rats were given bilateral fimbria-fornix lesions or control operations at either 7, 45, or 90 days of age. Rats were tested at about 90-120 days of age. Rats with control operations at any age learned the task to a criterion of 19/20 consecutive correct tests within an average of about 30 tests. Rats given fimbria-fornix lesions at 90 days showed the expected pattern of performance: their performance remained at chance levels up to the end of testing (150 tests, 30 test days). The pattern of errors made by the 90 day rats indicated no evidence of perseveration within a test: the rat was as likely to return to the already chosen arm as to choose the unchosen arm. In rats given fimbria-fornix lesions at 7 or 45 days, some but not all of the rats were able to learn the task, though not as efficiently as controls. In part, the ability of a rat to meet the criterion was a function of the extent of brain damage, and also a function of the age at surgery. These results suggest that, under certain circumstances, sparing of function after hippocampal system damage is possible. The mechanisms which underly this sparing are not yet known.

97.7 RATS WITH FIMBRIA-FORNIX LESIONS CAN USE A COGNITIVE MAPPING STRATEGY. J.T. Becker & D.S. Olton, Dept. Psych., The Johns Hopkins University, Baltimore, Maryland, U.S.A.

In order to determine the role of the hippocampal system in cognitive mapping, rats with lesions in the fimbria-fornix were trained to discriminate between two three-dimensional objects based on the location of these objects in the test environment. The rats were then given a series of transfer tests to determine: 1) the extent to which the rats used cognitive mapping strategies, and 2) the nature of the stimuli which controlled the rats' choice behavior.

The location discrimination was designed so that a rat could not predict the correct response based on either the stimulus characteristics of the objects themselves, or the location of the objects relative to himself as he entered the test arena. Only the location of the objects in the test environment accurately predicted the correct object.

The rats in the operated control group learned the discrimination in a mean of 50 trials, making 14 errors prior to reaching criterion performance. In contrast, the rats with fimbria-fornix lesions required 81 trials, and made 30 errors to criterion; both values significantly more than those of the rats in the control group.

The rats performed a series of transfer tests, called new door transfer tests, which required the rats to approach the goal locations from a new starting location, and travelling on a new path. The ability to perform accurately on the transfer trials is a critical test of the ability of the rat to use a cognitive mapping strategy. All of the rats, including those with lesions in the fimbria-fornix performed accurately on these transfer tests indicating that they were able to use a cognitive mapping strategy

A second series of transfer tests, called stimulus control transfer tests, was designed to determine the nature of the stimuli which controlled the rats' behavior. The rats with lesions in the fimbria-fornix differed from those in the operated control group in that these rats rarely (1 of 11) used the stimuli outside the arena to guide their choices.

These data demonstrate that although the rats with lesions in the fimbria-fornix were able to use a cognitive mapping strategy, the nevertheless used different cues to identify the correct location than did rats in the operated control group. These data are discussed in terms of the role of the hippocampal system in spatial information processing.

97.8 DELAYED RESPONSE (DR) PERFORMANCE OF FERRETS WITH FRONTAL LESIONS. Ausma Rabe, Yvette Zatz*, Raef Haddad and Donna Snow*. New York State Institute for Basic Research in Mental Retardation, 1050 Forest Hill Road, Staten Island, NY 10314.

Adult male ferrets with bilateral aspiration lesions in the dorsolateral frontal area (n=6) and sham operated controls (n=6) were trained in a Nencki box to select one of two positions signalled by a tone. They were food deprived and learned for a food reward (canned cat food), at the rate of 10 to 20 spaced noncorrection trials a day, five days a week. (1) Discrimination or predelay training: the ferrets were trained to choose the position at which the tone was sounded while the tone was on. The criterion was 45 correct responses in 50 consecutive trials (90%). (2) Delay training: the ferrets were permitted to make their choice following a 0,3,9 and 27 second delay after the presentation of the tone. The delays were randomized within the daily trials. Seventy trials were given with each delay interval. (3) Delay with distraction: an opaque cover was put over the restraining cage during the delay periods. Thirty six trials were given with each the 9 and 27 second delay.

The frontal ferrets mastered the initial discrimination at the same rate as the controls. The frontals were significantly poorer than controls at 0 delay (76% vs 85%, $p < .01$), but were not significantly impaired at 3,9, and 27 second delays; their performance did not deteriorate with the longest delay (76%, 79%, and 77%), while that of the controls dropped slightly (82%, 82%, and 77%). When distraction was introduced during the 9 and 27 second delays the performance of the controls, as compared to their own performance at these delays without distraction, showed only a small and statistically insignificant drop from 78% to 76%, while the performance of the frontal ferrets deteriorated appreciably from 81% to 62% ($p < .001$). The performance of the frontals was also significantly worse when compared to that of the controls (76% vs 62%, $p < .02$).

97.9 EFFECTS OF US PARAMETERS ON CLASSICAL CONDITIONING OF CAT HIND LEG FLEXION. A. G. Romano*, J. E. Steinmetz* and M. M. Patterson (SPON: M. P. Charlton). Dept. of Psych., Ohio Univ., Athens, OH 45701.

Using a new restraint system, we recently found rapid and stable conditioning of cat nictitating membrane and hind leg flexion responses which was relatively free of nonassociative responding. Brain correlates of cat nictitating membrane conditioning have already been examined. A comparison of brain correlates of conditioning for the two different response systems will be undertaken after identifying those parameters which produce similar conditioning performances in both response systems. Thus, two US parameters, intensity and duration, were manipulated to determine the minimal shock parameters necessary for reliable hind leg conditioning. Seventy-two cats were randomly distributed among nine experimental groups in which US intensity was either 2, 3, or 4 mA and US duration was 25, 50 or 100 msec. The CS was a 77 dB, 1000 Hz tone. The CS-US interval was 300 msec with the CS overlapping and coterminating with the US. The intertrial interval (ITI) was randomly varied among 50, 60, or 70 sec and averaged 60 sec. Nonassociative responding was assessed in eight additional cats who received unpaired presentations of the CS and the most severe US (4 mA, 100 msec), at an average 30 sec ITI. Each cat was placed in a plexiglas restraining device that allowed the hind legs to hang down. Conditioning took place in a converted, ventilated refrigerator shell. Two wound clips affixed to the right hind leg delivered the US while a third wound clip allowed the leg to be mechanically coupled to a potentiometer. All leg movements were transduced to dc signals and recorded on a polygraph. Time intervals and stimulus control were programmed by standard behavioral equipment. One day of adaptation to restraint, seven days of acquisition and two days of extinction were run. Acquisition for the experimental groups consisted of 45 CS-US paired trials and six CS-alone test trials while the control group received 51 CS trials randomly intermingled with 45 US trials. For all groups, no USs were administered during extinction.

Over the seven days of acquisition, the control group evidenced no reliable increase in their low level of responding while the experimental animals demonstrated significant conditioning with the level of conditioning increasing with increasing US duration. Although a significant US intensity effect was not observed, the level of conditioning appeared to increase with increasing US intensity at the 100 msec US. In general, the minimal shock level necessary for reliable conditioning appeared to be dependent upon shock duration rather than intensity with the 50 msec US producing the most consistent results.

98.1 ALGORITHMS FOR THE CHARACTERIZATION OF PULSATILE HORMONE SECRETION
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The secretion of most anterior pituitary and many other hormones is episodic; for some hypothalamic peptides, such as LHRH, pulsatile secretion may be necessary to maintain agonist effects. The detailed cybernetics of pulsatile hormone levels have not been extensively studied, in part because objective criteria for identifying secretory pulses have been unavailable. We have developed algorithms for carrying out this process. There are three steps in the procedure: removing long-term baseline trends, such as diurnal rhythms, from the series of points; selecting secretory peaks in the residual series; and resolving each peak, if appropriate, into overlapping secretory episodes.

In the first step, we have employed a robust smoothing technique, using the method of Cleveland (J Am Stat Assoc 74: 829, 1979). This generates a smoothed series which omits peaks or trends with time constants less than 8-12 h; the characteristic smoothing time can be set freely. The smoothed series is subtracted from the original, and in the second step, their difference, the residual series, is examined for the presence of pulses. A robust estimate of the variance of the residual series is calculated, and points are rescaled in terms of variance units; if desired, the variance of the assay can be used as the criterion instead. Peaks are identified as individual sub-series elevated above the baseline, of duration t, all the points of which have magnitude at least G_t, where the G_t are cutoff criteria based upon the width of the peak. Thus the algorithm will select both narrow, high peaks, and broader peaks which may be lower. The user selects the G_t for each hormone based upon its half-life and other criteria. For example, for bovine growth hormone data collected at 15 min intervals, criteria of G_1=3.4 for peaks 1 point (15 min) wide, G_2=2.7 for 2-point peaks, G_3=1.7, G_4=1.33, and G_5= 0.55 have been used. Points which meet these criteria are identified as belonging to peaks and flagged. In order to assure that the smoothing process is not influenced by long runs of closely spaced peaks, these flagged points are then temporarily removed from the series, and the smoothing is repeated; the revised residuals are then re-examined. After these two steps are iterated until there are no further changes, each peak is examined in the third step, to determine whether it can be resolved into more than one overlapping peak. Finally, the process collects statistics on the average frequency and amplitude of the peaks in the series. This method appears to offer the flexibility to deal with a variety of kinds of episodic secretion. Computer programs to carry out this process have been developed.

98.2 ACCENTUATION OF THE DIURNAL PERIODICITY OF SLEEP AND ACTIVITY IN ADRENALECTOMIZED RATS BY CORTICOSTERONE REPLACEMENT.
D.J. Micco, Jr., Jerrold S. Meyer and Bruce S. McEwen. The Rockefeller University, New York NY 10021.

Glucocorticoid hormones have been reported to modify the temporal distribution of specific sleep sub-stages and activity. Previous work failed, however, to use the predominant glucocorticoid of the species being examined or else administered hormones by injections so as to produce fluctuating hormone levels. In the current study, we employed the predominant glucocorticoid for the rat, corticosterone (Cort), in s.c. pellet implants in order to produce constant serum levels of the hormone in previously adrenalectomized (ADX) rats. These implants produced plasma Cort levels comparable to normal resting afternoon levels (19 g%). Recording of sleep stages and activity was done automatically (Winson, Electroenceph. Clin. Neurophys. 41:179, 1976). Rats were maintained in an isolation chamber on a 12:12h light: dark cycle. In ADX subjects, the peak and trough of sleep and activity within the 12h day and 12h night were not pronounced although a definite 24h rhythm was observed. With Cort replacement therapy, a conspicuous enhancement of the amplitude of the daily rhythms in paradoxical sleep (PS) and activity occurred. In particular, Cort enhanced activity in the last 3h of dark and produced a greater change in PS during the first 3h of the lights-on period. The Cort effect on activity is reminiscent of the action of hippocampal ablation to increase activity during the last 3-4h of the dark phase (Iuvone and van Hartesveldt, Behav. Biol.19: 228, 1977). The results are consistent with our working hypothesis that the hippocampus, a primary cephalic target of glucocorticoids, may figure prominently in Cort effects on the brain. The notion of a net inhibition of the hippocampus by Cort is also consistent with previous work from our laboratory demonstrating a rather subtle modulating influence of glucocorticoids (in the same direction as the effects of hippocampal ablation) on behaviors believed to involve the hippocampus for normal expression (Micco, McEwen and Shein, J., Comp. Physiol. Psych. 93: 323, 1979, Micco and McEwen ibid. 94, in press, 1980). The current findings provide additional evidence for a modulatory, rather than determinant, role of glucocorticoids on neural activity and behavior. (Supported by grants from NSF GB43558 and NIH (NS07080). J.S.M. was a USPHS Postdoctoral Fellow (NS05040) and D.J.M. has received support from theAlfred P. Sloan Foundation.)

98.3 EFFECT OF SURGICAL OR PHOTOPERIODIC CASTRATION, TESTOSTERONE REPLACEMENT OR PINEALECTOMY ON MALE HAMSTER WHEELRUNNING RHYTHMS
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1) Hamsters which had spontaneously recovered gonadal function after prolonged exposure to constant darkness (DD) achieved by blinding were castrated or sham operated. Half the castrates were given testosterone in a silastic capsule. All animals were then given access to running wheels. 2) Spontaneously recovered blind hamsters with access to wheels received the following treatment sequentially: sham operation; castration; blank capsule; testosterone capsule. 3) Photosensitive hamsters were pinealectomized or sham-P_x and placed in DD for 6 wks, to induce gonadal regression in to the sham-P_x animals. Animals were then given free access to running wheels. 4) Photosensitive hamsters were castrated or sham-operated, then placed in DD with access to running wheels. Subsequently, the animals received serial treatment of castration or sham-operation, blank and testosterone capsules. 5) Photosensitive animals were pinealectomized or sham-P_x, then placed in DD with access to running wheels.

The results showed that photoperiodic or surgical castration of animals prior to wheel access induced large increases in the variation of wheelrunning onset, a decrease in the number of wheel revolutions or minutes of running per day, a decrease in the number of activity bouts longer than 60 min, an increase in the number of activity bouts less than 6 min in duration, an increase in the number of total activity bouts and an increase in length in the active phase of the circadian locomotor rhythm compared to results from groups of animals with mature testes or testosterone replacement. Pinealectomy prevented DD-induced gonadal atrophy and the associated behavioral changes seen in sham-pinealectomized DD-exposed animals, changes which are best explained by the loss of testicular androgens in the latter animals. When the endocrine manipulations were performed sequentially in animals with prior access to wheels the effects of castration or hormone therapy on the above measures were not as clean as when the manipulations occurred prior to wheel access. The data suggest that access to wheels may influence the behavioral response to hormones, with animals tending to persist in the pattern which existed at the time of manipulation. There was no evidence that testosterone affected the circadian period of wheelrunning. The data suggest that if testosterone affects the circadian system of male hamsters, it does so by tightening the constraints on the activity phase, perhaps by slight changes in the phases of multiple oscillators regulating the locomotor rhythm.

98.4 THE RELATIONSHIP BETWEEN CORTICOSTERONE AND AVOIDANCE LEARNING.
J. H. Peck, S. Girelli* and M. Cohen*. Dept. of Psychology, Ithaca College, Ithaca, N.Y. 14850.

Some years ago a relationship was suggested between shuttle box avoidance and adrenal steroids such that as avoidance learning increased, blood levels of corticosterone decreased (Coover, et.al. JCPP, 1973, 82, 170-174). This study is an attempt to determine if there is a causal relationship between the levels of corticosterone and avoidance learning.

Avoidance rats (A) received 10 trials/day with a 60 second ITI. Each trial consisted of a tone followed 5 sec. later by 1 ma of scrambled foot shock. For each A rat which was trained in the shuttle box, there were 4 controls: 1) A yoked (Y) rat which received the same shocks as the avoidance rat, but with no opportunity to escape or avoid. 2) An all-shock (AS) rat which received 5 sec. of shock on each trial. 3) A handled rat (H) which was placed in a strange cage. 4) A basal rat (B) which was left in its home cage. The A rats (and their controls) were sampled after day 1 or upon reaching a 50%, 90% or overtrained (OT, 90% for 5 consecutive days) criterion. Upon reaching criterion, the rats were immediately decapitated, trunk blood was collected and corticosterone was later measured using a competitive protein binding assay. The first replications were run at the trough of the adrenocortical rhythm.

The greatest problem with the study was the failure of the rats to reach criterion and the replications were terminated after 30 days of training. This produced a new group of rats, the no-criterion rats (NC). Of the rats who reached criterion (about 30% beyond day 1) there was no evidence of a decline in corticosterone as a function of learning. Additionally, there was no decline in the NC rats nor any differences between the A and Y rats. All the control groups had about the same corticosterone values as rats killed on day 1.

Another set of replications was done at the peak of the adrenocortical rhythm. In fact about 50% of the rats reached criterion at the peak and adrenocortical values declined as the rats reached stricter criterion levels. But NC rats also showed a significant decline and there were no differences between A and Y rats.

Because of the small number of rats reaching criterion, no causal inferences can be drawn from these studies. It appears, however, that the decline in adrenal steroids is not related to learning because of the similarities of the A, Y, and NC groups at both the peak and trough of the adrenal rhythm.

98.5 CYCLIC NUCLEOTIDE LEVELS IN THE PITUITARY, HYPOTHALAMUS, PINEAL AND CEREBELLUM OF FEMALE RATS DURING THE ESTRUS CYCLE. G. Rufus Sessions, G. Jean Kant, Robert H. Lenox and James L. Meyerhoff. Depts. of Microwave Rsch and Med. Neurosciences, Walter Reed Army Institute of Research, Washington D.C. 20012 and Dept. of Psychiatry, Univ. of Vermont College of Medicine, Burlington, VT 05405

Current research has suggested that adenosine 3'5' monophosphate (cyclic AMP) and guanosine 3'5' monophosphate (cyclic GMP) function as "second messengers" in the CNS, mediating the effects of neurotransmitters. In several studies involving drug administration or certain types of stress, we have remarked on the exceptional responsivity of the pituitary cyclic AMP system (Kant et al. Biochem. Pharmacol. 29, 369-373, 1980). Since neurotransmitter turnover has been shown to change dramatically during proestrus in the female rat, we hypothesized that hypothalamic and pituitary cyclic nucleotide levels might fluctuate during the estrus cycle in response to these neurotransmitter changes. Changes in pituitary cyclic nucleotide levels might then have functional significance as regards pituitary hormone output during the cycle.

Forty-five female rats were maintained on a 14-10 hr light-dark cycle. Vaginal smears were obtained daily and rated by 2 observers to determine stage of estrus. Rats were sacrificed by microwave irradiation on the morning and afternoon of proestrus, at night during estrus and diestrus and the morning or afternoon of metestrus, diestrus 1, and diestrus 2. Cyclic AMP and cyclic GMP were measured by radioimmunoassay in the anterior and posterior pituitary, the hypothalamus, pineal, and cerebellum (Lenox et al, Neuroendocrinology, in press).

Cyclic AMP and cyclic GMP levels did not vary in any region examined as a function of the stage of the estrus cycle. However, the time of day at which the animals were sacrificed affected levels of cyclic AMP in the hypothalamus and cerebellum and levels of cyclic GMP in the cerebellum. Levels of cyclic AMP were as follows (pmoles/mg wet weight $\pm$ SEM): cerebellum 10 AM (.463 $\pm$ .037), 5 PM (.664 $\pm$ .033), 11 PM (.677 $\pm$ .064); hypothalamus 10 AM (.615 $\pm$ .029), 5 PM (.793 $\pm$ .037), 11 PM (.896 $\pm$ .055). In contrast anterior pituitary cyclic AMP levels were similar regardless of time of sacrifice: 10 AM (1.027 $\pm$ .051), 5 PM (.983 $\pm$ .071), 11 PM (1.057 $\pm$ .057). Cerebellar cyclic GMP levels (pmoles/mg wet weight $\pm$ SEM) were: 10 AM (1.338 $\pm$ .173), 5 PM (1.662 $\pm$ .154), 11 PM (2.257 $\pm$ .274). The increased nocturnal levels of cerebellar cyclic GMP may be a result of increased nocturnal activity (Meyerhoff et al., Life Sci 24, 1125-1130, 1979).

Cyclic nucleotide levels in the brain of female rats appear to be more influenced by circadian rhythms than by the estrus cycle.

98.6 THE EFFECTS OF ACUTE AND CHRONIC OVARIECTOMY ON PITUITARY-ADRENAL FUNCTION ON PROESTRUS IN RATS. S. Baron* and F. R. Brush. Psychology Research Lab., Syracuse Univ., Syracuse, NY 13210.

Female Long-Evans hooded rats were subjected to bilateral ovariectomy, sham ovariectomy, or no surgical manipulation between 0900 and 1100 hours on diestrus-2. Twenty-four hours or 21 days later, all rats were exposed to a 2-minute ether stress between 0900 and 1100 hours on proestrus (as determined by controls). Blood samples were collected from the external jugular vein within 3 minutes of onset of ether for the determination of resting concentrations of plasma corticosterone and by decapitation at 15 minutes after the onset of ether for the determination of stress-induced concentrations of plasma corticosterone.

The normally elevated resting concentrations of plasma corticosterone seen in proestrus rats were decreased 24 hours and 21 days following ovariectomy. However, a reduction in the stress-induced concentrations of plasma corticosterone was only observed at 21 days following ovariectomy.

Because ovariectomy resulted in a decrease in resting concentrations of plasma corticosterone, a subsequent study was designed to determine whether ovariectomy results in the elimination of A.M.-P.M. differences on proestrus. Using procedures described above, it was found that while ovariectomy resulted in a decrease in resting concentrations of plasma corticosterone in both the morning (1000 hours) and evening (1730 hours) of proestrus , the evening concentrations were higher than those observed in the morning. Therefore, the diurnal excursion of plasma corticosterone, while reduced, was not abolished by ovariectomy.

It appears that the ovarian hormones are responsible for the elevated resting concentrations of plasma corticosterone seen in the morning and evening of proestrus. Furthermore, the ovarian hormones contribute to the elevation of plasma concentrations of corticosterone observed in response to stress on proestrus, although ovariectomy does not affect the adrenal response to stress immediately. Therefore, the effect of ovariectomy on the pituitary-adrenal axis can be seen as a function of time after the removal of ovarian hormones.

98.7 A RETINAL PROJECTION TO THE LATERAL HYPOTHALAMUS IN THE RAT. J.N. Riley, J.P. Card, and R.Y. Moore, Dept. Neurology, State Univ. New York, Stony Brook, NY 11794.

A retinohypothalamic pathway terminating in the suprachiasmatic nucleus (SCN) of the hypothalamus is a consistent feature of the mammalian visual system. Although the existence of this pathway to the hypothalamus had been suggested in earlier studies, this pathway was not established until the development of newer anatomical methods, such as the autoradiographic method and electron microscopy of degenerating terminals. However, each of these methods is limited in its capacity to trace axonal connections. The autoradiographic method defines the course of a pathway but terminals occurring along the course of a pathway may not be identified. Electron microscopy is limited in its usefulness in screening for terminal fields. Usually other methods must be used to define areas of probable axonal termination prior to ultrastructural study.

During the course of a study of the organization of the hypothalamus neurons with dendrites extending into the optic tract were observed in Golgi-Kopsch material. In addition to neurons in the SCN and anterior hypothalamic area (Silver and Brand, Am. J. Anat., 155, 1979), some neurons in the lateral hypothalamic area (LHA) had dendrites extending into the optic tract. We have supplemented these observations with electron microscopy of normal material and experimental material prepared following enucleation or intraocular injection of horseradish peroxidase (HRP).

The presence of dendrites of LHA neurons in the optic tract was confirmed by electron microscopy. Axon terminals making conventional synaptic complexes with dendritic profiles were found within the optic tract. Following enucleation, some of the preterminal axons and terminals in synaptic contact with dendrites in the optic tract demonstrated dark degeneration. Intraocular injection of HRP resulted in the labeling of preterminal axons and synaptic terminals in the optic tract. Both Gray Type I and Type II synapses were observed.

These observations indicate that some LHA neurons receive a direct retinal projection from terminals making synaptic contact with LHA neuron dendrites that extend into the optic tract. The functional significance of this additional retinohypothalamic pathway remains to be determined.

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98.8 RETINAL AND CNS INPUT TO THE SUPRACHIASMATIC NUCLEUS OF THE GOLDEN HAMSTER. A.J. Silverman and G.E. Pickard, Department of Anatomy, Columbia University, P&S, New York, NY 10032.

The temporal organization of hormonal secretions and behavior is the consequence of a neural timekeeping process. Numerous lesion studies have suggested that the suprachiasmatic nucleus (SCN) represents the endogenous clock responsible for maintaining hormonal and behavioral circadian rhythms. A direct retinal projection to the SCN photicly entrains these rhythms. To complete our understanding of the other neural inputs to the SCN a horseradish peroxidase (HRP) tracing study was undertaken.

HRP (30% solution in 0.05 M Tris, pH 7.6) was iontophoretically injected unilaterally into the SCN through 15-35 um (o.d.) micropipettes using positive current of 2uA for 2 min. After survival periods of 24-72 hrs the animals were perfused with 0.9% saline followed by 2.0% glutaraldehyde. During the saline perfusion both eyes were removed and the retinae were dissected free from the pigment epithelium. The retinae were fixed briefly in 1.5% glutaraldehyde; the brains were fixed overnight. HRP in the retinae and 60 um brain sections was demonstrated histochemically using the TMB procedure.

HRP labeled retinal ganglion cells were identified in retinal whole mount preparations. 85% of the 160 labeled ganglion cells measured after HRP injections into the SCN were classified as "large" (11-17um diameter). The number of labeled ganglion cells observed in each retina was equal; there was no tendency for the contralateral retinae to be more heavily labled.

Labeled neuronal perikarya were found throughout the diencephalon and midbrain. Neurons of the vLGN afferent to the SCN were principally located in the most dorsal aspect of the nucleus clustered beneath the fiber plexus separating the ventral and dorsal LGN. A few labeled neuronal perikarya were observed in the region of the vLGN-zona incerta and in the lateral terminal nucleus. A large descending ipsilateral input from the lateral and medial septal nuclei and the medial preoptic area was noted. Scattered cells were also found throughout the anterior hypothalamic area, the periventricular region, the ventromedial nucleus and the arcuate nucleus. A reciprocal connection to the contralateral SCN was also observed. A large number of labeled cells were found in the parasubiculum and the contiguous ventromedial portion of the subiculum, bilaterally. Labeled cells in similar numbers were seen in the dorsal raphe nucleus and in the nucleus centralis superior. Labeled cells were also seen in the dorsal and ventral tegmental nuclei and in the dorsal nucleus of the lateral lemniscus. Knowledge of the input to the SCN will aid in the understanding of the neural substrate underlying circadian rhythms. (Supported by PMAF (GEP) and HD-10665 (AJS).

99.1 MANIA: CONTINUOUS AND CATEGORICAL MEASURES OF SEVERITY. R.C. Young*, R.W. Nysewander*, M. Schreiber* (SPON: C. Shamoian). Dept. of Psychiatry, Cornell Univ. Med. Coll., Westchester Division, White Plains, NY 10605.

Forty hospitalized psychiatric patients who on admission fulfilled diagnostic criteria for mania (Feighner, J.P. et al., Arch. Gen. Psychiat., 26:57, 1972) were each studied twice. On each occasion, severity of illness was judged independently by two interviewers based on a brief semistructured joint interview using a rating scale of established reliability, validity, and sensitivity (Young, R.C. et al., Brit. J. Psychiat., 133:429, 1978). The scores assigned by the two interviewers were averaged. The assessments were done within the first week after admission and again two weeks thereafter. Treatment consisted of neuroleptics and lithium carbonate, usually in combination.

The initial median total score was 26.2 out of a possible 60 (range 9.3 - 45.0); the median total score on the second rating was 16.1 (range 2.0 - 31.5; $p < .001$). There was also a significant decrease between the first and second rating on each of the eleven item scores ($p < .05$).

Item scores were converted to categorical data by considering as "positive" those values ≥ 1.0 (≥ 2.0 for items 5,6,8,9). The most frequently positive ($\geq 80\%$) items on initial rating were "mood," "energy," "speech," "language-thought disorder," "content," and "insight." The least frequently positive ($\leq 40\%$) items were "disruptive-aggressive behavior" and "appearance." At the second rating, the "mood" and "insight" items were still frequently positive. Psychotic mental content (≥ 7.0 on item 8) was present in 25% on initial rating and 5% on the second rating ($p < .05$). When selected categorical data were examined with respect to criteria for mania, 95% of patients were manic at the first rating, while 48% were manic at the second rating ($p < .001$). Total rating scale scores were significantly different in the manic and hypomanic groups at both the first ($p < .05$) and second ($p < .001$) ratings. At the first rating, 63% of patients were categorized both euphoric and irritable.

99.2 LITHIUM ACCUMULATION IN VITRO: EVIDENCE FOR POSSIBLE NEURON-GLIA INTERACTION. R. P. Saneto*, S. K. Srivastava*, J. R. Perez-Polo (SPON: J. S. Kittredge). Dept. Hum. Biol. Chem. & Genet., Univ. Tex. Med. Br., Galveston, Texas 77550.

Lithium (Li) accumulation and the components of Li influx in cultured clonal cell lines of human neuroblastoma (SY5Y) and human glioma (A_1B_1) were studied to determine possible neuron-glia interaction. The probable target tissue for the efficacious effect of Li in manic depression is neuronal tissue. On the other hand, glia may play an important role in the regulation of Li concentration within the neuron by acting as an ion buffering system for neuronal tissue. To better understand this interrelationship, we used the ion transport inhibitors ouabain and phloretin to dissect Li influx components in neuroblastoma and glioma cells. Neuroblastoma cells have a ouabain but not a phloretin-sensitive component of Li influx; 0.1 mM ouabain significantly inhibited the influx ($n = 6$, $P < 0.005$) while 0.1 mM phloretin had no effect. Since inhibition by ouabain was only partial (49%), probably neuroblastoma have other component(s) of Li influx. In contrast, glioma does not have either a ouabain or phloretin-sensitive influx components; both 0.1 mM ouabain and phloretin have no effect on Li influx. However, since the accumulation of Li influx is saturable, it is likely that an active component(s) of Li influx exists. Glioma cells accumulated about 4-fold more intracellular Li ($n = 6$, $P < 0.001$) than the neuroblastoma cells. In addition, the affinity of entry was 3-fold and velocity of entry was 2-fold greater in the glioma cell. Although our studies are with glioma cells in culture the data suggest that glia may regulate the level of Li by preferentially accumulating and establishing the level of Li in the extracellular environment of neurons. Thus, the glia may play an important role in therapeutic action of Li.

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99.3 THE EFFECTS OF THE PARASITE TOXOCARA CANIS ON BEHAVIOR IN THE MOUSE. Z. S. Dolinsky, R. G. Burright*, and P. J. Donovick. Dept. of Psychology and Center for Neurobehavioral Sciences, SUNY Binghamton, Binghamton, N.Y. 13901 and L. T. Glickman*, R. H. Cypess*, and J. Babish*. Dept. of Preventive Med., Cornell Univ., Ithaca, N.Y. 14850.

Toxocara canis, a parasitic roundworm commonly infects dogs. The eggs of the parasite are excreted in the animal's feces and may be ingested by children. The consequent symptoms include fever, leukocytosis, eosinophilia, hypergammaglobulinemia, and hepatomegaly. In addition, this parasite is known to migrate to the central nervous system in humans.

We examined the behavioral effects of the parasite Toxocara canis in mice. Binghamton Fuller Heterogeneous stock (HET) adult male mice were intubated with 1000 eggs (one gram of canine feces contains between 50-60 thousand eggs) of the parasite Toxocara canis. Control animals were intubated with an equal volume of physiological saline. Seven weeks later a battery of behavioral tests were begun. Tests included: home cage behavior; open field and water dip activity; performance on a passive avoidance task; ability to remain on a rotorod; and activity in running wheels. These tests required 8 weeks. Animals were then sacrificed and brains examined for larvae.

Through most of the behavioral testing there was no evidence of body weight or gross physiological disturbance as a result of Toxocara administration. However, Toxocara mice were less likely to explore the vertical aspects of their environment than controls as measured by ascending to another cage by chain or by standing up in an open field. Toxocara mice also exhibited altered behavior in water and dry open fields. Furthermore, Toxocara animals required more trials to learn a shuttle passive avoidance task.

Not until 11 weeks post-intubation did the Toxocara animals begin to lose weight relative to controls and continued to do so through the remainder of the behavioral testing. Behavior continued to reflect the effects of Toxocara. These mice left the rotorod sooner than controls. In addition, Toxocara animals were less active in the running wheels than controls.

Histological examination revealed larvae in cerebellum, brain stem, hippocampus, basal ganglia, colliculi and cerebral cortex. The number of larvae per weight of region was greater in these areas than in olfactory bulbs or hypothalamus.

These results suggest that Toxocara canis can alter motor activity in mice and may have important implications for children who have a known history of pica for dirt.

99.4 HOST DETERMINANTS OF THE BEHAVIORAL EFFECTS OF HERPES ENCEPHALITIS IN THE LABORATORY MOUSE. D. McFarland* and J. Hotchin. Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201.

Previous work in this laboratory (McFarland, Baker, and Hotchin, Physiology and Behavior, in press) has indicated that host and viral genetic factors greatly influence the nature of the behavioral effects of scrapie viral encephalopathy. In order to determine whether similar dependencies exist with other forms of viral encephalitis, the influence of host genetics on the behavioral consequences of experimental herpes encephalitis was examined in the present investigation. Nylar and Swiss mice were given immunizing footpad inoculations followed two weeks later by intracerebral herpes inoculations. The effects of the viral infection on open field and Y-maze exploration were subsequently examined. A significant interaction between host genotype and the effects of viral infection was obtained. These results suggest that organismic factors may be important determinants of the effects of CNS viral infections and suggest one possible source of the diversity of sequela observed in clinical populations.

99.5 DOUBLE-BLIND EVALUATION OF THE POTENTIAL THERAPEUTIC EFFECTS OF HEMODIALYSIS IN SCHIZOPHRENIA. D.P. van Kammen, S.C. Schulz*, J.E. Balow*, G. Mundinger*, W. Flye*, W.E. Bunney, Jr. *Biological Psychiatry Branch, NIMH, Bethesda, MD 20205.*

INTRODUCTION: To evaluate the reported improvement in schizophrenia with hemodialysis (Wagemaker and Cade, 1977), we designed a double-blind, sham-dialysis-controlled protocol to study the effects of hemodialysis on healthy, well-diagnosed schizophrenic patients. METHODOLOGY: 3 male and 5 female schizophrenic patients (RDC) (average age 30 yrs.) who had been ill for an average of 9 yrs. completed 20 weekly dialysis sessions: 10 consecutive active dialyses and 10 sham dialyses. They had signed informed consent forms prior to participation and formation of the arteriovenous fistula needed for blood access. We used an opaque EX-23 dialyzer coil with a cuprophan membrane of 18μ thickness and a surface area of 0.8 m^2, identical to the dialyzer used by Dr. Wagemaker. Blood-flow rate was maintained at 200 ml/min. and a dialysate-flow rate of 300 ml/min. Sessions lasted for 5 hrs. The double-blind was maintained by using an identical-looking opaque dialyzer containing tubing rather than a membrane and by administering fluids intravenously and orally. Double-blind behavioral evaluations were performed extensively by psychiatrists and nurses using the Brief Psychiatric Rating Scale (BPRS) and the Bunney-Hamburg Global Behavioral Rating Scale. RESULTS: Analysis of the double-blind psychiatrists' ratings revealed that there was no significant difference for the patient group between active and sham dialysis. No single patient responded to 10 wks. of active dialysis with a decrease in global psychosis or in affective symptoms. The double-blind nursing ratings corroborated the psychiatrists' observations. No individual symptoms such as hallucinations or thought disorder were decreased by active hemodialysis; 4 patients showed unequivocal worsening at the end of their active trial; complications and side effects were minimal. DISCUSSION: These data will be compared to other negative and positive reports of nonblind evaluations in the literature since 1960. At this time hemodialysis cannot be recommended as a treatment for schizophrenia. Inasmuch as only 8 patients completed the trial, further double-blind evaluations of other schizophrenic patients are needed to determine the effects of hemodialysis in schizophrenia.

100.1 COLLATERAL ARBORIZATION OF AXONS OF RAT STRIATAL NEURONS IN THE GLOBUS PALLIDUS STUDIED BY INTRACELLULAR HORSERADISH PEROXIDASE LABELING. H. T. Chang, C. J. Wilson* and S. T. Kitai, Dept. of Anatomy, Michigan State University, E. Lansing, MI 48824.

Studies employing retrograde axonal transport of horseradish peroxidase (HRP) have established that the majority of striatal neurons are projection neurons. Combined electrophysiological studies and intracellular labeling have further demonstrated that medium spiny neurons are striatal projection neurons and in some cases their axons could be traced to the globus pallidus (GP). In order to determine more precisely the pattern of termination of striatal efferents, we have examined in detail the collateral arborizations of striatal axons in GP.

Following intracellular recording of striatal neurons in the rat, cells were labeled by intracellular injection of HRP. After fixation in aldehydes, sagittal sections containing striatum and GP were processed for demonstration of HRP and prepared for light and electron microscopic analysis using the section-embedding procedure of Wilson and Groves (1979). After giving off numerous local collaterals, the axons of some very heavily stained medium spiny neurons could be followed for distances of several millimeters and were traced into GP. In all these cases, the main axons did not appear to end in GP, but were seen to give off various patterns of collateral arborization in GP. In some cases, the axons of the medium spiny neurons appeared to be unbranched throughout their course in striatum and gave rise to only a few collaterals terminating in GP. In other cases, axons from medium spiny neurons divided within striatum to form two or three branches which took a parallel course toward GP. These branches gave rise to extensive and highly overlapping axonal arborizations which involved large areas of GP and were oriented primarily in the rostro-caudal and dorso-ventral planes. These striatal efferent plexuses in GP consisted of thin unmyelinated fibers and boutons packed with large and moderately pleomorphic vesicles resembling to those formed by the intrinsic collaterals of spiny neurons within striatum. These terminals formed symmetrical synapses on dendritic shafts and somata of GP neurons and were similar to those described by Kemp (1970).

These observations indicate that: 1) At least some strio-pallidal projection neurons are medium spiny neurons, 2) The pattern of strio-pallidal terminal arborization is highly organized and involves a large area of GP, and 3) Individual striatal projection neurons may vary in their patterns of collateral arborization in their target areas. (Supported by USPHS Grant NS 14866).

100.2 PROCESSING OF SOMATOSENSORY INFORMATION IN THE GLOBUS PALLIDUS OF CATS. T. I. Lidsky and J. S. Schneider, SUNY at Stony Brook,N.Y.

Several different investigators have reported that high proportions of neurons in the globus pallidus (GP) changed firing rate during eating and drinking. However, the functional significance of these neural responses remains obscure. In an effort to better understand the nature of pallidal involvement, a series of experiments was begun with the general aim of assessing the role of the GP in the various sensory and motor processes which are important in ingestion. This abstract presents data concerning information processing in the GP with specific reference to those sensory stimuli important for ingestion.

Units were recorded from awake, partially restrained cats. Von Frey hairs,blunt wooden probes and an electromechanical device (that produced events of reproducible magnitude and duration) were used to present somatosensory stimuli.

48% of sampled GP cells responded to somatosensory stimulation of the face. Receptive fields varied from moderate in size (e.g. a vibrissa field) to quite large (e.g. the entire face). 68% of receptive fields were confined to areas served by one branch of the trigeminal nerve, 16% were from areas innervated by two branches and 16% from areas innervated by all three branches. 65% of the receptive fields were contralateral, 6% ipsilateral and 29% bilateral.

Response latencies were short (mean, 10.2 msec) and thresholds were quite low. The majority of cells were activated by Von Frey hairs calibrated at 1 gram. Increasing response magnitude resulted in more vigorous neuronal responding. However, given the variability in unit response on successive trials, only gross differences in force were being encoded.

42% of responsive cells were solely or best activated by rapid brushing of the hairs rather than indentation of the skin. Half of these cells responded primarily to stimuli moving in a particular direction.

Several aspects of GP sensory responses seemed particularly relevant for the control of ingestional behavior. 53% of the receptive fields included perioral tissue. Moreover, when stimuli were applied within various portions of the receptive field, the most pronounced responses were evoked from zones closest to the mouth. Finally, the majority of directionally sensitive neurons responded best to stimuli moving along a path directed toward the mouth.

(Supported by USPHS grant NS15328 from the NINCDS)

100.3 STRIATAL NEURONAL ACTIVITY AND THE CONTROL OF HEAD MOVEMENT IN CATS. J.S. Schneider and T.I.Lidsky, SUNY at Stony Brook,N.Y.

Previous work from this laboratory has shown that neurons in the head of the caudate and the putamen are involved in ingestive behavior. The present work was intended to further analyze the properties of cells in these areas with respect to sensory and motor processes which are components of ingestion.

Striatal neurons were recorded from chronically prepared cats. During testing, animals were restrained in a device which allowed rotational head movements in the horizontal plane.

37% of striatal cells changed firing rate in relation to some aspect of head movement. These movement-related cells could be grouped into one of three categories based on response characteristics. Type 1 (21% of movement units) fired only if postural fixation of the head was disrupted. When rotational force was applied to the cat's head by the experimenter and the animal resisted displacement, firing of these cells increased. However, type 1 cells did not change firing rate during self-initiated movements.

Types 2 and 3 movement cells (respectively 54% and 25% of movement cells) fired in relation to self-initiated head movements. Type 2 changed firing rate at the onset of head displacement and well after onset of neck muscle EMG activity. Type 3 cells changed firing rate before onset of EMG response. Lead times as long as 1200 msec were encountered although these latencies varied considerably on successive movements. While all type 2 and type 3 cells fired in relation to movements in either direction, roughly half of these cells showed augmented responding to particularly directed movements. Unit activity did not relate to other parameters of head movement such as magnitude, velocity or absolute position. No striatal cells were found which fired in specific relation to jaw or tongue movements.

24% of striatal cells (both those related and those unrelated to head movement) were responsive to cutaneous somatosensory stimuli. Receptive fields were moderate to large but always included perioral tissue. When stimulation was applied within various portions of the receptive field, the largest responses were evoked at the front of the mouth. Some cells were only responsive to moving tactile stimulation; stimuli which moved along a path directed to the front of the mouth evoked enhanced responding.

The sensory properties of striatal cells suggested the encoding of a tactile stimulus' position with respect to the front of the mouth. The motor concomitants of striatal activity suggested a role in the control of axial muscles. Taken together, these data indicate striatal involvement in the somatosensory control of head positioning.

100.4

Withdrawn by Author

100.5 TOPOGRAPHICAL STUDY OF THE STRIATOFUGAL PATHWAYS IN THE CAT AND THE MONKEY. M. Giguère and L.J. Poirier. Centre de recherche en neurobiologie, Hôpital de l'Enfant-Jésus, Québec, Qué. G1J 1Z4.

The organization of the striatofugal fibers was studied with the autoradiographic technique. The course and distribution of these fibers were compared in different animals following the injection of ^{3}-H leucine in various parts of the caudate nucleus and putamen. Their course and pattern of distribution are to a certain extent different in the two species.

In the monkey, the striatal efferents are topographically related to the corresponding parts of both divisions of the pallidum along the dorsoventral and rostrocaudal axes. In the sagittal plane, however, the caudato-pallidal and putamino-pallidal fibers end in the dorsomedial and the ventrolateral parts of both divisions of the pallidum, respectively. The striatopallidal fibers represent the most important projection from these structures. The striato-nigral fibers terminate in the substantia nigra in a reversed dorso-ventral pattern. The caudato-nigral fibers reach approximately the medial half of the substantia nigra and terminate in both the pars compacta and reticulata along the whole rostrocaudal extent of this structure. The putamino-nigral fibers follow a more lateral course and terminate more laterally and also over the entire rostrocaudal extent of the substantia nigra. Striatal efferents however do not apparently reach the most lateral part (pars lateralis) of the substantia nigra in the monkey.

The striatopallidal efferents have a similar topographical distribution in the dorsoventral and rostrocaudal axes in the cat and monkey. Caudato- and putamino-pallidal fibers, however, overlap at the level of the pallidum in the sagittal plane of the cat brain. In this species the entopeduncular nucleus receives fibers from the caudate nucleus but not from the caudal part of the putamen. Putamino-nigral fibers from the caudal part of the putamen, however, end in the most lateral part (pars lateralis) of the substantia nigra. Therefore the caudal half of the putamen end exclusively in the pallidum and the pars lateralis of the substantia nigra. Caudato-nigral fibers end over the whole pars compacta and reticulata of the substantia nigra. Fibers originating in the rostral part of the caudate nucleus project more medially in the substantia nigra and those originating more caudally terminate more laterally in this same structure. Moreover the pattern of distribution of the caudato-nigral fibers in the substantia nigra is more diffuse along the rostro- caudal or sagittal plane. (Supported by grant MT-732 of the Med. Res. Council of Canada).

100.6 CELLS OF ORIGIN AND AXONAL BRANCHING PATTERNS OF THE CORTICO-STRIATE PATHWAY IN RAT. S.E. Knowles*, C.S. Lin, J.K. Chapin and D.J. Woodward. (SPON: D.C. German). Dept. Cell Biology, Univ. of Texas Health Science Center, Dallas, Texas 75235.

The aim of this study was to determine the organization of neural projections from the cerebral cortex to the striatum of rat and in particular to establish: a) which cortical layer(s) project to the striatum; b) the soma size and axonal morphology of corticostriatal cells, and c) the degree of overlap of the striatal projections from different cortical areas.

HRP uptake and transport as a method for study of pathways was augmented by adding 1-3% lysophosphatidyl choline to a 20-30% solution of HRP prior to injection (2-3 μA, 15 min). The DAB reaction was enhanced by preincubation in 0.5% $CoCl_2$. In addition to retrogradely labeling cell somata, this technique also permitted visualization of the morphology of the somata, dendritic trees, axons and axon collaterals of both ortho- and retrogradely filled cells.

Small groups of retrogradely labeled cells were observed in cortex after discrete (usually less than 1 mm diam) HRP injections into striatum. A topographical organization was observed, with each cortical area examined (prefrontal, MI and SI) projecting to its striatal target. HRP filled corticostriatal cell bodies were found mainly in ipsilateral layer V. Most corticostriate cells fell within the small to medium size range (5-15 μM diam).

HRP injections were also made into electrophysiologically identified MI and SI forepaw and vibrissae areas and anterogradely filled axons in the striatum were examined. Termination zones found in the lateral striatum were clearly distinguished from larger mediodorsal areas containing heavy parallel bundles forming the internal capsule. Axon collaterals branched from the major corticofugal bundles and traveled for 500 to 1000 μM before arborizing into diffuse networks which contained varicosities. Major termination sites from the MI forepaw area were ~ 1mm rostral to those of the SI forepaw area. Overlap was observed in the peripheries of the striatal projection zones of these homotopic cortical regions.

In summary, a heterogeneous population of cortical layer V cells project to the striatum of rat. At least some of their axons are collaterals of projections to other subcortical sites. A topographical organization with a small degree of overlap between somatic sensory and motor areas is present in the corticostriate pathway.

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100.7 MONOLAYER CULTURES OF NEWBORN RAT CORPUS STRIATUM by Anne Messer, Div. of Labs & Research, NYS Department of Health, Albany, NY 12201.

Monolayer cultures of corpus striatum cells from newborn rats have been established using techniques similar to those previously described for rat and mouse cerebellum. Cells can be grown on media supplemented either with horse serum, or with an "artificial serum", a modification of the hormone mix first described by Bottenstein and Sato, and seem to exhibit a variety of rather similar neurochemical properties under the two conditions.

Striata dissociated with trypsin at room temperature are plated on to polylysine coated glass coverslips and incubated with 10% horse serum for 24 hours; then the coverslips are inverted and the media is changed to include either 10% horse serum or an artificial serum containing insulin, transferrin, progesterone, putrescene and selenium.

After 21 days in vitro, the majority of the cells have rounded, 9-12μ cell bodies, with scant cytoplasm and 2-5 processes. A few larger neurons and a substantial number of flatter cells (probably atrocytes) are also observed in all cultures.

Biosynthesis of γ-aminobutyric acid (GABA) and acetylcholine has been demonstrated by incubating whole cells with precursors, and measuring products using high voltage electrophoresis. Differences in transmitter synthesis between horse serum and artificial serum-grown cultures are not significant.

Thirty to 50% of the neuronal-looking cells in the culture, as well as many of the flat cells, show high-affinity uptake of GABA. Quantitatively, both the levels of GABA uptake, and the proportion of the total uptake which can be inhibited by the glial uptake inhibitor β-alanine are similar under the two culture conditions.

The presence of leu-enkephlin in the serum-free cultures can be demonstrated by immunocytochemistry.

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100.8 ENTOPEDUNCULAR NUCLEUS PROJECTIONS TO THE POSTERIOR MESENCEPHALON. E. Garcia-Rill, Dept. Anatomy, Univ. Arkansas for Medical Sciences, Little Rock, AR 72205.

We reported previously the existence of a small projection from the entopeduncular nucleus (EN) to the mesencephalic locomotor region (MLR) (Skinner et al., Neurosci. Abst. 5, 1979). We were able antidromically to activate EN neurons (less than 6%) from the same mesencephalic site stimulation of which, following precollicular-postmamillary transection, elicited locomotion on a treadmill in the same animal. This site was invariably located in the postero-medial cuneiform nucleus (CF). Recent studies reported similar (Larsen and Sutin, Brain Res. 157, 1979) and much greater (Filion and Harnois, J. Comp. Neurol. 181, 1978) numbers of antidromically activated EN neurons by stimulation of more anterior mesencephalic sites, from which locomotion cannot be induced.

In order to determine the synaptic effects of EN terminals on neurons in the posterior mesencephalon, intracellular recordings of neurons in CF and posterior nucleus tegmenti pedunculopontinus (NTPP) were carried out in locally anesthetized, paralyzed cats after suction ablation of the occipital cortex and removal of the tentorium. Stimulating electrodes were placed in EN (n=3), caudate nucleus (CN) (n=4), internal capsule (IC) (n=2) and motor cortex (n=4). Almost 10% of neurons in CF and posterior NTPP were found to respond to EN stimulation. Only cells which were driven from EN and, at longer latency, from CN, and did not respond to IC stimulation, were included in this group. All of the responses elicited in this area following EN stimulation were EPSP or EPSP-IPSP sequences. The mean latency to the beginning of the EPSP was 6.8 ± 1.7 (S.D.) ms following EN stimulation, and 11.2 ± 1.2 (S.D.) ms following CN stimulation. A similar proportion (10%) of neurons which responded to EN stimulation, but not to CN or IC stimulation, showed a mean latency of 12.5 ± 6.02 (S.D.) ms. These longer latency effects could be mediated in CF and NTPP via the substantia nigra. Although a large portion (30%) of neurons in the posterior mesencephalon responded to IC stimulation, none of these were found to respond to motor cortex stimulation. These results support our previous report describing terminal labeling only anterior to the MLR following injections of tritiated amino acids into motor cortex.

These findings support the suggestion that the EN-MLR pathway may be involved in the triggering, via the MLR, of locomotion generators. In the diseased state, lack of activity along this pathway may be partly responsible for the inability to initiate sequences of movements in which locomotion is necessary.

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100.9 PALLIDAL AND HYPOTHALAMIC INFLUENCES ON LATERAL HABENULAR NUCLEUS. Russell L. McBride. Dept. Anatomy, Emory Univ. Sch. of Med., Atlanta, GA 30322.

The entopeduncular nucleus (feline homologue of the primate medial segment of globus pallidus) and lateral hypothalamus-preoptic region project to the lateral habenular nucleus (LHB). While this indicates the LHB is a site of convergence of basal ganglionic and limbic system output, retrograde transport studies have shown that the region of LHB receiving pallidal input projects to the ventral tegmental area, while the region receiving hypothalamic input projects to raphe, paramedian reticular formation and central gray. This study was designed to determine the influences of pallidal and hypothalamic activation on the activity of LHB projection neurons in different regions of the nucleus.

In barbiturate anesthetized adult cats, bipolar stimulating electrodes were placed in the lateral hypothalamus and entopeduncular nucleus to orthodromically influence LHB neurons, and in the fasciculus retroflexus to antidromically activate LHB projection neurons. Extracellular recordings were made from LHB neurons with micropipettes filled with 4 M NaCl. Neurons were considered to be antidromically activated if they followed high frequency stimulation with constant latency and showed collision. Latency of antidromic activation of LHB neurons averaged 5 msec. Locations of stimulating and recording electrodes were verified histologically.

Both hypothalamic and entopeduncular stimulation resulted in inhibition with short latency and long duration of antidromically identified LHB neurons. Stimulation completely inhibited the activity of LHB neurons for 75 to 300 msec, with most neurons inhibited for about 150 msec. Some neurons responded with a single 3 to 5 msec latency spike followed by inhibition. The short latency spike followed high frequency stimulation with constant latency and therefore may be an antidromic response.

While a few LHB cells responded only to lateral hypothalamic or entopeduncular stimulation, most responded in the same manner to both stimuli. The similarity of responses from the two stimulation sites may be due to activation of entopeduncular axons as they pass through the lateral hypothalamus. Since entopeduncular stimulation should not activate lateral hypothalamic neurons, however, these studies indicate the entopeduncular nucleus influences LHB neurons in the same manner regardless of the location of the neurons.

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100.10 EFFECT OF CORTICAL ABLATION AND OF TEGMENTAL MIDBRAIN LESIONS ON HEAD TURNING INDUCED BY ENTOPEDUNCULAR AND BY NIGRAL STIMULATION IN THE CAT. R. Hébert* and M. Filion. Lab. Neurobiologie and Dépt Physiologie, Fac. Méd., Univ. Laval, Québec, Qué., Canada G1K 7P4.

In previous studies we have shown that the majority of medial pallidal (entopeduncular) neurons in the monkey and in the cat send branches of their axon to the ventrolateral thalamus and to the midbrain pedunculopontine nucleus. Nigral neurons also branch to the thalamus and to the midbrain. This suggests that pallidal and probably nigral activity can use thalamocortical as well as midbrain pathways to control motoneurons. To clarify this point, the entopeduncular nucleus and the substantia nigra were stimulated unilaterally with bipolar concentric electrodes in cats. Pulses of current of 0.1 ms duration were delivered in trains of 40 pps during 3 s. The stimulation was applied when the animal was sitting quietly and looking straight ahead. A light emitting diode was fixed to the head of the animal and filmed from above to record horizontal head movements. The intensity of the light was increased at the time of stimulation to measure the latency of the response.

Unilateral entopeduncular and nigral stimulation elicited contraversive head turning as described in the litterature. Threshold intensities were in the range of 0.05 to 0.5 mA. The latency of the movement was around 1000 ms at threshold and never shorter than 100 ms at maximum intensity of stimulation. When the responses to stimulation were stable, as shown by latency/intensity curves, two operations were performed under anesthesia at a month interval: 1) the rostral portion of the stimulated hemisphere (including frontal, somatosensory, motor and parietal cortical areas) was completely disconnected from the brain by suction of a slab of tissue in an oblique plane from above the rostral thalamus to the middle of the olfactory tract; 2) electrolytic lesions were made in the midbrain to destroy the pedunculopontine nucleus and tectal efferent fibers on the stimulated side. To date, our largest midbrain lesion destroyed these structures extensively but not completely. Yet, after these large cortical and midbrain lesions, the threshold and the latency of head turning were similar to those recorded before the lesions. Therefore, extensive lesions of cortical and of tectal efferent fibers and of the pedunculopontine nucleus spare a sufficient number of efferent fibers for head turning to be produced by entopeduncular and by nigral stimulation as easily and as rapidly as in intact animals. The effect of more complete midbrain lesions and of commissural lesions is under study.

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100.11 THE RAT OLFACTORY TUBERCLE: ITS CONNECTIONS AND RELATION TO THE STRIO-PALLIDAL SYSTEM. Richard B. Goldschmidt* and Lennart Heimer (SPON: M. West). Anatomy Inst. B, Univ. of Aarhus, 8000 Aarhus C, Denmark.

The olfactory tubercle is a heterogenous structure in the basal forebrain containing several different cell groups which are more or less intermixed. Although the rat olfactory tubercle is typically divided into layers like a cortical structure, Heimer and Wilson (1975) have suggested that many connections of this region are unlike any other cortical region and that some of its connections seem to resemble those of the striatum and globus pallidus. This suggestion was examined in more detail by comparing the experimentally observed projections of the olfactory tubercle with those of the striatum, nucleus accumbens, globus pallidus, and the neighboring pyriform cortex.

Connections of the superficial part of the tubercle were studied using laminar heat lesions. Degeneration was stained with the deOlmos cupric silver method. Medium-sized cells of the "pyramidal" layer project dorsally and caudally to terminate in the polymorph layer of the tubercle and more dorsally in the area called "ventral pallidum". In contrast to neighboring allocortical areas, no association pathways from these "pyramidal" cells to other cortical areas have been identified.

The suggested "ventral pallidal-thalamic" projection originates from neurons deeper in the tubercle, in the polymorph layer and "ventral pallidum". These large neurons were retrogradely labeled after injections of HRP or fluorescent tracers into the mediodorsal thalamic nucleus. Injections of fluorescent tracer into the subthalamic nucleus were used to evaluate the pallidal nature of this region. Many neurons were labeled in the main, dorsal part of the globus pallidus (external segment), but very few neurons were labeled in the "ventral pallidum". Since the "ventral pallidum" shares a common iron positive neuropil (Switzer & Hill, 1979) and is continuous with the external segment, and like the internal segment (entopeduncular n.) has a thalamic projection and no subthalamic projection, it shares some features of both these parts of globus pallidus. It should also be emphasized that there are important differences between the main, dorsal parts of the striatum and pallidum and the "ventral strio - pallidal system" to which the olfactory tubercle contributes.

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100.12 WIDESPREAD PROJECTIONS TO THE STRIATUM FROM THE LIMBIC MESOCORTICES IN THE MONKEY. C.L. Barnes,* G.W. Van Hoesen and E.H. Yeterian (SPON: A. Applebaum), Depts. of Anat. & Neurol., Univ. of Iowa, Iowa City, IA 52242.

The identification of structures responsible for the initiation of motor behavior has been elucive. In terms of cortex, early thinking assumed that motor cortex was connected directly to sensory cortex and that this linkage provided a means for triggering motor movement. With the exception of somatic sensation, anatomical results have not supported this seemingly logical supposition. In fact, physiological studies suggest that cortical influences on motor cortex may be channeled through the basal ganglia and cerebellum. Their influence on the thalamus then heralds a response in motor cortex. Thus, corticostriate and corticopontine projections may be viewed as early components in multisynaptic pathways enabling widespread parts of the cortex to influence motor behavior. Corticostriate projections are thought to arise from all parts of the cerebral cortex. However, little information is available on corticostriate projections from the cortical areas that constitute the limbic lobe. We have observed that the cingulate, retrosplenial, parahippocampal, temporal polar and orbitofrontal cortices all have powerful projections to the caudate nucleus and putamen, and thereby, provide a limbic influence on the striatum in consort with the more well-known sensorimotor influences. The brains of 25 rhesus monkeys were available for study. In each, tritiated amino acids (^{3}H-leucine, lysine and proline) had been injected into the various areas of the limbic lobe. Autoradiography was used to assess terminal labeling. It was observed that all limbic cortices project to the striatum, and in particular, to the caudate nucleus. Projections from the parahippocampal cortices terminate over all parts of the caudate nucleus (head, body, bend and tail). Cingulate projections terminate over the head, body and tail as well, with anterior areas (areas 24 & 25) projecting in addition to the putamen. Temporal polar and posterior orbitofrontal areas project mainly to the head and tail of the caudate nucleus, although orbitofrontal projections also terminate over the anterior part of the putamen. Overlap in topography has been observed, and in general, the heaviest projections are directed toward the periphery of the striatum. For all cases, terminal labeling was patchy, forming an overall mosaic-like pattern. These results provide evidence for a strong limbic influence on the striatum in the monkey, and may represent a structural basis for affect-related influences on motor behavior. (supported by grant NS 14944).

100.13 STRIATONIGRAL RELATIONSHIP IN MACAQUES. John Hedreen, Mahlon DeLong, and Glenn Holm. Departments of Cell Biology and Anatomy and Physiology, Johns Hopkins Medical School, Baltimore, MD 21205

According to current views of the striatonigral relationship in primates, derived principally from fiber degeneration studies, the striatonigral projection ends almost wholly in the pars reticulata of the substantia nigra (SN). The topographic interrelationships have not been completely worked out, although an exchange of connections between rostral striatum and rostral SN, and between caudal striatum and caudal SN, has been commonly accepted. Our studies with axonal transport methods require that these concepts be revised.

We made injections of combined tritiated amino acids and HRP, or HRP alone, into the neostriatum in 10 hemispheres of 5 Macaca cynomolgus monkeys. Stereotaxy was guided by multiple prior penetrations with a recording electrode.

The HRP-tetramethylbenzidine sections, and to a lesser degree the autoradiography + HRP-diaminobenzidine sections, revealed striatonigral terminals and nigrostriatal cells in the same sections. In many places the heavy terminal fields in the pars reticulata were seen to extend up and engulf groups of labeled pars compacta cells.

The labeled terminals occurred in dense patches within a broad strip extending the entire length of the SN, located along the medial edge after injections of rostralmost striatum, and more and more lateral with more and more caudal injections. The strips of terminals were not exactly parallel to the midline, especially more laterally, but paralleled instead the course of fibers in the underlying cerebral peduncle. The nigral projection fields from different regions of striatum showed considerable overlap, although whether there is convergence at the single cell level is not certain. Dense populations of labeled cells were seen in approximately the same longitudinal strips as the labeled terminals in the same case, and were sometimes immersed in a sea of such terminals. More scattered labeled cells extend medial to the terminal strip. Differences between dorsal and ventral, or medial and lateral injections were less apparent.

These findings provide anatomic evidence for a striato-nigrostriatal loop, and yield a new concept of the relationship between striatum and SN in primates.

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100.14 GLOBUS PALLIDUS: NEURONAL RESPONSES TO ARM LOADING. M.H. Branch* M.D. Crutcher, and M.R. DeLong. Dept. Physiol., Johns Hopkins University Sch. Med., Baltimore, Md. 21205

Neurons in the basal ganglia of behaving primates have been shown to alter their activity in a variety of motor tasks. This study examines quantitative responses of neurons in the globus pallidus of monkeys to load pertubations of the arm. Monkeys were trained to grasp a torquable manipulandum and maintain a steady position using visual feedback of arm position. Step loads were then applied to the handle, displacing the limb either toward or away from the animal. The animals were required to recover and maintain their initial limb position against the sustained load.

Neurons were selected for study in the task only if their activity was correlated with movements or manipulations of the arm. 91% of the neurons studied (n=33, 2 hemispheres) responded vigorously to the application of the load; 80% of these cells altered their activity at latencies of less than 100 msec after load onset. The median latency was within 40-60 msecs, with less than 7% of the neurons responding at latencies in the 20-40 msec range. 73% of the activity changes consisted of increases in discharge rate. In some cases, reciprocal changes in rate were observed with load application of opposite direction. In addition to the short latency responses observed, 53% of the neurons maintained an altered discharge rate during the static phase of the task (i.e. after recovery of initial limb position) suggesting a tonic neuronal relation to muscle tension.

The response latencies observed in globus pallidus neurons were sufficiently brief to suggest a proprioceptive input. However, responses to limb pertubations at similar latencies (40 msec) in motor cortex (1) and dentate (2) appear to be related to the "motor set" of the animal rather than to the kinesthetic input. Therefore, the responses observed in globus pallidus neurons may be related to the ensuing movement rather than to the afferent input. We are currently investigating this problem in the putamen, subthalamic nucleus and globus pallidus in order to clarify the processing of proprioceptive input within the basal ganglia.

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100.15 SINGLE UNIT ACTIVITY IN THE SUBSTANTIA NIGRA (SN) OF AWAKE RATS DURING CONDITIONED FORELIMB MOVEMENTS. Lawrence R. Huntoon and Samuel L. Liles. Dept. of Physiology, Louisiana State Univ. Med. Ctr., New Orleans, LA 70119.

Extracellular single-unit recordings were obtained using a chronic recording technique which has been previously described (Neurosci. Abstr. 3:40, 1977). Specially-designed concentric Pt-Ir microelectrodes were used to eliminate EMG contamination from orofacial muscles. A total of 218 cells were isolated in the ventral midbrain of 5 chronic rats: 26 in pars compacta region of SN, 115 in pars reticulata of SN, and 77 in regions adjacent to SN. All recording sites were verified histologically. Four categories of response types were found: (limb) movement-related, orofacial-related, modulated (e.g. related to posture or to a non-monitored movement), and cells which displayed a gating function (turned on or off during a series of limb movements). Within the (limb) movement-related category there were three subcategories of responses: (i) non-spontaneously firing cells that discharged only when the rat was making forelimb movements; (ii) spontaneously firing cells that were inhibited prior to the onset of forelimb movements; and (iii) spontaneously firing cells that increased their firing rate prior to the onset of movement. Analysis of limb EMG activity and peri-movement histograms of unit activity showed that many SN cells exhibited statistically significant firing changes more than 100 msec before the onset of limb movement. Present findings indicate that 10% of the cells in SN are related to contralateral forelimb movements and 4% are related to orofacial movements. The majority of the cells isolated in SN were small amplitude, rapidly firing cells which were predominantly located in pars reticulata. The very slowly firing dopaminergic nigrostriatal Type I cells, which are prevalent and easily isolated in acute preparations, were conspicuous by their absence in the awake rat.

The study concludes that there are cells in SN and in adjacent regions of the midbrain tegmentum of the rat which display movement-related activity, and that these cells play a role in the initiation of movement. Unlike in the monkey, however, where most of the cells in SN appear to be related to orofacial movements (Delong and Georgopoulos, Neurosci. Abstr. 4:42, 1978), most of the SN cells in the present study were related to limb movements. The great diversity of single cell responses reported in this study provides a basis for more specific examination of individual SN cells in relation to motor function. (Supported in part by NIH Grant NS-15485).

100.16 KITTENS: ONTOGENY OF EVOKED UNIT ACTIVITY IN THE SUBSTANTIA NIGRA. R.S. Fisher, M.S. Levine, N.A. Buchwald and C.D. Hull. MRRC and BRI, Sch. Med., UCLA, Los Angeles, CA 90024

Extracellular single unit recording was used to describe the ontogeny of the responses of units in the substantia nigra to stimulation of the caudate nucleus (CD) (monosynaptic input), the precruciate cerebral cortex (CX), and the dorsum of the central lateral forepaw (external sensory input).

Nigral units responded to each of these inputs at the earliest ages tested (2 days postpartum). There were, however, a number of significant indices of postnatal maturation; e.g., decreases in latency to response, increases in the proportion of units responding, and shifts in response type.

Recordings were obtained from more than 200 nigral units in 23 cats and kittens ranging from 2 days of age to adulthood. The results are summarized as follows: CD stimulation - 1) The proportion of units responding to CD stimulation increased from 29% (46/161) in the 1-40 day age range to 48% (14/29) in adults. 2) At all age ranges, CD stimulation predominantly evoked an initial inhibition in nigral units. There were, however, occasional instances of initial excitation in kittens. In adults, all initial responses were inhibitory. 3) Latency to onset of inhibition decreased with age ($\bar{X} \pm$ s.e.m. = 58 $\pm$ 14 msec (1-10 days) vs. 24 $\pm$ 3 msec (adults). 4) Postinhibition excitatory firings (rebound) occurred to a greater extent in adult nigral units (71%) than in kitten nigral units (25%). 5) Latency to antidromically activated spikes evoked by stimulation of CD or medial forebrain bundle decreased markedly with age (for CD stimulation, 40-60 msec (1-10 days) vs. 6-10 msec (adults)). CX stimulation - 1) At all age levels, about a third of the nigral neurons responded to CX stimulation. 2) At all age levels, CX stimulation produced a mix of initial excitation and inhibition in nigral units. 3) Latency to CX stimulation decreased with age (approximately 80 msec (1-10 days) vs. 17 $\pm$ 4 msec (adults)). 4) Rebound occurred more frequently in older cats (13% (1-40 days) vs. 67% (adults)). Forepaw stimulation - 1) As with CD stimulation, the proportion of nigral neurons responding to forepaw stimulation increased with age (48% (12/25) in adults vs. 30% (25/78) at 1-10 days). 2) Responses shifted from a predominant initial inhibition to a predominant initial excitation with age (16% (4/24) excitation at 1-40 days, 75% (9/12) excitation in adults). At all age levels, a high proportion of units responded to each of the three inputs tested (CX, CD, and paw). (Supported by USPHS HD 05958 and 1T32MH15345).

100.17 EFFECTS OF DOPAMINERGIC, CHOLINERGIC AND GABERGIC DRUGS UPON THE ACTIVITY OF RAT GLOBUS PALLIDUS NEURONS. D.A. Bergstrom* and J.R. Walters (SPON: D.B. Tower). NIH, NINCDS, Bethesda, MD 20205.

Dopamine (DA), acetylcholine (ACH) and GABA are known to be neurotransmitters in the basal ganglia. Extensive pharmacological studies have been performed on the effects of several agonists and antagonists of these transmitters upon the activity of neurons in the rat substantia nigra and neostriatum. The present study examines the responsiveness of cells in the globus pallidus (GP) to the effects of systemically administered pharmacological agents which are commonly used to explore DA, ACH and GABA influences.

Extracellular single unit recordings were made from GP cells in rats paralyzed with gallamine, locally anesthetized and artificially respired. Spontaneously active cells with biphasic action potentials in the frequency range of 10-140 spikes/sec were recorded. One cell was monitored per rat; drugs were given i.v. To investigate the effects of DA receptor stimulation and blockade, 3 doses of apomorphine (APO) were given at 3-5 min intervals; haloperidol (HAL) was administered alone or after APO. Five out of 15 cells showed a dose-related, HAL-reversible increase in firing to an average of 15%, 47% and 92% after 5, 35 and 280 µg/kg APO, respectively. The remaining 10 cells were not significantly affected. HAL (0.025-1.6 mg/kg), in rats pretreated with APO, produced a dose-related inhibition (90-100%) in 10 out of 11 cells. If HAL was given alone, however, a cumulative dose regimen of 1.6 mg/kg caused only a slight 13% decrease (n=8); a cumulative dose of 3.2 mg/kg HAL produced a maximum of 36% decrease in GP neuronal firing (n=6). Thus it appears that APO may be causing a subtle change in the modulation of dopaminergic influence on GP transmission that is greatly enhancing the ability of HAL to inhibit firing.

In 6 out of 8 cells recorded in atropine methyl nitrate pretreated rats, logarithmically increasing doses of carbachol (4 µg/kg to a total dose of 256 µg/kg) caused dose-related increases in firing (at 32 µg/kg: 50-100%). Two cells showed no change. The GABA agonist muscimol caused a dose-dependent, picrotoxin (PIC) reversible inhibition of the GP cells recorded (n=7). An average dose of 1.5 mg/kg muscimol produced a 50% inhibition in neuronal firing. PIC, given alone in 1 mg/kg increments every 2 min caused dramatic dose-related increases (4 mg/kg: 250%) in the firing of these cells (n=6).

These observations demonstrate that there are populations of neurons in the rat GP that appear to be sensitive to alterations in DA, ACH and GABA-related transmission. Whether these changes are direct effects on the neurons themselves or mediated indirectly through inputs to the GP remains to be determined.

100.18 d-AMPHETAMINE-INDUCED CHANGES IN NEURONAL FIRING IN RAT GLOBUS PALLIDUS. J.R. Walters and D.A. Bergstrom*. NIH, NINCDS, Bethesda, MD 20205.

Several investigators have shown that systemic administration of d-amphetamine (AMP) produces a decrease in the spontaneous firing rates of neurons located in the rat substantia nigra pars compacta (SNpc) and neostriatum. This effect has been shown to be reversed by haloperidol (HAL) and, in the SNpc, blocked by pretreatment with α-methyl-p-tyrosine (AMPT). We have been interested in determining whether AMP, administered systemically, affects the activity of cells in another nucleus of the basal ganglia, the globus pallidus (GP).

Extracellular single unit activity was recorded from spontaneously active cells in the GP of rats paralyzed with gallamine, locally anesthetized and artificially respired. AMP was given i.v. at 2 min intervals in logarithmically increasing doses (0.2 to 6.4 or 12.8 mg/kg). The firing rates of 17 out of 19 cells were significantly increased (>30%), one cell was unaffected (<30%) and one cell showed a dose-related decrease. Two types of AMP-induced increases were observed. Half of the cells showed a dose-related increase in rate and the rest responded markedly to the lower doses of AMP (0.8 m/kg or less) and maintained this enhanced rate throughout the remaining drug regimen. Lower frequency cells (<50 spikes/sec, n=13) were significantly more sensitive to the rate-increasing effects of AMP than were the higher frequency firing cells (>50 spikes/sec, n=4).

To examine the effects of HAL (i.v.) on AMP-induced increases in pallidal activity, rats were given AMP (cumulative dose: 0.8 to 3.2 mg/kg) until the monitored firing rate of the GP cell was 50-100% above baseline (n=5). HAL (0.2-0.4 mg/kg) antagonized the rate-elevating effect of AMP and returned the firing rates to baseline control. However, HAL, in doses up to 1.6 or 3.2 mg/kg, did not antagonize AMP's effect on 2 cells in rats given a cumulative dose of 12.8 mg/kg AMP.

To investigate the dependence of AMP's effect on catecholamine (CA) synthesis and release, GP cells were recorded in rats pretreated with reserpine and AMPT. Results (n=4) indicated that interruption of CA synthesis and release attenuated the rate-elevating effects of AMP.

This study indicates that systemically administered AMP can markedly enhance the firing rates of a population of GP cells in gallamine-paralyzed rats. This effect is reversed by HAL and attenuated by CA depletion. The effect of AMP differs from that of systemically administered apomorphine (Bergstrom & Walters, this vol.). AMP caused a greater and more consistent increase in pallidal firing rates and did not enhance the ability of HAL to inhibit pallidal firing to below predrug activity as did apomorphine.

101.1 MORPHOLOGY OF ELECTROPHYSIOLOGICALLY IDENTIFIED TYPES OF MYENTERIC NEURON: INTRACELLULAR RECORDING AND INJECTION OF LUCIFER YELLOW IN THE GUINEA-PIG SMALL BOWEL. S.M. Erde*, M.D. Gershon, and J.D. Wood. Dept. Anat., Columbia Univ. P.&S., New York, 10032. Dept. Physiol., Sch. of Med., Univ. Nevada, Reno, NV 89557.

The morphology of physiologically identified neurons of the myenteric plexus has not yet been described. Hodgkiss and Lees (J. Physiol. 285:19P, 1978) reported the intracellular injection of cells with Procion Yellow; however, they did not inject the dye through the electrode that was used for electrical recording but attempted to re-impale cells. In the present study electrical activity of myenteric ganglion cells was recorded intracellularly with glass microelectrodes filled with the fluorescent dye, Lucifer yellow, and beveled to resistances of 80 to 100 megohms. Presynaptic fibers and the processes of the neurons were activated by electrical shocks delivered by a 20 um Pt electrode positioned on an interganglionic connective. Neurons were identified electrophysiologically by their responses to injection of depolarizing current and to stimulation of their synaptic input. Lucifer Yellow was injected into the cells by iontophoresis from the recording electrode, and the morphology of injected cells was studied by fluorescence microscopy. Three types of neuron were identified on the basis of electrophysiological criteria. These were AH/Type 2 neurons, S/Type 1 neurons (Hirst et al., J. Physiol. 236:303,1974; Nishi and North, J. Physiol. 231:471, 1973) and a third type that had a relatively low input resistance, a resting potential of 65mV, but which did not discharge action potentials when injected with a depolarizing current. These latter cells showed large amplitude EPSPs (15mV) without spikes in response to stimulation of their synaptic input. There were two morphological classes of S/Type 1 neurons. One class had short club-like dendrites with a single long axon that projected via interganglionic connectives through two or more ganglia in the aboral direction. The second class of S/Type 1 neuron had oval somas with many fine, varicose processes that projected circumferentially around the bowel both inside and outside of the ganglion of origin. AH/Type 2 neurons had oval somas with two or more processes that projected both longitudinally and circumferentially. Processes ramified extensively within the ganglion of origin but also projected to other ganglia. Some of the processes of AH/Type 2 neurons did not exit the ganglion in fiber tracts; instead, they passed through the periganglionic sheath as single processes and terminated as swellings a short distance from the ganglion. These may be projections to other intestinal layers that were severed during the dissection of the myenteric plexus. The morphology of the third type of neuron was similar to that of AH/Type 2 neurons. Supported by NIH grants NS 12969, AM16813. NS07062.

101.2 ULTRASTRUCTURAL IDENTIFICATION OF NORADRENERGIC AXONS IN THE ENTERIC PLEXUSES OF THE GUINEA-PIG ILEUM. I.J. Llewellyn-Smith*, A.J. Wilson*, J.B. Furness*, M. Costa* and R.A. Rush* (SPON: P.R. Wilson). Centre for Neuroscience, Flinders Univ. Sch. of Med., Bedford Park, S.A. 5042 AUSTRALIA.

Noradrenergic axons in the myenteric and submucous plexuses of guinea-pig ileum were identified by electron-dense deposits in their vesicles after loading with 5-hydroxydopamine (5-OHDA; Tranzer, J.P. and Thoenen, H., Experientia 23:743, 1967), localization of dopamine-β-hydroxylase (DBH) with horseradish peroxidase-conjugated antibodies (Rush, R.A. et al., In Catecholamines:Basic and Clinical Frontiers, Usdin, E.et al., eds., p. 331, 1979) or the chromaffin reaction (Tranzer, J.P. and Richards, J.G., J. Histochem. Cytochem. 24:1178, 1976).

All three cytochemical techniques showed that noradrenergic axons in myenteric plexus contained many flattened vesicles (usually more than 30% of the total number of vesicles) in addition to oval or irregularly shaped vesicles. Cytochemically labeled profiles were absent in myenteric plexus from surgically denervated segments of ileum and pretreatment with 6-hydroxydopamine greatly reduced the number of labeled profiles.

In normal submucous plexus after the chromaffin reaction and 5-OHDA loading electron-dense deposits were found in the vesicles of some axon profiles which contained many small round and a few flattened and large round vesicles. Other profiles with similar types and proportions of vesicles were not cytochemically marked. After anti-DBH labeling, noradrenergic axon profiles contained more flattened vesicles than after the other treatments. All reactive axon profiles disappeared from the submucous plexus after extrinsic denervation. Axon profiles which contained small granular vesicles with rings of electron-dense material lining the inner aspects of vesicle membranes after conventional processing were not cytochemically labeled and persisted after extrinsic denervation.

In the myenteric plexus noradrenergic axon profiles were found frequently near the edges of ganglia, sometimes near nerve cell bodies and rarely in the internodal strands. In the submucous plexus noradrenergic profiles occurred randomly throughout submucous ganglia. No synapses between noradrenergic axons and nerve cell bodies or processes were observed in the myenteric plexus. Synapses between noradrenergic axons and nerve cell processes were occasionally found in the submucous plexus.

In conclusion, noradrenergic axons in myenteric plexus can be distinguished in conventionally processed tissue on the basis of morphological criteria alone whereas noradrenergic axons in the submucous plexus can only be identified by cytochemical staining procedures.

101.3 LONG TERM SURVIVAL OF SEROTONERGIC NEURONS IN TISSUE CULTURES OF MYENTERIC PLEXUS DISSECTED FROM GUINEA PIG ILEUM AND DUODENUM. M.D. Gershon, S.M. Erde and C.F. Dreyfus, Department of Anatomy, Columbia University, P&S, New York, NY 10032.

The enteric nervous system has been shown to contain serotonergic neurons. These neurons have been found to survive in organotypic tissue cultures derived from hemisections or muscularis externa of mouse intestine (Dreyfus et al., Brain Res.128: 109-123, 1977). The enteric serotonergic neurons are thus intrinsic to the gut. The present experiments were designed to determine if enteric serotonergic neurons would survive in cultures of longitudinal muscle with adherent myenteric plexus (LM-MP) stripped from guinea pig duodenum or ileum. Guinea-pig fetuses were removed just prior to the expected date of delivery; the gut was removed and the LM-MP was dissected. Flat sheets of tissue were explanted onto collagen-coated coverslips and grown as lying-drop preparations in Maximow depression slide chambers. After 2 weeks' growth in culture the cultures were exposed to glyoxylic acid for examination by histofluorescence, or incubated with ^{3}H-serotonin (0.9 uM; 60 min) and fixed (2.5% glutaraldehyde, containing 3% sucrose in 0.2M phosphate buffer at pH 7.4) for light and electron microscopic radioautography. Numerous cells were found showing the characteristic glyoxylic acid-induced fluorescence of serotonin. More such cells were found in cultures of ileum than duodenum. Uptake of ^{3}H-serotonin was found in axons and in some mast cells in all cultures. Ganglion cells survived well in culture and retained synaptic connections; however, after 2 weeks smooth muscle cells were no longer discernible. It is concluded that intrinsic enteric serotonergic neurons can be grown in culture even from relatively mature guinea pig small intestine. The presence of these neurons in cultured LM-MP strips confirms that the myenteric plexus contains serotonergic perikarya. As has been observed previously, the serotonin uptake mechanism in these neurons is probably confined to their axons. These long term cultures provide a stable preparation of myenteric plexus, devoid of inputs from the CNS or submucosal plexus, that should prove useful for further neurochemical or electrophysiological characterization of the myenteric plexus. (Supported by NIH grants NS12969 and NS07062.)

101.4 BIOSTRUCTURE AND BIOCHEMISTRY OF THE HAMSTER SUPERIOR CERVICAL GANGLION (SCG). A. C. Black, Jr., J. Jew, J. K. Wamsley, D. Sandquist*, J. R. West, and T. H. Williams. Department of Anatomy, University of Iowa College of Medicine, Iowa City, Iowa 52242.

Electronmicroscopy of the small, intensely fluorescent (SIF) cells of the hamster SCG indicates that they receive putative cholinergic afferent synapses. They also give rise to axodendritic and somatodendritic efferent synaptic contacts with the dendrites of principal ganglionic neurons (PGNs). Quantitative Fluorescence microscopy (FM) of five hamster SCG demonstrated that there were 223 ± 31 SIF cells per ganglion, equivalent to 140 ± 26 SIF cells per mg tissue (wet weight). SIF cells with long axons are found among clusters of SIF cells near blood vessels in hamster SCG, as in rat and guinea pig SCG.

Cyclic AMP has been shown to be a second messenger in SCG function. Incubation of hamster SCG in vitro with 50 μM L-isoproterenol under conditions producing maximal cyclic AMP production increased cyclic AMP values by 522% over controls, but no response to 50 μM dopamine (DA) was noted. Thus, like rat and guinea pig SCG, the hamster SCG contains a β-adrenergic receptor–adenylate cyclase complex, but lacks a dopamine receptor–adenylate cyclase complex. DA and norepinephrine (NE) levels were determined in hamster SCG. The ratio of DA to NE in the hamster SCG was 0.08. This low ratio is similar to values obtained in guinea pig SCG (where the SIF cells contain NE as a transmitter). By comparison, the rabbit SCG (where SIF cells contain DA as a transmitter) exhibits a DA to NE ratio of 0.678. Thus, while not conclusive evidence, the low DA to NE ratio in the hamster SCG suggests that NE may be the SIF cell transmitter in the hamster SCG. Comparison of these results with those obtained in other species suggests that the SCG of three rodents - the hamster, guinea pig, and rat - differ significantly from those of other species in their morphological and biochemical properties. (Supported by N.I.H. grants NS-11650 to T.H.W., HL-24351 to A.C.B., and HL-21914 to J.J.)

101.5 THE NUCLEUS TRACTUS SOLITARIUS OF THE CAT: A GOLGI ANALYSIS. T.J. Mullett*, B.E. Maley and R.P. Elde. Department of Anatomy, University of Minnesota Medical School, Minneapolis, MN 55455.

In a series of adult cats, the Golgi-Kopsch method was employed to impregnate a number of neurons within the nucleus tractus solitarius (NTS). Subsequent to routine histological procedures, representative cells from the commissural, intermediate, lateral, medial and ventrolateral nuclei of the NTS, which were named according to Loewy and Burton ('78), were drawn at a final magnification of 400x utilizing a drawing tube attached to a light microscope.

Cell soma size in most areas of the NTS ranged from 10-20 µm. In addition to the many small neurons, the ventrolateral nucleus contained a few large cells which measured over 25 µm in diameter. Most NTS neurons were multipolar and possessed short, proximal dendrites that were relatively smooth and most often lacked spines. These dendrites, in turn, gave rise to numerous secondary branches, which in many cases were longer than their parent structure. The dendritic arbor of most NTS neurons extended over 150 µm, while the dendritic spread of the large cells in the ventrolateral nucleus was greater. Although the majority of dendrites remained within the confines of the nucleus, a small number of peripheral neurons sent their dendrites outside the NTS into the surrounding areas. In addition, several NTS neurons bordering the tract of solitarius possessed dendrites which extended into the tract. These latter neurons were most often elongated and exhibited dendrites arising from the two opposite poles. Spines, when encountered, were restricted to the shafts of secondary dendrites and many times occurred in clusters at branching points of dendrites. The spine form included sessile, pedunculated and complex varieties. Somatic spines were not readily evident on NTS neurons. Axons of NTS neurons were found to arise from the cell soma and they could be followed for varying distances. Initially, these structures possessed a smooth contour, however, with increasing distance from their origin they became beaded. At no time could collaterals be seen arising from the parent axon.

The morphological aspects of this Golgi study will form a basis for analyzing the structure of neurons possessing one of the putative neurotransmitters within the NTS as visualized with immunohistochemistry. These studies should form a cytoarchitectonic basis for the neurotransmitter-specific synaptological studies now in progress.

R.P.E is a recipient of a Scholar in Neuroscience Award from the McKnight Foundation.

101.6 CAROTID SINUS NERVE AFFERENT PROJECTIONS TO THE NUCLEUS OF THE TRACTUS SOLITARIUS AND THE REGION OF THE NUCLEUS AMBIGUUS. R. O. Davies and M. Kalia. Dept. of Animal Biology, University of Pennsylvania, Philadelphia, PA 19104 and Dept. of Physiology, Hahnemann Medical College, Philadelphia, PA 19102.

As part of a study of peripheral sensory inputs to the pontomedullary respiratory complex we studied the distribution of carotid sinus nerve (CSN) afferent fibers (chemoreceptors and baroreceptors) using the transganglionic transport of horseradish peroxidase (HRP). The CSN was carefully dissected in anesthetized cats and a piece of parafilm was placed between it and the underlying tissue. 10 µl of 5-10% HRP solution in Triz buffer (pH 8.6) was injected into the uncut CSN using a 10 µ tip, glass micropipette coupled to a Hamilton Syringe. This procedure of "intraneural" injection of a peripheral nerve avoided problems associated with HRP application to a cut nerve: surreptitious uptake by surrounding nerve fibers and the effects of axonal degeneration on the transport process. Following a 48 hour survival period the brainstem and petrosal ganglion were removed and processed for demonstration of HRP reaction product using tetramethyl benzidine (TMB) as the substrate. HRP reaction product was visualized in sensory nerve terminals (extraperikaryal), nerve fibers (sensory and motor) and perikarya. Sensory nerve terminals were found in the nucleus of the Tractus Solitarius (nTS) bilaterally, with the ipsilateral side showing more intense labeling. Within the nTS the distribution of extraperikaryal labeling was non-uniform. The dnTS, dlnTS, mnTS and ncom showed heavy labeling. This corresponds to the general distribution previously reported (Neurosci. Lett. 14:153, 1979). Significant labeling was also seen in vlnTS, sg, nI and dorsal third of the area postrema. Another region showing moderate sensory nerve terminal labeling was identified ipsilaterally in the vicinity of the nucleus Ambiguus (nA) at the level of the obex to 2mm caudal to the obex. HRP labeled fibers from the nTS could be followed to this region of extraperikaryal labeling. HRP labeled perikarya were found in the rostral region of the nA (retrofacial nucleus) 5-6 mm rostral to the obex, thus confirming previous reports of brainstem efferents in the CSN. No sensory terminal labeling was found at these rostral levels. Furthermore the location of these efferent perikarya was congruent with the anatomically and physiologically identified Bötzinger Complex which projects to the vlnTS. (This research was supported by USPHS grants HL-17800, HL-23961, HL-08805 and RCDA HL-00103 to MK.)

101.7 BRAIN STEM CONNECTIONS OF GLOSSOPHARYNGEAL AND VAGAL AFFERENT FIBERS IN THE CAT. F. R. Calaresu, A. W. Hrycyshyn*, B. A. Flumerfelt and J. Ciriello. Departments of Physiology and Anatomy, University of Western Ontario, London, Canada N6A 5C1.

Brain stem projections of the glossopharyngeal and vagus nerves in the cat were studied by exposing these nerves to crystalline horseradish peroxidase for 4-10.5 hours. After a survival period of 24-120 hours transverse and horizontal frozen sections of the brain stem were processed according to the tetramethylbenzidine method. Labelled fibers from both nerves were found bilaterally in the solitary complex, and ipsilaterally in the ventral region of the external cuneate nucleus and in the medial portion of the nucleus praepositus hypoglossi. Within the solitary complex labelling was observed in the parvocellular, lateral, ventrolateral, medial, and commissural solitary nuclei. In addition, the glossopharyngeal nerve was found to project ipsilaterally to the rostral dorsal motor nucleus of the vagus, the nucleus insulae cuneate lateralis, the ventrolateral portion of the medial cuneate nucleus, the dorsal part of the nucleus interpolaris and caudalis of the trigeminal complex, and the dorsolateral nucleus medullae oblongata centralis. Finally, the area postrema was found to receive a distinct bilateral projection of vagal and an ipsilateral projection of glossopharyngeal fibers. These findings point to the existence of projections to the brain stem which are more widely distributed for the glossopharyngeal compared to the vagus nerve and provide useful information on the wiring diagram of visceral and tase projections to the central nervous system.

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101.8 CENTRAL AFFERENTS TO THE INTERMEDIATE NUCLEUS TRACTUS SOLITARIUS AND THE COMMISSURAL NUCLEUS OF THE VAGUS: A RETROGRADE TRANSPORT STUDY IN RAT AND RABBIT. D.A. Ruggiero, C. Ross, and D.J. Reis, Laboratory of Neurobiology, Dept. of Neurology, Cornell University Medical College, New York, NY 10021.

Baroreceptor and other cardiovagal afferents terminate in the intermediate third of the nucleus tractus solitarius (NTS) and the commissural nucleus of the vagus (CNV). We sought to compare the central projections to both of the cardiovascular subdivisions of the solitary complex and to areas of the medullary reticular formation (RF) underlying NTS.

Fifty to 100 nl of a 40% HRP solution (0.9% saline) were injected in the medulla of rats and rabbits. After 24 to 48 hrs the animals were perfused with 1.25% glutaraldehyde and 0.4% paraformaldehyde followed by a 15% solution of sucrose in phosphate buffer. Brains and selected segments of the spinal cord were sectioned at 50 µ and processed using the Mesulam technique.

Afferents to NTS in rat are similar to those of CNV. Projections arise from the posteromarginal zone and lamina X of spinal cord (SC), lateral reticular nucleus, A5 area of Dahlstrom and Fuxe (Acta Physiol.Scand. 62, 1964), locus ceruleus, paraventricular hypothalamus (PVN), and parabrachial nucleus (PBN). Afferents from PBN to NTS and RF are, however, primarily derived from the medial PBN and Kollicker-Fuse nucleus--an area which projects to the spinal cord (Ross, Ruggiero and Reis, Neurosci. Abstr., 1979). Projections to RF are more widespread and, for most nuclei, quantitatively greater than those to the overlying NTS. Major afferents to RF arise from SC, other parts of RF, cranial nerve nuclei, deep cerebellar nuclei, zona incerta, PVN and the central nucleus of the amygdala. Projections into NTS and RF of rabbits were similar.

To control for diffusion of the enzyme, HRP was injected into the nucleus gracilis (NG). Afferents to NG are different from those to NTS and are derived from SC lamina IV and V, spinal trigeminal nucleus, ventral hindbrain RF, red nucleus and cerebral cortex.

Projections from NTS/RF identified by anterograde transport of HRP primarily ascend via the central and lateral tegmentum. Labeled efferents from NTS/RF to the PBN mainly terminate in the dorsal PBN in contrast to the major source of PBN afferents to the NTS.

We conclude that sites of baroreceptor projection in NTS and CNV, are richly innervated by projections from restricted regions of brain and spinal cord. Some of these projections may serve to modulate baroreflex activity.

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101.9 **Afferent Projections to the Dorsal Motor Nucleus of the Vagus; HRP and Electrophysiological Studies.** R.C. Rogers, H. Kita, S. Shibata and D. Novin. Center for Ulcer Res. and Ed., UCLA, Los Angeles, CA. 90073; Department of Physiol. Kyushu Univ. Sch. Med. Fukuoka,812 Japan.

The afferent projections to the dorsal motor nucleus of the vagus (DMN) of the rat were determined using a combination of HRP histochemical and electrophysiological techniques. With bipolar stimulating electrodes palced on the cervical vagus, the dorsal medulla was exposed by removing the occipital skull plate. Recording electrodes filled with 2% HRP (Sigma, type VI), .3M NaCl and .2M tris buffer (pH 8.6) were directed under visual guidance toward the DMN. When clear antidromic activation of neurons occured following cervical vagal stimulation, small amounts of HRP were iontophoretically ejected through the recording electrodes. Subsequent histological processing of the HRP-injected brains revealed bilateral projections from the nucleus of the solitary tract, the parvo- and giganto- cellular reticular nuclei of the medulla, the nucleus raphe obscuris and the paraventricular nucleus of the hypothalamus. A weak ipsilateral projection from the principal trigeminal sensory nucleus was also observed. Control injections of HRP into the solitary nucleus revealed only local reticular inputs. In seperate experiments, the physiological nature of projections from the paraventricular nucleus to the DMN was examined. Neurons in the paraventricular nucleus of the hypothalamus were antidromically activated by stimulating the DMN. Antidromic activation of the paraventricular nucleus followed the medullary stimulus with a latency of about 50msec. and these neurons would follow stimulus frequencies of up to 200/sec. Identified neurons in the DMN were, in turn, orthodromically activated by paraventricular stimulation with a latency of about 50 msec.

These data verify and extend the results of previous investigators (Saper, et al, Brain Res. 117:305,1976; Swanson and Hartman, Neurosci. Abs. 5:235,1979.) with regard to direct hypothalamic - autonomic projections.

This project was supported by NSF/JSPS exchange grant #NSF-INT 78-17733.

101.10 PROJECTION OF BUFFER NERVES TO THE SOLITARY COMPLEX IN THE CAT. J. Ciriello, A. W. Hrycyshyn* and F. R. Calaresu. Departments of Physiology and Anatomy, University of Western Ontario, London, Canada N6A 5C1.

Previous experiments have suggested that primary afferent fibers of the buffer nerves project primarily to the middle third of the nucleus tractus solitarius. However, the precise distribution of buffer nerves within this region remains controversial. The location of second order neurons of carotid sinus (CSN) and aortic depressor (ADN) nerves in the solitary complex of the cat was determined in this study using anatomical and electrophysiological methods. Using the anterograde transport of horseradish peroxidase it was found that both the CSN and ADN had a wider distribution in solitary complex than previously suggested. Within the solitary complex both buffer nerves projected bilaterally to the dorsal portion of the lateral and medial solitary nuclei, the commissural solitary nucleus and the medial aspect of the parvocellular solitary nucleus along the lateral edge of the area postrema, with a predominant ipsilateral projection. Exclusive projections of the CSN were found bilaterally to the intermediate solitary nucleus, and ipsilaterally to the ventrolateral solitary nucleus and the area just ventral to the solitary complex. These areas receiving primary afferent fibers within the solitary complex were explored for single units responding to stimulation of the buffer nerves in chloralosed, paralyzed and artificially ventilated cats. Of 177 responsive units, 80 responded only to stimulation of the carotid sinus nerve, 44 only to stimulation of the aortic depressor nerve, and 53 to both nerves. These data demonstrate that second order neurons have a wide distribution in the solitary complex and receive inputs from either one or both buffer nerves, pointing to the existence of separation of central pathways carrying CSN and ADN afferent information.

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101.11 DISTRIBUTION IN THE BRAINSTEM OF NEURONS WITH EFFERENT PROJECTIONS IN THE CERVICAL VAGUS: AN HRP STUDY IN THE DOG. C.L. Chernicky, K.L. Barnes, J.P. Conomy and C.M. Ferrario. Division of Research and Department of Neurology, Cleveland Clinic Foundation, Cleveland, Ohio 44106.

The distribution of cell bodies with axons in the cervical vagi was examined in the brainstem of the dog using retrograde axonal transport of horseradish peroxidase (HRP). The sheath of the cervical vagosympathetic trunk was opened 3-4 cm distal to the nodose ganglion and the proximal cut end of the vagus was slipped into silastic tubing filled with 30% HRP (Sigma, Type VI) in acrylamide gel; the sheath was closed around the package with a continuous 6-0 suture. After 5-7 days the dogs (wt. 6-9 kg) were perfused for HRP histochemistry. Serial 60 µm sections from C_1 to the caudal pons were processed for HRP with the tetramethylbenzidine procedure (Mesulam, J. Histochem. & Cytochem. 26: 106, 1978) and examined for HRP reaction product using both bright and dark field illumination.

Only a few lightly labelled neurons could be found in the brainstem after 5 days survival. On the other hand, numerous cells containing HRP reaction product were observed after 7 days. Thus the rate of transport of HRP by axons in the dog's vagus appears to be slower than that reported for other species. Although heavy labelling was observed in neurons within the nodose ganglion, no evidence could be found within the brainstem of transganglionic transport of HRP.

Labelled neurons were seen in the brainstem ipsilateral to the HRP-treated vagus. The majority of cell bodies containing HRP reaction product were found in either the dorsal motor nucleus of the vagus (DMV) or the nucleus ambiguus (NA). Labelled cells were distributed quite homogenously throughout the rostral-caudal extent of the DMV. On the other hand, the majority of cells seen within NA were found between 0.5 and 3.0 mm anterior to the obex. In addition, a few labelled cells were observed in the intermediate zone between DMV and NA described by Geis and Wurster (Brain Res. 182: 19, 1980). A few small neurons containing HRP reaction product were also seen ventrolateral to NA, approximately 3 mm anterior to the obex.

These studies reveal that localization of neural pathways using HRP in the dog may require longer survival times than in other species. Nevertheless, the distribution within the dog's brainstem of neurons with axons in the cervical vagus appears similar to that previously reported in the cat (Geis and Wurster, 1980; Sugimoto et al, Neurosci. Lett. 12: 53, 1979).

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101.12 SITES OF ORIGIN OF EFFERENT FIBRES OF VAGUS, GLOSSOPHARYNGEAL AND CAROTID SINUS NERVES IN THE CAT. A. W. Hrycyshyn*, J. Ciriello, B. A. Flumerfelt and F. R. Calaresu (SPON: J. Kiernan). Departments of Anatomy and Physiology, University of Western Ontario, London, Canada N6A 5C1.

Cells of origin of vagus, glossopharyngeal and carotid sinus nerves were localized utilizing the retrograde horseradish peroxidase (HRP) tracing method. Following application of crystalline HRP for 4 to 10.5 hours to the proximal end of cut nerves, cats were allowed to survive from 24 to 120 hours and their brain sections were processed according to the tetramethylbenzidine method. Following application of HRP to the left vagus transected at lower cervical levels, almost all the cells within the ipsilateral dorsal motor nucleus of the vagus nerve were labelled. No such labelling occurred on the contralateral dorsal motor nucleus. HRP labelled cells were also observed within the ipsilateral nucleus ambiguus and bilaterally within the retrofacial nucleus. When HRP was applied to the right cervical vagus, labelling of cells was observed bilaterally within the dorsal motor nucleus of the vagus and nucleus ambiguus with a predominant projection to the ipsilateral side. Labelling of cells in the retrofacial nucleus was similar to that seen when HRP was applied to the left vagus. Following application of HRP to the cut glossopharyngeal nerve at a level distal to the emergence of the carotid sinus nerve, labelled cells were found ipsilaterally within and about the rostral nucleus ambiguus and within the facial and retrofacial nuclei. A few labelled cells were seen within the ipsilateral gigantocellular-tegmental field. When only the carotid sinus nerve was exposed to HRP, cell labelling occurred bilaterally in the nucleus ambiguus, facial and retrofacial nuclei, with labelled cells predominating on the ipsilateral side. Few cells were also seen in the ipsilateral gigantocellular-tegmental field. These results demonstrate that the right vagus nerve contains preganglionic fibres originating from both dorsal motor nuclei of the vagus and nuclei ambiguus, while the left vagus nerve originates only from the ipsilateral side. Furthermore, the finding of labelled cells in the medulla after application of HRP to the carotid sinus nerve suggests a possible route by which brain stem structures may modulate activity of carotid baro- and chemoreceptors.

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101.13 HISTOCHEMICAL ANALYSIS OF VAGAL EFFERENT NEURONS BY DUAL STAINING FOR HORSERADISH PEROXIDASE AND ACETYLCHOLINESTERASE.
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Several recent studies have shown that the vagal innervation of the heart originates not only from the dorsal motor nucleus (DMN) but also from the nucleus ambiguus (AMB) and neurons located between these nuclei or ventral to AMB. Although it is well accepted that the DMN and AMB contain cholinergic neurons, the neurochemical characteristics of the extranuclear vagal efferents are not established. To examine this problem, rat vagal efferent neurons were labeled with horseradish peroxidase (HRP), and tissue sections through the medulla oblongota were subsequently stained for both HRP and acetylcholinesterase (AChE).

The loading of neurons with HRP was accomplished by sectioning the vagus nerve just below the larynx and placing the proximal end in a small trough containing 30% HRP (type VI) in 0.85% NaCl. After 45-60 min. the nerve was removed from the HRP solution, and the animal was allowed to survive for 24 h. The brain was then fixed and processed according to methods described by Mesulam (J. Histochem. Cytochem 24:1281-1286, 1976). The HRP was visualized as blue granules, and AChE positive neurons stained reddish brown. The vast majority of HRP-labeled neurons were found in the DMN, and these also contained AChE. Similar dual-labeled neurons were found in the nucleus commissuralis and nucleus intercalatus. The amount of AChE stain in all these neurons ranged from light to very dark. Several neurons within the AMB proper also stained for both HRP and AChE, and the AChE stain in these cells was quite dark. A few cells were found outside of the major nuclear groups. In general these stained only lightly for AChE or not at all. The absence of AChE stain in some of these neurons raises the possibility that they may not be cholinergic. The present findings indicate that cholinergic neurons constitute the vast majority of vagal efferents, but a small number of non-cholinergic medullary neurons may also contribute axons to the vagus nerve. (This study has been supported by a grant from the American Heart Association, Tennessee Affiliate.)

101.14 THE SEGMENTAL ORGANIZATION OF PARASYMPATHETIC PREGANGLIONIC NEURONS AND SOMATIC NEURONS IN THE SACRAL PORTION OF THE MAMMALIAN SPINAL CORD.
I. Nadelhaft, W. C. deGroat, C. Morgan, and S. Ames* VA Hosp. & Depts. of Neurosurg. & Pharmacol., Univ. of Pittsburgh Med. Sch., Pittsburgh, Pa.

What is the relationship between the location of a cell body within the spinal cord and the root through which its axon leaves the cord? In a recent study, Rubin and Purves (1) showed that, contrary to earlier reports, sympathetic preganglionic axons in the thoracic rami arise from cell bodies located in the corresponding cord segments. In the course of our study of the innervation of the pelvic viscera in the cat, we have attempted to increase the labelling efficiency of the sacral parasympathetic nucleus (SPN) by applying HRP to the central cut ends of sacral ventral roots. These experiments also allowed us to study the segmental organization of the SPN and the ventral horn motor nuclei. In adult cats anesthetized with dial-urethane, ventral roots (single or alternate on opposite sides of the cord) were exposed to HRP. Subsequently, appropriate lumbar, sacral, and coccygeal spinal cord segments were sectioned and processed for HRP reaction product. Labelled neurons were always located ipsilateral to the exposed root and, on the basis of morphology (size and shape) and position, could be separated into two major groups (parasympathetic and somatic) each of which was further divided into subgroups; parasympathetic: dorsal band, lateral band (2); somatic: lateral motor group, Onuf's group X, medioventral motor group. The rostrocaudal position of all those cells labelled via a particular root was found to be shifted in a rostral direction relative to the segmental boundaries (defined by gross spinal rootlet entry zones). On the average (16 cases) the cells first appeared 0.9 mm rostral to the caudal boundary of a segment and extended into the next rostral segment by 1.1 mm. We found no evidence that axons travelled more than one segment before exiting through a spinal root. Our results support the hypothesis that in the sacral region the spinal cord is organized so that cell bodies, whose efferent axons lie within a particular spinal root, are located ipsilateral to that root and, except for a slight rostral shift, within the cord segment associated with that root.

1. Rubin, E., and Purves, D., Neurosci. Abst. 5, 49 ('79)
2. Nadelhaft, I., deGroat, W. C., & Morgan, C., J. Comp. Neurol., in press

101.15 FLUORESCENCE MICROSCOPY/HORSERADISH PEROXIDASE HISTOCHEMISTRY: A METHOD FOR STUDYING TWO NEURONAL SYSTEMS IN THE SAME SECTION.
Bang H. Hwang* and Terence H. Williams. Department of Anatomy, University of Iowa, Iowa City, IA 52242.

Although fluorescence microscopy (FM) has yielded much information about the extensive projections of CA neurons in the CNS, it does not provide detailed information about distribution of CA terminals in relation to the innervated cell bodies. Retrograde axonal transport of horseradish peroxidase (HRP) can be used to visualize HRP-positive cell bodies whose terminals have taken up HRP. Using FM and HRP techniques together can be valuable for demonstrating the neuroanatomical relationships between CA terminals and the neurons innervated by these CA terminals.

The steps in this procedure are as follows: (1) 24 hrs. after HRP administration, rats are pretreated with Nialamide (500 mg/kg, i.p.) 2-5 hrs. before sacrifice; (2) the animals are perfused through the aorta with 250 ml of 2% glyoxylic acid in Krebs-Ringers solution containing 10% sucrose at pH 7.2, saturated with a $95\%O_2$-$5\%CO_2$ mixture; (3) after dissecting out the tissue, immersing for 10 min. in Krebs-Ringer solution containing 10% sucrose, transferring to a pre-cooled chuck without mounting medium, and putting the tissue in a cryostat set at -25°C until the tissue becomes solid, the tissue is cut into 20 μm sections; (4) after mounting on a glass slide, the section is immersed in the fixative (1.5% paraformaldehyde-0.025% glutaraldehyde in phosphate buffer, pH 7.3) for 20 seconds; (5) the slide is then run through 3 changes of 1% glyoxylic acid in phosphate buffer at pH 7.2, containing 6.8% sucrose (de la Torre, 1979), before drying for 5 min. under a hair dryer; (6) the slide is then placed in a pre-warmed beaker containing mineral oil in an oven at 95°C for 5 min.; (7) after mounting with fresh mineral oil and cover slip, the section is ready for FM observation; (8) after photographing CA terminals and recording coordinates, the slide is immersed in acetone to remove mineral oil, rinsed in distilled water and processed for HRP localization according to Mesulam (1978); and (9) with the aid of the coordinates, the locations of previously photographed CA terminals are observed for HRP localization in the cell bodies. By matching up the FM image and HRP localization in the same section, the precise anatomical relationships of CA terminals to target neurons are established.

(Supported by NIH grant NS11650 to T.H.W.)

102.1

Withdrawn by Author

102.2 EFFECT OF PHENYTOIN ON 2-DEOXYGLUCOSE UPTAKE AND IONIC CHANNELS. I. Yokoi* and T. Yanagihara. (SPON: M. D. Muenter). Dept. of Neurol., Mayo Clinic and Mayo Med. Sch., Rochester, MN 55901.

Phenytoin (DPH) is a commonly used anticonvulsant. However, the anticonvulsive mechanism is not clearly elucidated. Although DPH binds to various membrane structure, high affinity or saturable binding has not been demonstrated. In the present study, the effect of DPH on the uptake of 2-deoxy-D-glucose (2-DG) into isolated nerve endings was investigated with and without depolarization of membrane by 25 μM veratridine or 60 mM K^+. We also evaluated the contribution of Ca^{2+} for depolarization and/or the effectiveness of DPH. Since synaptosomes have a high affinity uptake system for 2-DG and our investigation has shown the alteration of uptake by membrane depolarization, this model will be useful for the above purposes. Synaptosomes were prepared by Ficoll density gradient ultracentrifugation from rat cerebral cortex (Cotman and Matthews, Biochim. Biophys. Acta 249:380, 1971) and were incubated in the presence of [^{3}H]2-DG (0.1-2 mM) under various ionic conditions for 5 min at 25°C. After incubation, particle-associated 2-DG was recovered on filter membrane, and the radioactivity was determined. The data was analysed using Lineweaver-Burk plot. The effect of DPH was evaluated in the presence of 100 μM DPH.

In the presence of 1.8 mM Ca^{2+}, the Km and Vmax of 2-DG uptake was 0.45 mM and 1.07 pmol/mg protein/min, respectively. The elimination of Ca^{2+} enhanced the uptake by 35%. Ca^{2+} and 2-DG appear to be competitive in this system. Uptake of 2-DG was inhibited by 60 mM K^+ (20%) and by 25 μM veratridine (40%) in the presence of Ca^{2+}. The inhibition was 25% by 60 mM K^+ and 30% by veratridine from the enhanced uptake level in the absence of Ca^{2+}. Uptake of 2-DG was inhibited by 15% in a competitive manner by DPH in the presence of Ca^{2+} and 20% in the absence of Ca^{2+}. Depolarization by veratridine was significantly suppressed by DPH but not by depolarization with 60 mM K^+. This was also true in the absence of Ca^{2+}. The present investigation suggested that DPH exerts its effect through sodium channel and that Ca^{2+} facilitates the effect of DPH on depolarization caused by veratridine. (Supported by the grant NS-52327 from NIH).

102.3 BINDING OF DIAZEPAM AND PHENYTOIN IN RABBIT BRAIN IN VITRO. T. Yanagihara and I. Yokoi*. Dept. of Neurol., Mayo Clinic and Mayo Med. School, Rochester, MN 55901.

Previous investigation from this laboratory demonstrated binding of phenytoin to many subcellular and cellular fractions after incubation with brain slices in vitro but the binding was not high affinity or saturable (Soc. Neurosci. 5:578, 1979). With diazepam, under a similar experimental condition, the binding was observed in many subcellular fractions, but displacement by clonazepam and saturable binding were observed only with the synaptosomal and microsomal fractions. In the present investigation, binding characteristics and drug interaction of these two anticonvulsants were evaluated using isolated subcellular elements from rabbit brain in vitro. For this purpose, nuclei, synaptosomal membranes and microsomes were prepared by sucrose or Ficoll density gradient ultracentrifugation. Synaptosomal membranes were prepared according to Whittaker et al (Biochem. J. 90:239, 1964) and Cotman and Matthews (Biochim. Biophys. Acta 249:380, 1971). The binding assay was carried out by incubation of a subcellular element in the presence of [^{3}H]diazepam or [^{3}H] phenytoin and subsequent membrane filtration. The results were analysed by Lineweaver-Burk or Scatchard plot.

With [^{3}H]phenytoin, there was no specific high affinity binding in any of these subcellular fractions investigated. With [^{3}H]diazepam, the binding in nuclei was nonsaturable and nondisplaceable by clonazepam. The binding to other subcellular fractions studied here was displaced by clonazepam. The synaptosomal membranes showed higher binding capacity than microsomes. The synaptosomal membrane fraction prepared by the method of Cotman and Matthews showed the highest specific activity. With the thoroughly washed membrane fraction, the analysis suggested the existence of two separate binding sites with different affinity. The dissociation constant was tentatively calculated as 10.3 and 82.2 nM respectively, and the corresponding maximum binding site was 0.46 and 1.45 pmol/mg protein. This finding may indicate the presence of benzodiazepine receptor in more than one subcellular location or the presence of more than one endogenous ligands for benzodiazepine receptor. Phenytoin (100 μM) inhibited binding of diazepam up to 25% but only at a low concentration (less than 5 nM) of diazepam. Phenobarbital failed to inhibit binding of diazepam. There is no evidence at present that phenytoin exerts anticonvulsant action through benzodiazepine receptor. (Supported by the grant NS-52327 from NIH).

102.4 HARMALINE-INDUCED TREMOR: INTERACTION WITH THE BENZODIAZEPINE RECEPTOR AS A POSSIBLE MECHANISM OF ACTION. H. A. ROBERTSON. Department of Pharmacology, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

Harmaline (1-methyl-7-methoxy-3,4-dihydro-β-carboline), at doses of 10-30 mg/kg, i.p., produces tremor. Diazepam antagonizes both the tremorogenic effect of harmaline and the increase in cerebellar 3',5'-cyclic GMP elicited by harmaline. Inhibition of GABAergic neurotransmission also produces increases in cyclic GMP (Costa et al, 1975). I now report that harmaline displaces 3H-flunitrazepam from the benzodiazepine receptor. Furthermore, the concentration of harmaline in brain during tremor is sufficient to occupy at least some benzodiazepine binding sites.

3H-flunitrazepam binding assays were done in vitro on membrane fractions from mouse brain using 2nM 3H-flunitrazepam. Brain harmaline concentrations were determined by spectrophotofluorimetry. Although the affinity of harmaline for the benzodiazepine receptor is low (IC_{50}=600 μM), brain concentrations of harmaline produced by tremorogenic doses fali within a range (20-70 μM) where at least a small percentage (5-20%) of benzodiazepine receptors would be occupied. The time course of harmaline-induced tremor was also studied. A dose of 30 mg/kg i.p. produced tremor within 5 minutes at which time the brain concentration was 35 μM. Peak brain concentration (80 μM) was achieved at 30 minutes but tremor continued for more than 2 hours. At 4 hours the brain concentration had fallen to 12 μM and no tremor was evident. The results indicates that occupancy of only a small percentage of receptors may be necessary for production of tremor, suggesting either "spare receptors" or a specific population of receptors preferentially affected by harmaline. Paul et al (1979) have also shown that only a small fraction (25-30%) of benzodiazepine receptors need be occupied by diazepam to completely abolish pentylenetetrazol convulsions. These results suggest that harmaline might act by displacing an endogenous ligand for the benzodiazepine receptor, a ligand whose role is to facilitate GABAergic neurotransmission.

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Supported by the MRC of Canada.

102.5 THE ACTION OF AVERMECTIN Bla ON THE NEMATODE ASCARIS. I. S. Kass*, A. O. W. Stretton and C. C. Wang. Dept. of Zoology, Univ. of Wisconsin, Madison, WI 53706 and Merck Inst., Rahway, NJ 07065.

Avermectin Bla (AVM) paralyzes nematodes without causing hypercontraction or flaccid paralysis. We have investigated the site of action of AVM in Ascaris by using selective stimulation and differential drug application techniques. When a single excitatory motorneuron is stimulated directly, AVM does not affect its activity; however if the motorneuron is stimulated indirectly through interneurons, AVM inhibits transmission between interneurons and the motorneuron and no activation of the motorneuron occurs. This inhibition is reversed by picrotoxin. We believe that AVM acts via a gabanergic mechanism. This is supported by the fact that muscimol and piperazine, GABA agonists in Ascaris, both mimic the action of AVM; furthermore the effects of muscimol and piperazine are reversed by picrotoxin. The site of action of these three compounds was investigated in experiments in which they were applied only to the ventral nerve cord. In each case the transmission between interneurons and the motorneuron was blocked, but could be reversed by picrotoxin. The effects of AVM in Ascaris are consistent with its action in other systems. In the lobster Fritz et al. (Proc. Nat. Acad. Sci., 76:2062, 1979) showed that AVM eliminated IPSP's; Pong et al. (J. Neurochem. 34:351, 1980) showed that AVM caused the release of GABA from mammalian synaptosomes. Our hypothesis is that in Ascaris AVM causes the release of GABA, causing an inhibition of the interneuron to motorneuron pathway.

102.6 TISSUE CULTURE STUDIES OF KAINIC ACID NEUROTOXICITY IN THE ABSENCE OF GLUTAMATERGIC FIBERS. F.J. Seil and W.R. Woodward. Neurology Research, V.A. Med. Ctr. and Depts. of Neurology and Biochemistry, Univ. of Oregon Health Sci. Ctr., Portland, OR 97201.

On the basis of results from both animal and tissue culture studies, it has been suggested that the presence of glutamatergic afferent fibers is necessary for the manifestation of kainic acid neurotoxic effects. Cortical glutamatergic afferent fibers can be eliminated in cerebellar cultures by exposing explants to cytosine arabinoside during the first 5 days in vitro. Such exposure results in destruction of cerebellar granule cells, whose axons (parallel fibers) normally contact all other cerebellar cortical neurons, and whose neurotransmitter is believed to be glutamic acid. The purpose of this study was to determine whether or not kainic acid neurotoxicity was expressed in granuloprival cerebellar cultures.

Cerebellar explants derived from newborn mice were exposed to 5 μg cytosine arabinoside per ml nutrient medium for 5 days after explantation and then cultivated in normal medium. After 16 days in vitro, granuloprival cultures were exposed for 6 days to nutrient medium incorporating either 10^{-4}M kainic acid or 10^{-3}M D-glutamic acid. The latter is a metabolically inactive excitatory isomer of L-glutamic acid that is not neurotoxic to normal cerebellar cultures. Granuloprival explants exposed to D-glutamic acid also demonstrated no neuronal destructive effects, and the cortices of such cultures contained abundant large neurons and numerous neurites. By contrast, large cortical neurons and their axonal processes were markedly reduced or absent in granuloprival cerebellar cultures exposed to kainic acid, and reactive gliosis was prominent. Effects were similar to those obtained by exposure of normal cerebellar explants to 10^{-4}M kainic acid, and were repeated upon application of kainic acid to granuloprival cerebellar explants cultivated in low-L-glutamate medium. The results of this study suggest that kainic acid neurotoxic effects can be manifested in the absence of glutamatergic afferent fibers.

102.7 SUBSTANCE P DECREASES MEMBRANE POTASSIUM AND SODIUM CONDUCTANCES OF MOUSE SPINAL CORD NEURONS IN CELL CULTURE. L. M. Nowak and R. L. Macdonald. Neurosciences Program and Department of Neurology, University of Michigan, Ann Arbor, MI 48109.

The undecapeptide, substance P (SP), is a neurotransmitter candidate for a subpopulation of small diameter dorsal root ganglion (DRG) neurons in which SP-like immunoreactivity has been localized in vivo and in vitro. We have examined the ionic basis of SP-produced depolarization of mouse spinal cord neurons grown in primary dissociated cell culture and report that SP decreased membrane conductance to potassium ions (g_K) and to a lesser extent, sodium ions (g_{Na}).

Spinal cords and attached DRGs were dissected from 12-13.5 day old fetal mice, mechanically dissociated, plated on 35mm collagen-coated dishes and grown in culture at 35° for 4-8 weeks prior to electrophysiological investigation. Intracellular recordings were made from neurons bathed in buffered saline on the heated (35-37°), modified, stage of an inverted phase-contrast microscope, using one or two 4M potassium acetate-filled micropipettes. Peptides were applied directly to the neuronal surface by pressure pulses (1 sec at .25-3.0 psi) from blunt glass micropipettes (2-10μ tips).

SP produced dose-dependent (.5-20μM), slow (.75 sec latency), reversible depolarizations accompanied by decreased membrane conductance. SP-response amplitude increased when membrane potential was depolarized and decreased when membrane potential was hyperpolarized with steady polarizing currents. SP-responses had reversal potentials (RPs) that were more negative than resting membrane potential, suggesting that SP reduced either g_K or chloride ion conductance (g_{Cl}). However, intracellular and extracellular tetraethylammonium, which blocks g_K, eliminated or reduced SP-responses respectively. Moreover, SP-responses recorded with 3M potassium chloride-filled micropipettes (which shift chloride equilibrium potential to about -20mV) were increased or unchanged. These data indicated that SP reduced g_K, rather than g_{Cl}, to depolarize membrane potential. This hypothesis was further tested by manipulating the extracellular potassium concentration ($[K^+]_o$). The RPs for SP-responses varied log linearly as a function of $[K^+]_o$ with a slope of -60 in high $[K^+]_o$ s (15, 20 and 40mM), however, in low $[K^+]_o$s (1, 5 and 10mM), RPs were more depolarized than predicted by the Nernst potential for potassium, indicating that another cationic conductance, probably g_{Na}, was also decreased.

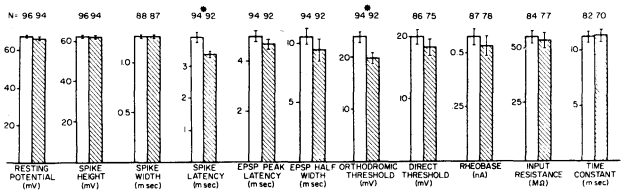
Thus SP depolarized membrane potential by reducing the univalent cation conductances of mammalian spinal cord neurons in cell culture.

103.1 COMPENSATION FOR LOSS OF SYNAPSES IN SENESCENT RAT HIPPOCAMPUS. C. A. Barnes and B. L. McNaughton. Institute of Neurophysiology, University of Oslo, Karl Johans Gate 47, Oslo 1, Norway.

Anatomical studies have revealed that the granule cells of the fascia dentata undergo a partial (27%) deafferentation as a result of senescence (Geinisman & Bondareff, Am. J. Anat., 145,129-136, 1976). The present report examines the physiological correlates of this process in order to determine how the input-output relations of the granule cells might be altered. The experimental techniques involved extracellular stimulation and recording in the awake unrestrained rat (male Long Evans; 31 aged 12 mo; 30 aged 30 mo), and both extra- and intracellular recording from granule cells in the in vitro transverse hippocampal slice preparation (male Wistar albino rats; 16 aged 10 mo; 16 aged 31 mo).

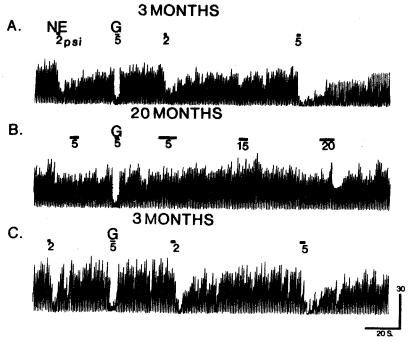
Compared with adult controls the senescent animals exhibited a significant reduction in both the perforant path excitatory synaptic field potential and in the afferent fiber response. Since there was no apparent change in the threshold for fiber activation these data support the anatomical literature indicating a loss of afferent synapses. For a given magnitude of afferent fiber response, however, the old animals exhibited a larger synaptic field potential, suggesting that the remaining synapses were in fact more powerful. Furthermore, the magnitude of the extracellular population spike was greater in the old animals when plotted as a function of extracellular EPSP amplitude.

Intracellular recording from a total of 190 stable cells revealed two statistically significant ($p < .05$) differences between age groups as seen in the Figure below (shaded histograms refer to the old population): a 17 percent reduction in the voltage threshold for synaptically elicited granule cell discharge, and a 13 percent reduction in the latency of the action potential. This pattern of results suggests that the locus of spike initiation may move into the proximal portions of the dendrites of old animals. Taken together these data indicate that the granule cells may compensate for a loss of synapses during senescence both by an increase in their electrical responsiveness to synaptic activation and by an increase in synaptic efficacy.



103.2 AGE-RELATED ELECTROPHYSIOLOGICAL CHANGES IN RAT CEREBELLUM. J. Marwaha, B. Hoffer*, R. Wyatt† and R. Freedman*. Departments of Pharmacology and Psychiatry, Univ. of Colo. Health Sciences Center, Denver, CO 80262, and †NIMH, St. Elizabeth's Hospital, Washington D.C. 20032.

The spontaneous discharge of cerebellar Purkinje neurons was studied in 3-, 12-, 15-, and 20-month-old rats. Fluphenazine and haloperidol administered intraperitoneally produced a dose-dependent increase in the spontaneous discharge in 3-month-old animals, but were ineffective in 12-, 15- and 20-month-old rats. Intraperitoneal administration of amphetamine resulted in a dose-dependent decrease in spontaneous Purkinje neuron discharge in 3-month-old rats without affecting neurons from the older animals. Disruption of norepinephrine afferents by 6-OHDA or reserpine increased spontaneous discharge rate in 3-month-old animals. This treatment did not affect Purkinje neurons in 12-, 15- and 20-month-old rats. Local application of drug with the same multibarreled electrode revealed that neurons from older rats were significantly less sensitive to inhibition by norepinephrine than were Purkinje neurons from 3-month-old animals. Similarly, application of GABA did not reveal a differential sensitivity (see figure). For locus coeruleus activation to produce 50% inhibition in Purkinje neuron discharge, significantly higher stimulation currents were required in 15-month-old rats than in 3-month-old animals. Our results suggest a decreased postsynaptic sensitivity to norepinephrine in 12-, 15 and 20-month-old rats as compared to 3-month-old animals.



Supported by DA 02429 and DA 07043.

103.3 A COMPUTER-ASSISTED STUDY OF NEURON NUMBER AS A FUNCTION OF AGE IN MOUSE BARREL CORTEX. C.A. Curcio* (SPONS: P.D. Coleman). Dept. of Anatomy, Univ. of Rochester Sch. Med. Dent., Rochester, N.Y. 14642

It is widely assumed that the aging process produces a substantial reduction of neurons in the brain. Studies showing cell loss in the cerebral cortex have actually demonstrated changes in packing density, since cortical volume changes with age are not well understood. The barrel is a unique feature of rodent somatosensory cortex, a morphological and functional subunit whose dimensions may be determined empirically; therefore cell counts in a barrel may indicate whether there is absolute cortical neuron loss with age. Thus, cells in one identified barrel, C3, have been counted in coded tissue from male C57Bl6 mice aged 4 to 30 months.

Barrels are most easily visualized in thick tangential sections at low power. In contrast individual cell types are best resolved in thin sections at high power. Therefore, a two-stage approach has been adopted in which data from a single barrel at different levels of histological processing and examined at different magnifications are integrated by a computer modelling and graphics system (Curcio and Sloan, in preparation).

Tangential 100 mu Vibratome sections through mixed aldehyde-fixed hemispheres are osmicated, dehydrated, and flat-embedded in plastic. Barrels are visible in these unstained preparations, and are photographed and mapped at low power. Barrel C3 is then resectioned at 1 mu for cell counts and is examined at high power. The barrel boundary seen in thick sections and profiles of neuronal nuclei drawn from thin sections are traced onto a digitizing tablet. The computer system then superimposes the barrel boundary on the cell map using common landmarks (blood vessels) and determines cell density within the barrel. Barrel cross-sectional area is also determined from the low power maps, and barrel height is determined from cortical tissue from the opposite hemisphere sectioned normal to the pial surface.

Supported by NIH training grant GM07136 to CAC and NIH grant AG1121 to PDC. Animals obtained from NIA colony.

103.4 FINE STRUCTURAL STUDIES ON THE AGING NEUROENDOCRINE AXIS: OBSERVATIONS ON THE PITUITARY AND ADRENAL. J.E. Johnson, Jr. Hitachi Scientific Instruments, Rockville, Md. 20850; Dept. Neurology, Johns Hopkins School of Medicine, Baltimore, Md. 21205; Section on Experimental Morphology, National Institute on Aging, GRC, NIH, Baltimore City Hospital, Baltimore, Md. 21224.

Several investigators have proposed that the hypothalamic-pituitary-target axis represents a key system in affecting the aging process. Dilman (1971) suggested that there is a gradual increase in the threshold of hypothalamic sensitivity to feedback suppression. A later study suggested that the pineal may modulate this feedback sensitivity (Walker and Bethea, 1977). If sensitivity loss as a function of age were affected by age related pineal deterioration, the pineal should have distinct morphological age changes which, indeed, it does (Johnson, 1980). Recently, Denckla (1974) proposed that the pituitary may modulate some aspects of aging by actively secreting a substance which decreases tissue response to thyroxine. Removing the pituitary (and thus removing the source of this unidentified hypothetical substance) has reduced age indices in the kidney (Johnson and Cutler, 1980). If the secretion of this substance is an actively maintained program for aging, one might predict that the pituitary would, itself, be protected from the more severe changes with age.

In the present study, male Fischer-344 rats, 4-36 mos of age were perfused with aldehyde fixative, the pituitary, adrenal and other tissues removed and processed for electron microscopy.

Cells in the adenohypophysis were found to show very little structural age change. Only occasional age pigment (probably lipofuscin) granules were seen. Cells in the neurohypophysis on the other hand, contained age pigments more frequently. In the aging adrenal gland, the situation appeared different. Both the adrenal cortical cells and the neuroectodermally derived cells of the medulla contained significant amounts of age pigment. Thus, the two glands, both containing hormone secreting cells as well as nervous tissue, show markedly different responses to age. The adenohypophysis, in fact, shows among the least structural age changes we have found in a variety of tissues examined.

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103.5 CAN RETINAL CELLS AGE GRACEFULLY? Ruth A. Trachimowicz and James W. Hinds. Dept. of Anatomy, Boston University School of Medicine, Boston, MA 02118.

In earlier studies (Lai et al., Invest. Ophthalmol. 17: 634, 1978; Weisse and Stötzer, Virchows Arch. (Path. Anat.) 362: 145, 1974) retinal aging was characterized both by a generalized thinning of the photoreceptor layer (ONL) throughout the retina and, in the far periphery, a selective loss of entire retinal layers; specifically, receptor inner and outer segments (IS+OS), ONL, and outer plexiform layer (OPL). The present study describes retinal aging in an age-graded series of males from 3 rodent strains: C57BL/6 mice (2 - 31 mos., median life span 31 mos.), AKR mice (4 - 14 mos., median life span 11 mos.), and COBS rats (6 - 31 mos., median life span 26 mos.). In general, the changes noted are mild compared to those previously described. No cysts were observed in either the central (C) or peripheral (P) loci sampled, and all the retinal layers, though reduced in thickness, were still intact at both loci. All 3 strains exhibit the most pronounced changes in the ONL, as decreased layer thickness (AKR: 40% C, 35% P; COBS: 26% C, 17% P; C57BL/6: no significant change) and/or decreased rod planimetric density (AKR: 43% C; C57BL/6: 14% C). Rod planimetric density has not yet been measured in COBS rats. A thinning of IS+OS was observed only in AKR mice. In none of the strains examined do the OPL, inner nuclear layer (INL), or inner plexiform layer change with age, and in the AKR and C57BL/6 mice INL cell counts at the central locus show no change in the planimetric density of either bipolar or amacrine cells. INL planimetric densities have not yet been measured in the COBS rats.

Preliminary results of synapse counts in the OPL of 3 young (4 mos.) and 3 old (14 mos.) AKR mouse retinas suggest a loss of rod synapses with age (from 883/1000 μm^2 to 488/1000 μm^2), while the number of cone ribbon synapses appears to remain constant (119 - 131/1000 μm^2). As a model for aging the retina thus differs from the olfactory bulb in its response to receptor cell loss: postsynaptic mitral cells in the olfactory bulb undergo a pronounced atrophy with advanced age (Hinds and McNelly, Soc. Neurosci. Abstr., in press), while retinal bipolar cells appear unaffected by a loss of receptor input. OPL synaptology will be analysed to determine what, if any, compensatory changes occur in receptor - bipolar contacts to maintain the bipolar cells following receptor loss (Do the sizes of the remaining rod spherules increase with age? Do the number of ribbon synapses/ rod spherule increase with age?

This research was supported by NEI grants EY-07022 and EY-01398 and NIA grants AG-00001 and AG-00020.

103.6 PURKINJE CELL DENDRITES IN AGED RATS: A MORPHOMETRIC GOLGI ANALYSIS. Joseph J. Pysh and Michael D. Benson*. Department of Anatomy, Northwestern University Medical and Dental Schools, Chicago, Illinois 60611.

The affect of aging on the structure of the neuron is of considerable neurobiological interest. The objective of this study was to examine the structure of Purkinje cell dendrites in inbred aged rats as demonstrated in Golgi-Kopsh preparations. The isoplanar nature of the dendritic tree makes the Purkinje cell uniquely suitable for morphometric analysis.

Four 3-month old and four 33-month old Fisher 344 female rats were used in this study. A total of 115 complete Purkinje cell dendritic trees were obtained for analysis. Fifty-one Purkinje cells from young animals and 64 cells from aged animals were evaluated.

Body weight and cerebellar weight were significantly greater in aged compared to young animals by 37% and 17%, respectively. Histometric analyses of sagittal sections of the cerebellar vermis revealed that vermis sectional area was 8.6% larger in aged rats which correlated with a 12% larger area of the granular layer and white matter. No differences were found in the thickness or sectional areas of the molecular layers of aged compared to young rats. This suggests possible atrophy of the molecular layer since previous studies in mice demonstrated continual growth, attributed primarily to glial hypertrophy, of both the granular and molecular layers from 3 to 8 months of age. The qualitative appearance of Purkinje cells in young and old animals were similar. Morphometric analyses of Purkinje cells revealed no significant differences in dendritic field size, branching density, total branch length or spine density. An analysis of frequency distribution histograms of Purkinje cell dendritic field size did not reveal any evidence of atrophy or hypertrophy in aged animals. A reduction of 10% in total numbers of spines per cell in Purkinje cells of old compared to young animals was found not to be statistically significant.

In conclusion, the present study establishes that aging is not associated with significant alterations in the dendritic tree of rat Purkinje cells.

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103.7 d-AMPHETAMINE BEHAVIORAL EFFECTS AS A FUNCTION OF AGE IN THE RAT M.M. Kilbey*, J.W. Moore, Jr.*, and W.C. Willson* (SPON: C.N. Jones). Psychology Dept., M.T.S.U., Murfreesboro, TN 37132. Behavioral effects of psychoactive drugs appear to be correlated with, and possibly to result from, action of drug upon neurotransmitter metabolism. Descriptive parameters of transmitter properties as a function of early development are relatively well known, and some recent work has begun to elucidate transmitter functions in aged animals. Differences in transmitter function might be expected to be expressed in differential behavioral effects in younger and older rats in comparison to those seen in young adult rats. In our laboratory, the effects of d-amphetamine upon general activity, startle response, stereotypy and stimulus properties have been measured in male and female Long-Evans rats as a function of age (3,12 and 24 months). Results from these experiments indicate a complex pattern of differences in d-amphetamine induced behavioral changes as a function of age. The degree of stereotypy induced by 8 mg/kg d-amphetamine was not found to vary as a function of age. Startle response to an air puff stimulus, in younger animals under drug conditions (0,4 and 3 mg/kg), were significantly augmented as a function of dose while aged animals showed no dose-response effect. Four mg/kg d-amphetamine significantly increased activity measures in aged animals, but not younger Ss. In the stimulus properties paradigm, age differences were found in the rate of acquisition of stimulus control of behavior by .5 and 1.0 mg/kg d-amphetamine as well as in generalization functions once stimulus control had been established. These results are discussed in terms of their implications for drug/behavior relationship differences in rodents as a function of age and the utility of age as a variable in animal models of abnormal behavior.

103.8 ELECTROPHYSIOLOGICAL ANALYSIS OF REACTIVE SYNAPTOGENESIS IN THE DENTATE GYRUS OF THE AGED RAT. E. W. Harris, S. W. Scheff and C. W. Cotman. Dept. Psychobiology, Univ. California, Irvine, CA 92717 and Dept. Anat., Med. Center, University of Kentucky, Lexington, KY 40536.

The hippocampal formation has been used extensively to study the response of the mammalian central nervous system to damage. After removal of the largest extrinsic input to the hippocampal dentate gyrus, arising from the ipsilateral entorhinal cortex, there is a pronounced increase in the terminal fields of remaining afferents. Using anatomical techniques, it has been shown that aged animals subjected to unilateral entorhinal lesion have a reduced capacity to sprout. The work presented here is an electrophysiological analysis of the normal and reorganized dentate gyrus in young adult compared to aged rats.

Conventional extracellular recording techniques have been used to examine two inputs to the dentate gyrus: the projection from the contralateral hippocampus (the commissural system) and the pathways arising from the ipsilateral and the contralateral entorhinal cortex (the ipsilateral and crossed temporodentate pathways). The maximum rate-of-rise and laminar distribution of the field potentials evoked by stimulation of each pathway have been determined in young (3 month) and aged (27 month) rats, and in animals with long-standing unilateral entorhinal lesions.

The commissural system of normal aged rats was not noticeably different from that in young adult rats, but the normal crossed temporodentate pathway was larger in the aged animals. Following entorhinectomy, both inputs exhibited an increase in evoked response amplitude in aged animals similar to that in young animals. However, the increase in commissural system response is less than that observed in young rats, in agreement with anatomical findings. The sprouted crossed temporodentate pathway is also smaller than in young animals. These data illustrate that there is a reduced functional reorganization of the fibers which are present in the aged animal. (Supported by NIH AG00538-04 and NINCDS NS06480-01.)

103.9 EFFECTS OF DIAZEPAM AND IMIPRAMINE ON BODY TEMPERATURE OF AGED SQUIRREL MONKEYS. S.M. Clark* and J.M. Lipton (SPON: J. Kirkpatrick), Depts. of Physiology and Neurology, University of Texas Health Science Center, Dallas, TX 75235.

Certain medications prescribed for elderly patients may increase their vulnerability to accidental hypothermia. We tested the effects of two drugs widely prescribed for the elderly, diazepam and imipramine, on rectal temperature of squirrel monkeys 2-16 yrs old. In a thermoneutral environment, diazepam (0.125-0.5 mg/kg, im) produced hypothermia in animals of all ages, with monkeys over 14 yrs showing a greater response with the highest dose. Hypothermia in the old monkeys was greatly augmented in a cold environment (15°C) but there was no difference between responses of old and younger animals in the heat (30°C). In initial studies, icv administration of diazepam in a thermoneutral environment produced hyper- rather than hypothermia in all animals, and the response was decreased in animals over 10 yrs of age. The responses of animals of all age groups to imipramine (1.5-12.0 mg/kg, im) given in a thermoneutral environment was variable, but animals over 14 yrs more consistently developed hypothermia. There was no clear age-related difference in response of old animals exposed to heat. Because of toxic effects, no experiments were carried out on imipramine in the cold. These results suggest that these commonly used drugs may increase the risk of accidental hypothermia in the elderly. Determination of the site of action of these drugs in producing hypothermia in aged primates (central vs peripheral site of action) must be determined in future experiments. (Supported by National Institute on Aging Grant AG 00109.)

103.10 AGING EFFECT ON THE NORADRENALINE CONTENT OF RAT BRAIN MICROVESSELS. Larry J. Embree, David W. Jackson*, Frederick Ordway*, and Isaac F. Roubein. Veterans Administration Medical Center and Department of Neurology, Louisiana State University Medical Center, Shreveport, Louisiana 71130.

There is both anatomical and physiological evidence that the intraparenchymal vasculature of the brain is innervated by noradrenergic nerve fibers. This noradrenergic system of vascular innervation has the potential for influencing cerebral blood flow and overall brain metabolism. Thus, its proper function would be critical to maintenance of normal neurologic activity, and derangements might correlate with aging and certain disease states, particularly those classified as metabolic or degenerative.

One aspect of the integrity of this central adrenergic system is the level of noradrenaline (NA) in the cerebral microvasculature, and while there are published studies on NA content of microvessels isolated from brains of young adult animals (Lai et al., Proc. Nat. Acad. Sci., USA, 1975, Reinhard, et al., Neusci. Abstracts, 1979), information is lacking on the effect of aging on the level of this monoamine in microvessels; hence this study was undertaken.

Aging male Sprague-Dawley rats (23-24 months old) were sacrificed by decapitation, the brains were removed immediately, stripped of meninges, choroid plexus, pineal body, and brain stem. Microvessels were isolated from forebrains and their NA content was determined using high pressure liquid chromatography and electro-chemical detector after homogenizing the tissues in 0.4 M perchloric acid. Protein levels were determined on the resolubilized pellet by the Lowry method. Microvessels isolated from young adult rat (10-12 weeks old) brains were used as controls.

Microvessel preparations from aging animals contained 128 ±17 pg NA/mg protein and similar preparations from young adult rats contained 243 ± 26 pg NA/mg protein. This difference was statistically significant ($P < .01$).

This decrease in microvascular related neurotransmitter may indicate a deficiency in the control of the cerebral vascular bed which could lead to metabolic disturbances of the neural tissues and clinical neurologic dysfunction. If similar changes in NA content of microvessels are found in aging human brain, the possibility exist for a pharamacological approach to ameliorate some of the signs and symptoms that are associated with aging of the brain.

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103.11 ULTRASTRUCTURAL CHARACTERISTICS OF THE SUPRAOPTIC NUCLEUS (SON) OF THE AGED RAT. T.H. McNeill, C.L. Clayton, R.J. Joynt. Department of Neurology, University of Rochester, Rochester, N.Y.

The ultrastructural characteristics of the SON of the 30 mo. old rat were examined. Five young (3 mo.) adult male Fisher 344 rats and five senescent (30 mo.) rats of the same strain were prepared for electron microscopy. Brains were fixed under pentobarbitol anesthesia by a vascular perfusion with 2% paraformaldehyde-2% gluteraldehyde in phosphate buffer, pH 7.2. Tissues were post-fixed in 1% osmium tetraoxide for 1 hour, dehydrated in increasing consentrations of ethanol and embedded in Spurr low viscosity resin. Ultrathin sections were mounted on uncoated 200 mesh grids and stained with uranyl acetate and lead citrate. Sections were analyzed in a Zeiss EM 10A electron microscope at an accelerating potential of 60kV.

Electron microscopic examination of the stained tissue sections suggest that the ultrastructure of SON perikarya is not significantly altered with age. Perikarya had a well defined nucleus with a large nucleolus. Cytoplasm exhibited diolated arrays of perinuclear Golgi, parallel arrangement of short cisterna of rough endoplasmic reticulum, lysosomes and mitochondria. Large neurosecretory granules were concentrated along the periphery of the cell and in the proximal axon. In contrast to SON perikarya in the 3 mo. old rat "aged" perikarya had numerous large lipofuscin granules with associated lipid bodies and an apparent increase in cytoplasmic polyribosomes. Although a morphometric analysis was not undertaken, axosomatic synapses were identified on SON cell soma. These data suggest that unlike other neuronal systems (i.e. Purkinje cells, hippocampus, spinal cord etc.), the ultrastructure of SON perikarya is not significantly altered with age and may possibly reflect a cellular hyperactivity in the 30 mo. old rat.

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103.12 ULTRASTRUCTURAL CHARACTERISTICS OF MEDIAN EMINENCE (ME) IN THE AGED MOUSE. Claudia J. Clayton,* David E. Scott and Tnomas H. McNeill. Dept. of Neurology and Anatomy. Univ. of Rochester, Rochester, N.Y.

Ultrastructural characteristics of the aged ME were studied in male C57BL/6 mice. Animals 4 months old and 29-30 months old were anesthetized with pentobarbital and perfused through the ascending aorta with 2% glutaraldehyde-2% paraformaldehyde, pH 7.2, in phosphate buffer. Medial basal hypothalami were blocked and post-fixed in 1% osmium tetroxide for 2 hours, dehydrated in graded ethanols and embedded in Spurr low viscosity resin. Ultrathin sections were mounted on uncoated 200 mesh grids and stained with uranyl acetate and lead citrate. Sections were analyzed on a Zeiss EM 10A electron microscope at 60 kV.

The fibrous zone of the aged ME demonstrated an increase in size and number of Herring bodies. These were usually unmyelinated or thinly myelinated and contained mitochondria, polymorphous autophagic granules, autophagic vacuoles and secretory granules of varying density. Glial elements in this zone often contained lipofuscin.

Several types of ultrastructural alteration were apparent in the external zone of the aged ME. Processes packed with clear microvesicles were common and were often very large,measuring up to 4.7um in diameter. Few dense core vesicles were observed in these processes. Other large processes exhibited lysosomes and mitochondria centrally which were surrounded by a dense accumulation of concentrically and longitudinally oriented microtubules. Unmyelinated Herring bodies were present and exhibited microtubule proliferation as well as autophagic granules and vacuoles, lamellar bodies and dense core vesicles. Other fibers contained numerous lamellar bodies in addition to mitochondria, microtubules and dense core vesicles. No degenerating fibers or terminals were observed. The majority of fibers in the external zone were normal in appearance.

Morphological changes in the aged ME may reflect an altered availability of releasing factors for transport to the pituitary. Immunocytochemical studies are underway to determine if these ultrastructural alterations are confined to distinct populations of fibers.

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103.13 INDUCTION OF PSEUDOPREGNANCY BY CERVICAL STIMULATION IN MINIMALLY ANDROGENIZED AND AGING FEMALE RATS. J.R. Lehman* and S. E. Hendricks, Department of Psychology, University of Nebraska at Omaha, Omaha, Nebraska 68182

The present study is a part of a series of experiments designed to assess the utility of using the androgenized female rat as a model system for investigating age related changes in reproductive function. Previous experiments have suggested that with increasing age, the capacity of cervical stimulation (CS) to induce a progestational response deteriorates; e.g., a greater number of intromissions are required for an aged female. Comparisons were made among groups of intact, multiparous females (3, 9, and 14 months of age) and adult female rats which were subjected to varying dosages of androgen during the neonatal period (oil, .05, .5, and 3.0 µg testosterone proprionate injected at five days of age). Two weeks prior to and following CS, vaginal smears were obtained daily. CS stimulation (30 sec. of 10, 30, or 50 V pulsed at 200/sec. with a duration of one msec.) was delivered during the evening of an estrous day. Increases in voltage of CS were consistently associated with an increasing tendency of the rat to exhibit a pseudopregnancy response within each of the age and androgenized groups. For androgenized females which were cycling prior to CS there was a negative correlation between rates of pseudopregnancy and dose of neonatally injected androgen. Aging female rats which were cycling prior to CS treatment did not reveal an age-associated difference in proportion of animals becoming pseudopregnant. However, for those aging females which were in a state of persistent vaginal estrus prior to treatment, there was an age-associated attenuation in tendency to exhibit pseudopregnancy in response to CS. These findings indicate that with respect to responsiveness of the neuroendocrine system to CS, the dosages of neonatally injected androgen given produce deficits which are not apparent in aging rats.

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103.14 EFFECTS OF AGING AND ESTROUS CYCLE ON THE HYPOTHALAMIC-PITUITARY-THYROID AXIS IN THE FEMALE GOLDEN HAMSTER. H.J. Chen. Neuroendocrine Research Laboratory, Barrow Neurological Institute, Phoenix, Arizona 85013

The purposes of these studies were aimed at determining the effects of aging and estrous cycle on basal and thyrotropin releasing hormone (TRH)-induced thyrotropin (TSH) release and on serum Thyroxine (T4) and Triiodothyronine (T3) concentrations in the female golden hamster (Mesocricetus auratus). Unlike the old female rat which enters a period of constant estrus, pseudopregnancy, and finally anestrus beginning at 10-14 months of age, 70 - 80 percent of female hamsters maintain regular 4-day estrous cycles at an older age (16-18months). This ability of the older female hamster to maintain regular estrous cycles renders it an ideal animal for study of the effect of aging on various endocrine functions during the estrous cycle. Young (3-4 months) and old (16-18 months) cycling female hamsters were used in the present experiments. The animals were injected subcutaneously with a single dose of TRH (100 ng/100 g BW) between 9:00 AM and 10:00 AM on various stages of the estrous cycle. Blood samples were collected one hour before and 10, 30, and 60 minutes after TRH injections. Serum TSH, total and free T4, and total T3 were determined by radioimmunoassays.

TSH response to TRH (100 ng/100g BW) stimulation was significantly greater in the young than in the old female hamsters even though basal TSH concentrations were comparable. Different stages of the estrous cycle did not affect basal or TRH-induced TSH release in either young or old hamsters.

Basal total and free Thyroxine concentrations were significantly higher in the young (3-4 months old) than in the old hamsters at all stages of the estrous cycle. In young females total T4 concentration was significantly ($p<0.01$) higher on estrus than on any other stages of the estrous cycle. Such a difference in T4 serum concentrations was not observed in the old (16-18 month) female hamster. Serum T3 concentration was significantly higher in the young than in the old hamsters, although different stages of the estrous cycle did not affect T3 concentrations in both old and young hamsters.

These results indicate that aging decreases TRH-induced TSH release in the golden hamster just as has been reported in female rats with similiar gonadal steroid influences (Chen and Walfish, J. Endocrin. 78: 225-232, 1978). Thyroid functions as indicated by circulating T4 and T3 concentrations diminish with advancing age in the female golden hamster. There is an increase in T4 concentrations in young female hamsters on the morning of estrus as compared with any other stages of the estrous cycle.

103.15 ESSENTIAL,DIETARY METAL IMBALANCE AND ALUMINUM INTOXICATION IN DEMENTED ALZHEIMER PATIENTS: INTERACTION WITH THE CATECHOLAMINE SYSTEMS. G. Wenk, Kettering Institute, Univ. of Cincinnati, Cincinnati, OH, 45267.

The distribution of Aluminum (Al) to certain catecholamine (CA)-rich regions of the brains of Alzheimer, demented, and elderly patients may be influenced by other trace metals in the diet. Clinical symptoms of depression, memory loss, and tremor, parallel elevated Al levels in the cortex, hippocampus, and cerebellum. A possible interaction of Al with the catecholaminergic systems in these regions is suggested. The neurotoxic effects of Al on the CA systems, and the influence of a dietary trace metal imbalance on the brain-Al distribution were investigated.

The results indicated that Al accumulation can be influenced by an imbalance in dietary copper, zinc, iron, and magnesium levels. The norepinephrine concentration in the cortex and hippocampus are depressed in animals eating diets with suboptimal amounts of copper or zinc, who also show elevated Al levels in these regions. Dopamine in Al-rich cortexes was also found to be lowered in animals on certain suboptimal diets.

103.16 CHOLINE TRANSPORT ACROSS THE BLOOD-BRAIN BARRIER IN AGED RATS. P. B. HICKS, C. ROLSTEN* and J.C. SCHOOLAR. Texas Research Institute of Mental Sciences, Houston, Texas 77030.

Choline transport across the blood-brain barrier (BBB) can be adequately described as the parallel sum of facilitated transport and passive diffusion processes (Cornford, et al, J. Neurochem. 30:229, 1978). Because of the possible benefits from the pharmacological use of choline in disorders that increase in incidence in geriatric patients, it is of considerable importance to characterize choline transport at the BBB in aged animals. We have previously reported that the brain uptake index (BUI) of choline decreases in aged rats. In order to characterize age-related changes in the kinetics of choline transport across the BBB, male Fisher 344 rats obtained from the N.I.A. were used at 3, 9 and 24 months. Choline transport was measured by the method of Oldendorf using ^{14}C-tryptamine as the diffusible reference substance and Tc^{99m}-DTPA as the non-diffusible vascular space marker. Choline transport (nmol/g·min) was determined at choline concentrations from 0.125mM to 10mM in five brain regions (cortex, striatum, midbrain, hippocampus and diencephalon). The kinetic constants of facilitated transport and passive diffusion of choline were estimated by a nonlinear regression fit of the data to the following equation:

$$V = \frac{Vmax \cdot (C)}{Km + (C)} + Kd \cdot (C)$$

where V = choline transport rate (nmol/g·min), C=choline concentration (M) in the injection mixture, Vmax = maximal choline transport rate (nmol/g·min), Km = choline concentration (µM) at half-maximal transport, and Kd = diffusion constant (µl/g·min).

Vmax values were higher in the cortex ($\bar{X}$= 8.6 nmol/g·min) than all other regions ($\bar{X}$= 5.8 nmol/g·min). Km values did not show regional variation ($\bar{X}$= 285 µM). Kd values showed marked regional variation being highest in cortex ($\bar{X}$= 5.27 µl/g·min), intermediate in hippocampus and midbrain ($\bar{X}$= 2.76 µl/g·min), and lowest in striatum and diencephalon ($\bar{X}$= 1.89 µl/g·min). In comparison to the 3 month old rats the 24 month old rats had lower Vmax values in the midbrain, but higher values in both the cortex and hippocampus. Km values were lower in the 24 month old rats only in the midbrain. Kd values for the 24 month old rats were higher than the 3-month old rats in the diencephalon and higher than the 9-month old rats in the cortex and striatum.

The results suggest that only minor changes occur in choline transport with age between 9 and 24 months. However, the physiological significance of these changes has yet to be evaluated. It appears likely that the efficacy of choline in the treatment of mental and motor disorders in geriatric patients is not related to BBB function.

103.17 NEUROCHEMICAL STUDIES IN AGING BRAIN: A. PROTEIN ALTERATIONS IN MYELIN. M. Malone, J. Greaney* and M. Szoke*. Geriatric Research, Education and Clinical Center, Veterans Hospital, Bedford, MA 01730.

Simple brain atrophy with dementia is a degenerative disorder characterized pathologically by atrophy of brain white and grey matter, the presence of neurofibrillary tangles, senile plaques and deposits of lipofuscin. Atrophy is the most consistent age-related finding and there is strong evidence that primary loss occurs in white matter. Such loss involves the myelin sheath. Myelin is a lipid-protein complex elaborated from the surface membranes of oligodendroglia and developmentally sub-stages of myelin have been isolated and identified. Our present studies have been made to characterize biochemically changes with age in central myelin.

Myelin was isolated from the brains of old (13 mo.), aged (19 mo.) and mature (2-4 mo.) Sprague-Dawley rats by the method of Norton and Poduslo (J. Neurochem. 21, 749, 1973) as modified by Zimmerman and Quarles (ibid 25, 749, 1975) to yield Heavy, Intermediate and Light myelin forms. After delipidation and solubilization (Quarles, et al, J. Neurochem. 466, 176, 1977), myelin proteins were separated on an acrylamide gel slab (10%) (Chan and Lees Biochem. 13, 2704, 1974), stained with Coomassie Blue and PAS and studied by densitometer with applied reference standards.

We have found a major decrement in a high molecular weight myelin glycoprotein in old myelin and an absence of this material in aged myelin isolates. This is a major aging change in the structure of white matter.

103.18 NEUROCHEMICAL STUDIES IN AGING BRAIN: B. STRUCTURAL CHANGES IN MYELIN LIPIDS. M. Szoke*, M. Malone and J. Greaney*. Geriatric Research, Education and Clinical Center, Veterans Hospital, Bedford, MA 01730

Loss of brain weight is a prominent feature of aging and regional atrophy is the most consistent pathological change in senile dementia. This atrophic change involves white matter and E.M. studies (Terry, et al, Am. J. Path. 44, 269, 1964) have reported primary changes in the organization of myelin. Central myelin is a complex lipid bimolecular-protein structure elaborated through a series of transitional forms from the primary plasma membranes of oligodendroglia. Detailed changes in lipid structure and composition characterize the process of myelination in early development. We have studied these changes in myelin isolates from the mature (2-3 mo.), old (13 mo.) and aged (19 mo.) rat brain.

Myelin was isolated from rat brains by fractionation on discontinuous ultracentrifuge gradients in methods designed to yield compact mature myelin and pre- or protemyelin forms. Myelin lipids were extracted by the Folch procedure (Folch-Pi, J. et al, J. Biol. Chem 226, 497, 1957), separated by preparative TLC (Seminario de Bohner, L. et al: J. Chromat. 17 154, 1965) and the glycolipids subjected to hydrolysis and derivatization, followed by Gas Liquid Chromatography (Kishimoto, Y.: Res. Methods in Neurochem. 1978). We found that compact myelin isolates from aged rat brain showed a major increase in the ratio of unsaturated (mono-enoic)/saturated long chain (C18-26) glycolipid fatty acids compared to isolates from mature or older animals. Such age-related changes suggest fundamental instability at the molecular membrane level.

103.19 STRUCTURAL CHANGES ASSOCIATED WITH AGING IN THE FORNIX OF THE RAT AND MOUSE. P.A. Paskevich* and J.N. Naranjo*. (SPON: J.F. Lipinski). McLean Hospital, Belmont, MA 02178 and the Department of Anatomy, Harvard Medical School, Boston, MA 02115.

It has been known for some time that age-related changes occur in the central nervous system. Recent studies utilizing newer techniques have confirmed these findings, and have enabled more accurate description of neuronal and glial degeneration. The present study is a histological and electron microscopic examination of the fornix in aged and young Fisher-344 rats and C57/USC mice. The tissue of 10 aged (28-32 months old) and 10 young (8 months old) rats and 8 aged (24-28 months old) and 8 young (6 months old) mice were analyzed using the following techniques: i) a reduced silver stain method (Naranjo and Greene, 1977) to ascertain gross degenerative changes throughout the pre- and post-commissural fornix, and ii) electron microscopy to analyze ultrastructural changes as a result of age.

Fiber degeneration, slight argyrophilia and a proliferation of oligodendroglia were noted throughout the entire extent of the fornix in the aged animals. The descending columns of the fornix as well as the commissure of the fornix were particularly impregnated, with densely stained degenerating fibers being the predominant feature. An absence of degeneration was noted at every level of the fornix in all young animals.

An ultrastructural analysis was used to enhance and confirm our histological data and further clarify the morphological changes associated with cellular aging in the central nervous system. It was noted that perivascular astrocytes contain membrane bound inclusion bodies that have the characteristic lamellar appearance of myelin. Furthermore, an analysis of myelinated fibers show numerous degenerative axoplasmic profiles, with a typical breakdown of microtubules.

This research was supported by the June Rockwell Levy Foundation and the Alfred P. Sloan Foundation.

104.1 OSCILLATORY EYE MOVEMENTS IN STROBE REARED CATS. G. Mandl, G. Melvill Jones, M. Cynader and J.S. Outerbridge*. Dept. of Physiol. and Biomed. Eng. Unit, McGill Univ., Montreal; and Dept. of Psychology, Dalhousie Univ., Halifax, Canada.

INTRODUCTION. Kittens reared from birth in stroboscopic light develop spontaneous eye oscillations. The present experiments set out to characterize these abnormal eye movements as recorded in complete darkness, in strobe light of various frequencies, after exposure to normal light, after attenuation of the vestibulo-ocular reflex (VOR) gain (eye vel./head vel.) by vision reversal, and during optokinetic stimulation.

METHODS. 2 cats were reared from birth to one year in 8 Hz stroboscopic light of 5 μsec flash duration. One normally reared animal was used as a control. Horizontal movements of the right eye were measured by the scleral eye coil method (resolution 5-10 min arc, 3 db at 1 KHz). Head fixation was achieved by means of a conventional acrylic block fastened to the skull. Power spectral analysis, by a method of modified periodograms using a Hanning data window, was performed on sections of relevant eye movement records, to ascertain their frequency content. VOR gain was estimated by rotating the animal sinusoidally in the dark, at 1/8 Hz and 5^{o}/sec amplitude.

RESULTS. (1) With head fixed in the dark, both strobe reared cats generated spontaneous eye oscillations of about $0.5\text{-}1.0^{o}$ amp. with each of the 2 animals exhibiting a "fundamental" frequency close to that of their original rearing environment. (2) Strobe light exerted a strong modifying influence upon the spontaneous "dark" eye oscillations, with pronounced entrainment of eye oscillations at a given "forcing" strobe frequency. In some cases, power spectral analysis revealed the presence of more than one distinct frequency component, indicating a mixture of several harmonics. (3) In the presence of normal light, the frequency of spontaneous eye oscillations, as measured in one of the strobe reared animals, dropped from its characteristic "dark" value of 9 Hz, to a new maintained low of 2.7 Hz. (4) Adaptive attenuation of the VOR to some 40% of control value caused a virtually total abolition of regular spontaneous eye oscillations in the dark, although transient responses to single strobe flashes could still be elicited. (5) The abnormal spontaneous eye movements did not interfere with, and were not abolished by, normal oculomotor activity.

Supported by the Canadian Medical Research Council.

104.2 SEX DIFFERENCES IN 6-HYDROXYDOPAMINE INDUCED HYPERACTIVITY AND AMPHETAMINE RESPONSIVENESS IN DEVELOPING RATS. J.T. Concannon and M.D. Schechter, Dept. of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

In an attempt to produce hyperactivity in developing rats, we utilized neonatal intracisternal injections of 6-hydroxydopamine (6-OHDA) and measured activity across maturation using a time-sampling procedure described by Shaywitz et al. (Science 191: 305, 1976). At 5 days of age, rat pups from litters consisting of an equal number of male and female rats were randomly assigned to either 6-OHDA or vehicle treatment using a littermate control design. Behavioral activity was measured at 12, 15, 18, 21, 24 and 27 days of age after an IP injection of either 0.5 mg/kg d-amphetamine or saline. An age-dependent increase in activity between 12 and 21 days of age was found for vehicle-treated animals injected with saline. Thereafter, males showed a decline in activity, while females remained at their peak of activity. The 6-OHDA treatment produced hyperactivity in males at 18 and 27 days of age, although it did not significantly alter activity in females. Although there was never a "paradoxical" calming effect of amphetamine, amphetamine responsiveness was altered by 6-OHDA in a sex-dependent manner. That is, male 6-OHDA treated rats showed less of an increase in activity associated with amphetamine at 12, 18, 24 and 27 days of age. Female 6-OHDA treated rats also showed less of an increase in activity associated with amphetamine at 12 and 18 days of age, but displayed more of an increase in activity than vehicle-treated rats at 24 and 27 days of age. Whole-brain dopamine (DA), assayed at 35-36 days of age, was depleted 38%, while norepinephrine (NE) was not reliably depleted. Furthermore, the 6-OHDA treatment did not significantly alter body weight gain. At a basic level, these results suggest that the failure to produce hyperactivity by 6-OHDA treatments in rat pups may result from failure to consider sex of animals within treatment conditions. At a clinical level, these results may provide a starting point for the laboratory examination of the source of the higher incidence of diagnosis of hyperactivity in male pre-adolescent children.

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104.3 CHRONIC MATERNAL ETHANOL CONSUMPTION THROUGH THE 2ND TRIMESTER OF GESTATION: SYNAPTIC PLASMA MEMBRANE PROTEINS & GLYCOPROTEINS IN OFFSPRING. A.B. Noronha* and M.J. Druse-Manteuffel Department of Biochemistry and Biophysics, Loyola University Medical Center, Maywood, Illinois 60153.

Female Sprague Dawley rats were pair-fed using either control or ethanol (6.6%) (v/v) liquid diets for 1½ months prior to conception and during the first two weeks of gestation. At 9,16 or 23 days of age, control and ethanol offspring were injected intraventricularly with 30 μCi ^{3}H-fucose. Eighteen hours later, the animals were sacrificed and synaptic plasma membranes (SPM) were isolated.

Body weights of control and ethanol offspring were comparable at all ages examined.

Although the total brain protein content of ethanol and control pups was similar at 10 to 24 days, the ethanol pups had less than 50% of the normal amount of SPM protein at 17 and 24 days of age. Similarly, total incorporation of ^{3}H-fucose into brain glycoproteins was normal in ethanol pups, although they demonstrated a significant decrease in fucose incorporation into SPM glycoproteins at all ages . Thus, it appears that SPM proteins and glycoproteins are severely affected by maternal ethanol consumption, despite the fact that the ethanol mothers terminated their consumption of ethanol after the 2nd trimester of gestation.

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104.4 LEARNING DEFICITS AND BRAIN MONOAMINE LEVELS IN RATS WITH CONGENITAL HYPERBILIRUBINEMIA. R.M. Swenson, J. Jew and T.H. Williams. Department of Anatomy, University of Iowa, Iowa City, Iowa 52242.

Rats with congenital hyperbilirubinemia (homozygous Gunn strain) were tested at 28 days of age on an active learning task (2 way shuttlebox avoidance) and a passive learning task (step down avoidance) and compared with controls (i.e., their heterozygous littermates). Pain sensitivity was measured by testing jump thresholds to painful electric footshock. One week after the behavioral testing, eight portions of brain (hippocampus, caudate, remaining telencephalon, hypothalamus, remaining diencephalon, midbrain, pons-medulla, and cerebellum) were assayed for norepinephrine and dopamine according to the method of Jacobowitz and Richardson (Pharmacol., Biochem. & Behav., 1978, 8, 515).

When measured against performance of the control animals, the hyperbilirubinemic rats demonstrated significant learning retardation. The latter failed to learn the active avoidance task although there were given twice the number of trials needed by control animals for mastering the task. Similarly, hyperbilirubinemic rats failed to avoid shock in the passive avoidance task: compared with control animals, hyperbilirubinemic rats stepped down faster onto the grid on which they previously were shocked. These differences in learning ability were not due to differences in sensitivity to electric footshock since jump thresholds to shock were similar in hyperbilirubinemic animals and their controls.

Biochemical studies showed that, compared to controls, hyperbilirubinemic rats have higher levels of norepinephrine in cerebellum, hypothalamus, and hippocampus, whereas dopamine was significantly lower in cerebellum, pons-medulla, and caudate. This is the first study to show changes in putative neurotransmitter levels in brains of congenitally jaundiced rats. Further work will correlate these data with the learning measures and with morphological observations of the brain areas that show biochemical changes. These multidisciplinary studies carried out at different stages in development may indicate whether any recovery from hyperbilirubinemia occurs. (Supported by N.I.H. grant HD11800.)

104.5 PERMANENCE OF KINDLING IN DEVELOPING RATS. Solomon L. Moshe* and Bruce J. Albala. Dept. of Neurology and Dept. of Psychiatry, Albert Einstein College of Medicine, Bronx, New York 10461.

The kindling phenomenon consists of repeated applications of focal intracranial stimulations at low intensities that lead to the development of electrographic afterdischarges (ADs), behavioral automatisms and eventually generalized seizures. Kindling has been induced in suckling and young rats as well as in mature animals. However, although the suckling rats developed generalized seizures, the kindling patterns differed from those seen in young (35 days) and older rats. Since in mature animals, kindling has been reported to be permanent, the present study was designed to investigate whether the kindling changes produced in developing animals are also enduring.

Male suckling rats were chronically implanted with bipolar electrodes in the left amygdala. At 18 days of age they were stimulated every hour for 1 second (400 μAmps, 60 Hz); ADs were consistently elicited in all. Though all animals were given a fixed number of stimulations approximately one half developed generalized seizures while the remaining were only partially kindled. Subsequently, the electrodes were removed and the animals left to grow to 60 days of age. The mortality rate during this period was 30 %. The surviving rats were then reimplanted in the amygdala and divided into two groups. One group included those animals that had developed generalized seizures (Fully Kindled), while the other consisted of those that had shown little or no behavioral manifestations (Partially Kindled). They were then stimulated daily using the same parameters as before and compared to age-matched naive controls. Data analysis included only those animals in which the amygdala placements were histologically verified.

There were no differences in the weights and AD thresholds among the three groups. However, the duration of the first AD elicited was longer in the Fully Kindled rats as compared to Controls (63.7 sec vs 19.7 sec, $p<0.05$). Furthermore Fully Kindled rats required less stimulations to develop generalized seizures than the Partially Kindled rats (4.3 vs 7.0, $p<0.01$) and Controls (4.3 vs 14.1, $p<0.001$). The Partially Kindled rats also had significantly shorter kindling rates than Controls (7.0 vs 14.1, $p<0.001$).

The above data indicate that despite continuous changes in brain size and progression of synaptogenesis and myelinization kindling is long lasting in the immature rat and carries over into adulthood. The ease of mature animals to develop generalized convulsions correlates directly to previous neonatal seizure exposure. The results are indicative that kindling can be employed as a developmental model of epilepsy.

104.6 FAILURE IN DEVELOPMENT: RECEPTOR SENSITIVITY CHANGES FOLLOWING POSTNATAL X-IRRADIATION OF HIPPOCAMPUS. G. C. Palmer, R. B. Chronister and L. K. Gerbrandt. Depts. Pharmacol. and Anat., Univ. S. Ala. Col. Med., Mobile, Ala. 36688 and Neurosci. Res. Program, MIT, Boston, MA 02116.

Numerous approaches have been used to investigate mechanisms involved in neuronal plasticity. One of the more challenging techniques in this regard is x-irradiation applied to postnatal neurogenesis in the dentate gyrus of hippocampus. Here it has been shown that a redistribution of norepinephrine fibers occurs while the amount of norepinephrine remains constant. The purpose of the following experiments was to examine the receptor mechanisms associated with these norepinephrine fibers--specifically, the adenylate cyclase activation induced by norepinephrine.

Neonatal rats (Long-Evans derived hooded) of both sexes were irradiated either bilaterally (N=6) or unilaterally (N=12) between the 2nd and 15th postnatal days. Sham irradiated animals and the non-irradiated sides serves as controls. All comparisons were made using the Students *t* tests.

At maturity (at least 200 grams wt) the hippocampi were removed, homogenized and analyzed for either adenylate cyclase activity or cyclic AMP phosphodiesterase activities (both high and low Km). Cyclase activity was expressed as pmoles of cyclic AMP formed/mg protein/min and phosphodiesterase activities as nmoles (low Km) and μmoles (high Km) of cyclic AMP hydrolized/mg protein/min.

In all cases irradiation produced a dramatic decrease in cyclase activation. This diminished response was noted through dose response determination to norepinephrine, dopamine and histamine (10^{-6}-10^{-4}M). The comparisons were significant across animals (bilateral x-irradiation) or within animals (unilateral x-irradiation). Basal activities were not different between the controls and the x-irradiated animals.

The high Km phosphodiesterase levels (both calmodulin independent and dependent) were not altered. However, the low substrate moiety (low Km) displayed a significant increase in the ability to hydrolyze cyclic AMP.

The results of the present study show that the consequences of x-irradiation are mixed. First of all, there is a redistribution of norepinephrine fibers. Secondly, the enzyme-metabolic-receptor mechanisms of the postsynaptic cell are either altered (such as transcription changes) or never develop properly. This suggests that the determination of receptor plasticity changes requires careful scrutiny. Further work is in progress to elaborate these mechanisms. (Supported by Epilepsy Foundation of Amer. and Scottish Rite Schizophrenic Foundation.)

105.1 DEVELOPMENTAL CHANGE IN CO_2 SENSITIVITY OF CHICK LENS INTERCELLULAR COUPLING. Stephen M. Schuetze and Daniel A. Goodenough.* Dept. Anat., Harvard Medical School, Boston, MA 02115.

Adult lens cells are extensively interconnected by gap junctions. Freeze fracture studies show that lens junctions differ from those in other tissues in that the connexons (subunits) are more widely spaced and disordered. To investigate the functional meaning of this structural difference, we are studying gap junctions in early embryonic chick lens cells (Hamburger and Hamilton stages 12-22). During stages 12-14, the presumptive lens begins as a thickened region of ectoderm that subsequently invaginates. Freeze fracture replicas of these lenses fixed in glutaraldehyde reveal small, infrequent gap junctions that contain tightly packed, ordered connexons, unlike their adult counterparts. Between stages 14-18, the lens completes invagination and pinches off from the ectoderm to form a hollow, spherical lens vesicle. At these stages, freeze fractured gap junction plaques contain small islands of tightly packed connexons within larger regions of loosely packed connexons. Between stages 18-22, the lens increases in size and lens fiber cells begin to elongate. Junctions in these older lenses appear similar to adult lens junctions.

Lens cells are electrically coupled at all stages, shown by paired intracellular recordings. Typical resting potentials are -60 to -70 mV. The cells are also dye coupled. One minute after iontophoretically injecting a single lens cell with the fluorescent tracer Lucifer yellow (MW 443), dye can be found in dozens of adjacent cells. There is no striking change in dye coupling with age. Both dye transfer and electrical coupling occur via gap junctions and not via intercellular cytoplasmic bridges: When a single cell at any stage is injected with horseradish peroxidase (MW 40,000), the tracer is confined to the injected cell. Studies reported in the literature on other tissues suggest that increasing the CO_2 tension of the bath reversibly blocks intercellular coupling by lowering intracellular pH. We find that incubating stage 15-22 lenses for 20' in medium equilibrated with 50% CO_2/50% O_2 has no detectable effect on dye transfer. In contrast, incubating stage 12-14 lenses for 10' in the same medium appears to decrease dye coupling, since only a few cells near the injection site contain dye. The appearance of CO_2-resistant coupling may be correlated with the appearance of loosely packed connexons in freeze fracture replicas (cf. Raviola et al. (1978) JCB 79:229a). Supported by NIH grants EY03011 and EY05337. We thank W. Stewart for the generous gift of Lucifer yellow.

105.2 ELECTRICAL UNCOUPLING OF VERTEBRATE SPINAL CORD NEURONS DURING DEVELOPMENT. N.C. Spitzer, Biology Dept. UCSD, La Jolla, CA 92093

Low-resistance intercellular junctions demonstrable by electrical coupling are a common feature in the development of many tissues. Neuronal development involves the appearance of electrical excitability, chemosensitivity and electrical uncoupling. The 200 Rohon-Beard neurons in the spinal cord of Xenopus tadpoles are sufficiently large and accessible to be visualized and impaled with microelectrodes at very early stages of development, and are thus suitable for studies of neuronal differentiation.

These cells are electrically coupled and do not make action potentials or respond to a variety of putative neurotransmitters just prior to the closure of the neural tube, ~6 hrs after their birthdate in gastrula. Coupling coefficients as large as 0.5 are seen between adjacent cells, but the low resting potentials recorded (~-50 mV) suggest that the coupling in vivo may be larger. This coupling does not show rectification, but is voltage-dependent: current injection that causes sufficient polarization of one cell for several seconds uncouples it from other cells. The apparent input resistance of the first cell increases, and the coupling coefficient decreases to ~10% of its initial value. The cells are usually rapidly recoupled following the current pulse, and the phenomenon can be repeated many times. Cells occasionally remain uncoupled after termination of the current pulse, probably as the result of a leak current arising from the impalement: the difference in membrane potential between the injected cell and other cells may be sufficient to keep it uncoupled. The voltage-dependent uncoupling is unaffected by replacement of Na^+ with Tris, Cl^- with isethionate, high Ca^{2+}(40 mM), addition of 30 mM Co^{2+}, or high K^+ that depolarizes the cells by as much as 30 mV. Lowering the pH from 7.4 to 6.5 with HCO_3^- buffer does not abolish electrical coupling, but appears to eliminate the voltage dependence. Other cells in the spinal cords of the same embryos show coupling that is not voltage dependent.

Rohon-Beard cells begin to make action potentials at the time of closure of the neural tube. These depend on an inward Ca^{2+} current and are several hundred ms in duration. The depolarization of a cell by this action potential spreads electrotonically to nearby cells, causing them to fire action potentials. Individual Ca^{2+} action potentials are not large and long enough to uncouple the cells. Over the next few days the ionic dependence of the action potential shifts from Ca^{2+} to Na^+ and its duration decreases to 1 ms. These cells become electrically uncoupled around the time of appearance of the Na^+ component of their action potential. Other, unidentified cells in the spinal cord are still coupled and show voltage-dependent uncoupling at this time. Rohon-Beard neurons appear to remain uncoupled during further development.

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105.3 AN ULTRASTRUCTURAL PROFILE OF DEVELOPING RAT SUBSTANTIA GELATINOSA. M. L. Kirby. Dept. of Anatomy, Medical College of Georgia, Augusta, GA 30912.

The substantia gelatinosa is involved in the central processing of nociceptive information. A study of the ontological development of the area should enhance our understanding of the adult morphology of the substantia gelatinosa and ultimately the complex processes involved in the perception of pain. Five Wistar rats were perfused and analyzed on each of the following days: 15, 17, and 19 through 22 day fetuses; 1, 3, 6, 10, 15, and 30 day neonates and > 90 day adults. All animals were perfused with 4% gluteraldehyde and the spinal cords were dissected from low cervical and upper thoracic regions and processed for electron microscopy. The neuronal cell body diameter and synaptic density in the substantia gelatinosa were analyzed from electron micrographs taken from 16 different fields in each section. Cell body areas in the micrographs were measured with a planimeter and converted to diameters. Synapses in each field were counted and multiplied appropriately to determine the number of synapses/1000μ^2. The cell body diameter increased from 5.5μ on day 15 of gestation to 9.7μ on day 15 postnatally and decreased thereafter to the adult mean diameter of 8.6μ. Synapses were seen first on day 17 of gestation. Synaptic density increased gradually to parturition. Between 1 and 3 days postnatally, synapses underwent a 2-fold increase from 23.5±1.8 to 41.3±4.6. A gradual increase occurred after day 3 and the adult synaptic density was 55.1±6.6. The population of synapses in the developing substantia gelatinosa was obtained by analyzing the synapses in 2 different fields averaging 7,000-10,000μ^2. The initial population of synapses at 17 days was 12.7% axosomatic, 81.7% axodendritic and 5.6% axoaxonic. Axosomatic and axodendritic synapses changed inversely in a generally linear fashion to reach the adult values of 1.3% axosomatic, 19.1% axoaxonic. The axodendritic presynaptic terminals were populated primarily by clear-spherical (CS) vesicles with very few clear-flat (CF) or dense-cored (DC) vesicles. However, the CF and DC containing terminals increased during development.

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105.4 A GOLGI ANALYSIS OF NEURONAL RELATIONSHIPS IN THE SPINAL CORD OF THE MOUSE EMBRYO. L.E. Wentworth. Department of Anatomy, Univ. of California, School of Medicine, San Francisco, CA 94143.

The initial development of neurons in the cervical spinal cord of the mouse embryo of 9 to 11 days gestation (E9-E11; E0=vaginal plug) has been reported previously (Anat. Rec., 193:717-718, 1979). By E10 the central process of dorsal root ganglion (DRG) cells has entered the spinal cord and by E11 the anlage of the dorsal funiculus (DF), (oval bundle of His), is forming. By E12 the DF is a well formed fiber bundle located just dorsal to the entering dorsal root fibers. Branches of dorsal root axons can be seen entering the intermediate (mantle) layer, although they do not penetrate deeply into this zone. A few commissural neurons (CN) and ipsilateral association neurons (AN) begin to sprout dendrites by E11, but the dendritic processes of these cells are not seen to be closely approached by dorsal root axons. On E12 the CN's are mainly located along the interface between the mantle and ventricular layers; some of the CN's, with cell bodies located close to the DF, have dendritic processes entering the DF. The axon of CN's pass through or close to some of the dendrites of the medial motor column neurons.

The AN's and CN's intermingle on E11; by E12 the AN's are primarily located ventromedial to the developing dorsal funiculus and lateral to the CN's. Whereas Sims and Vaughn found that many interneurons of E13 send axons into the ipsilateral lateral marginal zone and appear to provide an early synaptic input to dendrites of lower motor neurons (Neuroscience Abst., III:119, 1977), the most advanced AN's at E12 (in the present study) have dendritic processes entering the DF and their axons enter the lateral funiculus, which also contains dendrites of some of the more advanced lateral motor column neurons.

Cells first appearing at E12 include multipolar neurons located along the border between the mantle layer and DF with processes branching into both areas. The ventricular layer, especially in the alar plate region, still contains radially oriented cells forming a pseudostratified columnar epithelium with an apical and basal process attached to the lumen and external limiting membrane respectively.

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105.5 PRE-MIGRATORY MOTOR NEUROBLASTS HAVE AXONS IN THE PERIPHERY: AN HRP STUDY. Sally A. Moody and Marieta B. Heaton, Dept. Neurosci., College of Medicine, Univ. of Florida, Gainesville, FL 32610.

Pre-migratory trigeminal motor neuroblasts lie in a medial column near the developing MLF after leaving the mitotic cycle. At stage 14(Hamburger and Hamilton, 53 hours of incubation) a lateral migration is first observed. The cells are adjacent to marginal zone fibers and a small motor root is apparent.This lateral migration continues until day 6 of incubation when the main trigeminal motor nucleus is completed(Heaton & Moody,1980, JCN 189:61).

On incubation days 2.5,3,3.5,4,& 4.5, we have cut the motor root at the proximal edge of the V ganglion and applied crystalline horseradish peroxidase (HRP,Sigma type VI) to the wound in order to study whole-cell silhouettes during the temporal extent of this developmental process. In this experiment only those cells are labeled whose axons have been injured by the knife cut.

In day 2.5 embryos, V motor neuroblasts are labeled only if the cut is no more than 8μm from the brainstem. These cells are close to the pial edge and are without a primitive internal process. Migratory and lateral cells are labeled; medial column cells are not. In several embryos in which the cut is 10-12μm distant from the exit of the motor root, no metencephalic cells are labeled. In embryos which received HRP applications on days 3,3.5,&4, pre-migratory medial column cells, migratory cells and post-migratory lateral nucleus cells are labeled. Rarely are trailing processes observed. Medial cells exhibit only occasional somal filopodia and frequent axonal branches. As the cells progress laterally, somal filopodia increase. Lateral nucleus cells either have short stubby processes or only a long, slender one extending dorsally. Axonal processes are sinuous with frequent swellings. At day 4.5 of incubation only a few medial column cells are labeled; these are small with smooth perikarya. The number of migratory cells has decreased and the lateral nucleus cells morphologically resemble those of earlier ages.

Migrating cells are often labeled in groups, cell somas in intimate contact with axons of adjacent cells. Thus the fiber bundles on which these cells appeared to be migrating in Hematoxylin stained material(Heaton and Moody,ibid) were probably their own axons. In cases in which the ganglion sensory root is severed but not the motor root, the labeled fibers seen within the metencephalon, the central processes of the ganglion, are confined to the spinal tract of V. They do not extend into the migratory area.

These experiments demonstrate that axonal outgrowth and invasion of the periphery, at least to the level of the V ganglion, preceeds somal migration of the trigeminal motor neuroblasts in chick. This outgrowth may be important in the determination of the appropriate migration pattern of motor neuroblasts.

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105.6 DEVELOPMENTAL CHANGES IN DEGENERATION ARGYROPHILIA OF MEDIAL PREFRONTAL CORTEX EFFERENTS IN THE GOLDEN HAMSTER. James E. Crandall and Christiana M. Leonard. Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610.

The appearance of long lasting degeneration argyrophilia(LLDA) in retinal and olfactory bulb efferents has been previously suggested to be related to functional maturation of these systems. The development of reliable hoarding behavior between Day 21 and Day 25 in the hamster coincides with age related changes in mediodorsal thalamic and perirhinal cortical afferent input to medial prefrontal cortex as revealed by retrograde HRP methods. In order to test the hypothesis that the onset of LLDA in medial prefrontal cortex efferent output might also be related to the maturation of hoarding, lesions of medial prefrontal cortex were produced in hamsters ages 10, 21 and 25 days. Following survival times of either 24 or 72 hours, series of coronal 25 um sections were stained with a Fink-Heimer procedure for anterograde degeneration. In pairs of animals matched for lesion extent location, we found no differences between LLDA patterns in 21 and 25 day animals. LLDA was well established in major fiber tracts (internal capsule, cerebral peduncle and pyramidal tract) as well as principal terminal regions in the caudoputamen, mediodorsal thalamus and lateral tegmentum. Short survival degeneration argyrophilia disappeared by Day 21, but is clearly evident on Day 10. On the other hand, no LLDA was noticeable in Day 10 animals.

It appears that the occurrence of LLDA in medial prefrontal cortex efferents does not correlate with the ontogeny of reliable hoarding behavior. Dramatic changes in the afferentation of this region, by contrast, do coincide with the behavioral transition. These findings that efferent projections of a region are established before ingrowth is complete support a theory of the retrograde development of the neuron, i.e., axonal maturation precedes dendritic. Our HRP results suggest that between 21 and 25 days of age the afferent projections segregate while cortico-cortical input increases. Whether either of these changes is dependent upon or a stimulus for dendritic outgrowth remains to be determined.

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105.7 A GOLGI STUDY OF THE DEVELOPMENT OF PYRAMIDAL NEURONS IN THE RAT VISUAL CORTEX AFTER EYE OPENING. Janice M. Juraska, Cynthia Elliott* and Janice Wesa*. Psychology Dept., Indiana Univ., Bloomington, IN 47405 and Psychology Dept., Univ. of Illinois, Champaign, IL 61820.

In previous work, the dendritic development of pyramidal neurons in the rat visual cortex was quantified through day 15 (Juraska & Fifkova, J. Comp. Neurol. 183:247, 1979). At day 15 dendritic branching appeared very similar to that seen in the adult. This observation is quantified in the present study. Hooded rats of both sexes were sacrificed at 15, 30 and 60 days of age. The 30 and 60 day rats had previously been weaned at 23-25 days and were group housed with same sex littermates until sacrifice. All rats were intracardially perfused with 4% paraformaldehyde and their posterior cortex was stained with the rapid Golgi method according to Lund (J. Comp. Neurol. 147:455, 1973). The basilar dendrites of layer V pyramidal neurons were traced with the aid of a camera lucida at 500X and analyzed for number and length of branches at each order from the cell body. Fifteen neurons per animal from four rats (two male and two female) at each age were sampled from areas 17 and 18. There were no differences in either number or length of dendrites among the three ages. Thus the dendritic branching pattern of layer V pyramidal neurons in the rat visual cortex at day 15 (one day after eye opening) is indistinguishable from the adult pattern in socially housed animals. Another cell population--layer III pyramidal neurons--is currently being quantified. Spine counts on both layer III and layer V pyramidals are also in progress.

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105.8 ANATOMICAL EVIDENCE FOR DIFFERENTIAL RATES OF MATURATION OF THE MEDIAL DORSAL NUCLEUS PROJECTIONS TO PREFRONTAL CORTEX IN RATS. J.V. Corwin*, J.E. Crandall, C.M. Leonard, and T.A. Schoenfeld. Dept. of Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610.

Previous anatomical research in the visual and olfactory systems has suggested that the presence of long lasting degeneration argyrophilia (≥ 72 hours: LLDA) may be a sign of functional maturation. We have attempted to relate the presence of LLDA as an anatomical marker of maturation to behavioral data derived from lesion studies of prefrontal cortex in infant rats. Sahley (1979) has found evidence that sulcal prefrontal cortex is functionally mature before medial prefrontal cortex. Sulcal prefrontal cortex lesions resulted in behavioral deficits as early as 14 days of age, whereas medial prefrontal cortex lesions produced no behavioral impairments at that time. We examined the projections of the medial dorsal thalamic nucleus to sulcal and medical prefrontal cortex in the rat to determine if these cortices differed in the age at which LLDA is first seen. The behavioral effects of prefrontal cortex lesions in infancy might predict that LLDA should be present in sulcal prefrontal cortex before being found in medial prefrontal cortex. To test this hypothesis, unilateral medial dorsal thalamic electrolytic lesions were produced in rat pups at 13, 20, or 25 days of age. The pups were sacrificed at either 24 or 72 hours post lesion. The brains were cut at 25μm and stained using the method of Fink-Heimer (Procedure I). The results indicate an age dependent pattern of LLDA in the sulcal prefrontal and medial prefrontal cortices. In the 13 day, 24 hours survival group (n=5), terminal degeneration was found in Layers 1 and 3 in both frontal subfields. By 72 hours survival (n=4), although LLDA was found densely distributed in layers 1 and 3 of sulcal prefrontal cortex, there was no discernable degeneration in medial prefrontal cortex at this survival time. In contrast, in the 20 and 25 day, 72 hour survival groups (n=6) LLDA was found densely distributed in layers 1 and 3 in both sulcal and medial prefrontal cortex. These anatomical results indicate a differential rate of maturation between the medial dorsal nucleus efferents to the two frontal subfields. The earlier presence of LLDA in sulcal prefrontal cortex (at 13 days) as compared to medial prefrontal cortex is anatomical evidence for the earlier maturation of the sulcal prefrontal system. With regard to the prefrontal system in rats, there is corresponding behavioral and anatomical evidence that the sulcal prefrontal system matures earlier than medial prefrontal system.

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105.9 A FREEZE FRACTURE STUDY OF THE DEVELOPING AXOLEMMA IN RAT OPTIC NERVES G.M. Bray and B.J. Oldfield* Neurosciences Unit, The Montreal General Hospital and McGill University, Montreal, Canada.

The nodal, paranodal and internodal portions of the axon membranes of myelinated fibers show distinctive intramembranous features which are presumed to relate to the specific roles of these regions in impulse conduction. Although each of these regions is characterized by different axon-sheath cell relationships, the extent to which the ensheathing cell influences the development of the axonal specializations is unclear. In this study, the intramembranous structures in the immature axons of rat optic nerves were examined by freeze fracture and thin-section electron microscopy.

Sprague-Dawley rats, 5 to 21 days of age, were perfused with phosphate-buffered aldehyde solutions. Both optic nerves were removed and immersed in fixative at 4° C. For freeze fracture, whole optic nerves were glycerinated, frozen on gold discs, fractured at -119° C and replicated with platinum and carbon. Portions of the optic nerves from each animal were also post-fixed with osmium tetroxide and embedded in epoxy resin.

On post-natal day 7, when optic nerve axons were unensheathed, both the inner (P-face) and outer (E-face) leaflets of the axolemma contained few particles (10-100/μm^2) of the type seen in mature axons. On day 14, many axons were ensheathed and thinly myelinated but others were still unensheathed. Both the P and E-faces of the unensheathed axons as well as the P-faces of the ensheathed axons showed particle densities which approached 1000/μm^2. Accummulations of particles resembling those seen at nodes of Ranvier were not observed along the unensheathed axons.

Assuming that the responses of axon membranes to fixative solutions were similar at different stages of neonatal development, there appears to be a marked increase in the densities of axolemmal particles in rat optic nerves between 7 and 14 days postnatally. Because these increased numbers of particles were present in both the ensheathed and unensheathed axon membranes at day 14, this phase of intramembranous development may not depend on contacts between axons and sheath cells.

105.10 MORPHOLOGY OF THE ACOUSTICOLATERAL AREA IN THE TADPOLE, RANA CATESBEIANA. J. Jacoby*, K. Rubinson and R.H. Browner. Dept. of Physiol. Biophys., New York Univ. Med. Ctr., New York, NY 10016 and Dept. of Anatomy, New York Med. College, Valhalla, NY 10595.

During metamorphosis, R. catesbeiana undergoes a degeneration of its lateral line system and the continued maturation of its terrestrial auditory system. The dorsolateral medullary tegmentum loses the lateral line input but remains the target of auditory input after metamorphosis. This study examines the changing morphology of the region during premetamorphic and metamorphic states. R. catesbeiana tadpoles were perfused with Heidenhain-Susa fixative, embedded, sectioned, and stained with toluidine blue. Through late stage 28 (referred to R. pipiens, Witschi, 1956), no magnocellular elements could be discerned in horizontal or transverse sections. The small (<than 10μ) cells which will constitute the dorsal acoustic nucleus are distinguishable at stage 25 and have migrated to the lateral surface by late stage 26. The longitudinal nucleus then lies lateral to a prominent cell-free area, the dorsal island of Kingsbury. By stage 28, the cells appear to have been displaced progressively deeper from the lateral surface while still maintaining a sharp medial border adjacent to the reduced dorsal island. The dorsalward displacement of the acoustic nucleus occurs later in metamorphosis as does the differentiation of the magnocellular nucleus.

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105.11 CELL LINEAGE ANALYSIS OF PURKINJE CELLS OF C57BL/6J↔BALB/cByJ CHIMERIC MICE. M. L. Oster-Granite and J. Gearhart*. Dept. Anat., Univ. Md. Sch. Med., Baltimore, Md. 21201.

It is difficult to believe that a structure as exquisitely organized as the cerebellar cortex results from a random distribution of cells within each of its distinct cell populations. Several of the anatomic and physiologic properties which we observe in the development and organization of the mammalian cerebellum could be more readily explained if interconnected or contiguous cell populations shared a common or clonal origin.

To determine whether the cell populations of the mammalian cerebellum are randomly arranged or not, one must be able to follow unequivocally the fate of single cells or populations of cells throughout cerebellar development. To do this, one must use cell lineage analysis to construct fate maps for the various cerebellar cell populations.

We have adapted the cell marker system most commonly used for cell lineage studies in the mammalian embryo, the ubiquitous enzyme, glucosephosphate isomerase (GPI, E.C.5.3.1.9). We have purified the two most common electrophoretic variants of murine GPI, GPI-1A and GPI-1B. We have produced xenogeneic allozyme-specific antisera to one of these variants, GPI-1B. We use such antisera to visualize cells which contain the GPI-1B variant by immunocytochemical (PAP) staining techniques in histologic sections. We have produced murine chimeras between inbred mouse strains expressing different GPI variants (C57BL/6J(Gpi-1^b)↔BALB/cByJ (gpi-1^a)). We have examined the location and distribution of Purkinje cells which stain for the GPI-1B variant in the cerebella of a number of these chimeric mice. Other investigators, using different cell marker systems and different mouse strain combinations, analyzed a very limited number of chimeric mouse cerebella or only analyzed portions of one cerebellum. They found that Purkinje cell mosaicism was finely grained; the Purkinje cells tended to be randomly araanged.

In contrast, following extensive reconstructions of the cerebella of a number of our murine chimeras, we found that the Purkinje cells were not randomly arranged. Cells of like genotype tended to remain together in the adult cerebellar cortex. To determine how much randomization of the Purkinje cells does occur during the processes of cell migration and intermingling, cell selection, and cell death that occur during cerebellar development, we have examined progressively younger chimeric mice. We will discuss the developmental implications of our findings, as well as the chimerism exhibited by other cerebellar cell populations during cerebellar development. Supported by NIH (HD12685), NSF (PCM16763) the National Foundation-March of Dimes (JG), the Alfred Sloan Foundation (MLOG), and Paralyzed Veterans of America.

105.12 MORPHOLOGICAL ORGANIZATION OF PONTINE CATECHOLAMINERGIC NEURONS IN DEVELOPING RABBIT BRAIN. Christel Marschall. Abt. Molek. Gen., Max Planck Institut exp. Med., Gottingen, FRG.

The occurrence and distribution of catecholamine-containing (CA) perikarya in the pons of the rabbit during late gestation and at birth (gestation = 31 days) are described. CA perikarya were visualized with either the Falck-Hillarp paraformaldehyde method or with glyoxylic acid-induced fluorescence. Adjacent and serial sections were stained with luxol fast blue and/or cresyl violet.

CA perikarya form a bilateral rostro-dorsal continuum from the ventrolateral caudal pons medially to the aqueduct. Neurons are generally loosely arranged. In the developing rabbit the compact organization characteristic of the locus coeruleus (A6) CA neurons is limited to its dorsomedial extension. The locus subcoeruleus is composed of a widely circumscribed region in all gestational ages observed, extending from the ventrolateral pons and merging medially with the locus coeruleus. The scattered distribution of CA perikarya contrasts with the tightly-packed organization reported in the rat. This may, however, reflect a species - specific pattern rather than a developmental characteristic since Blessing, et al., (J. Comp. Neurol. 179: 407, 1978) have also pointed out the loose arrangement of CA neurons in older (4 mo.) rabbits.

The most ventrolateral extension of the CA continuum is found in the caudal pons. These CA perikarya probably represent the outermost aspects of the locus subcoeruleus rather than comprising a distinct cell group (M7, Garber and Sladek, J. Comp. Neurol. 159: 289, 1975) since they do not differ morphologically.

CA perikarya are generally bipolar, oval and oriented diagonally to the midline. This orientation is also apparent in cresyl violet sections. CA cells evidence a medium fluorescence intensity without drug pretreatment, however, the fluorescence is distributed unevenly in the cytoplasm. In sagittal sections CA fibers are seen to run both longitudinally and cross - wise, and are found both in areas containing CA neurons, as well as in CA neuron - free regions.Fiber tracts leaving the locus coeruleus and subcoeruleus were not visualized.

Although glyoxylic acid - induced fluorescence produces a sharper delineation of cell bodies and processes, general morphological relationships are retained preferentially with the Falck-Hillarp method.

Supported by a postdoctoral fellowship from the Max Planck Gesellschaft. Present address: Dept. of Zoology, Michigan State University, East Lansing, Michigan 48824.

105.13 PATTERNS OF SPONTANEOUS AND EVOKED DISCHARGES DURING PURKINJE CELL DEVELOPMENT IN CULTURE. M. C. Calvet and J. Calvet *. Lab. of Neurophysiology, INSERM U3, Hôpital de la Salpêtrière, 47 Bd. de l'Hôpital, 75651, Paris cedex 13, France.

Cerebellar explants from new-born rats and kittens were grown in Maximow assemblies with a drop of nutrient medium, renewed twice a week and which consisted of 30% Hank's BSS, 35% minimal essential medium (Eagle) and 35% foetal calf serum, supplemented with glucose (6mg/ml). After 8-66 days in vitro, extracellular recordings of the spontaneous and evoked discharges were taken from at least two neurons simultaneously. In some cases, the recorded neurons were stained by Horseradish peroxidase iontophoretic filling, in order to characterize their morphological features. Electrophysiological data from 95 Purkinje cells (Pc) and from 30 brain stem neurons were processed with a LSI 11 computer which was programmed to display standard interspike interval histograms and cross-correlograms.

The results statistically confirmed that Purkinje cells (Pc) and neurons from incorporated brain stem tissues had distinctive electrical patterns. When the P. cells developed in differently organized cerebellar explants, they showed different patterns of firing and two major types of histograms could be individualized. Cross-correlation analysis indicated that 80% of the Pc pairs had a tendency to fire synchronously when the cerebellar explant was grown with attached brain stem. No correlation could be evidenced between two Pc grown without their extracerebellar afferents. A high degree of synchronization of spontaneous firing was also found between Pc and brain stem neurons and between brain stem neuronal pairs from the same culture.

A comparable analysis was performed on the evoked activity recorded from the same neurons. Depending on the stimulating site, different types of Pc responses were individualized.

Our data suggest that the P. cell patterns of firing are modulated by the onset of synaptic connections arising from brain stem tissues and the role of climbing fiber function in Pc electrogenesis in vitro is specially discussed.

105.14 THE "MINI-LASER MICROELECTRODE": DETECTION OF CALCIUM SPIKES IN PROCESSES AND GROWTH CONES OF DIFFERENTIATED NEUROBLASTOMA CELLS UTILIZING FLUORESCENT VOLTAGE SENSITIVE PROBES. A. Grinvald* and I. Farber* (SPON: M. Devor) Dept. of Neurobiology, Weizmann Institute of Science, Rehovot, Israel.

Changes in fluorescence of neurons, stained with voltage sensitive dyes were used to record electrical responses in cell somata, processes and growth cones of neuroblastoma cells, N1E-115, grown in a monolayer culture. A 30μm microbeam from an inexpensive He-Ne laser, (5mW, H3025-P, Hughes Co.) was focused on the desired target. The cells were stained in normal medium containing 10μM oxonol dye WW-802,[1,3-dipentyl barbituric acid-(5)]-[p-sulfophenyl-3-methyl-5-pyrazolone-(4)]-pentamethin oxonol. Cells were stimulated either extracellularly or intracellularly with a microelectrode, and changes in fluorescence were recorded from the image plane of a 40X microscope objective with a photomultiplier. A fractional change of 5% in the fluorescence intensity was observed per 100 mV potential change. Action potentials were recorded from 3-6μm processes with a signal to noise ratio of ∿3-10 without averaging. A comparison of an electrical recording from the cell body with the simultaneous fluorescence change indicated that the optical response is linearly dependent on the membrane potential change. Action potentials in growth cones were recorded in medium containing 15mM TEA, 1μM TTX and 20mM Ca^{2+}. These spikes resemble the Ca^{2+} spikes recorded in neuroblastoma somata (Moolenaar and Spector, J. Physiol 292, 307, 1979). The following data suggest that active calcium channels are indeed present also in the processes: The spike in the growth cone is delayed by 2 to 6 msec compared to the one recorded simultaneously from the cell body with a microelectrode. The delay is a function of temperature. Frequently the different time course of the spike in the growth cone cannot be explained by different geometry or by passive conductance. Further evidence comes from an experiment in which the growth cone was at least 900μm away from the cell body. The amplitude of passively conducted 100 mV hyperpolarization was at least 3 times smaller than the amplitude of the Ca^{2+} spike both recorded in TTX-TEA medium. Unless the process had unusual rectification properties these results can be explained only if active Ca^{2+} spike zones exist in the process. The present technology is relatively simple, therefore, it should be useful in situations where the use of microelectrodes is difficult, especially for small or thin "targets" in monolayer cultures, where due to the absence of background fluorescence the signals are relatively large.

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105.15 DEVELOPMENT AND TRANSMITTER SENSITIVITY OF DISPERSED FETAL HIPPOCAMPAL NEURONS IN CULTURE. S.M. Rothman* (SPON.:J. Sanes), Depts. Anatomy and Neurobiology and Pediatrics, Washington Univ. School of Medicine, St. Louis, MO 63110.

Cultures of dissociated hippocampal neurons from 18-day-old rat fetuses were examined by scanning electron microscopy after periods between one hour and 20 days following plating on a poly-L-lysine coated substrate. Cell attachment was virtually complete within one hour after plating, and at that stage many cells could be seen which had started to extend processes with growth cones. By four hours process formation was well advanced and some cells had assumed a pyramidal configuration. After 16 hours in culture numerous contacts were seen between neighboring growth cones, and this frequently led to fasciculation of the interacting fibers. During the next three weeks the cell bodies enlarged considerably, and two distinct types of processes became evident: large, rapidly tapering dendrite-like processes and finer, more uniform axon-like processes. A dense plexus of processes was formed and many of the finer processes had "bouton-like" swellings as they traversed the neuronal surfaces. Non-neuronal elements, which comprised only about five percent of the cells initially plated, eventually formed a confluent monolayer beneath the neurons.

The sensitivity of these cells to various neurotransmitters was examined during intracellular recording with high impedance (>150 MΩ) microelectrodes. All cells used for physiology had stable resting potentials in excess of 50 mV., and most had action potentials greater than 60 mV. Glutamate (.5M), a potent in vivo excitant depolarized almost all cells when applied by iontophoresis. GABA, applied by iontophoresis (.5M) or microperfusion (10^{-3}M) to cell bodies produced hyperpolarizing or depolarizing responses with approximately equal frequency. Virtually all cells responded to GABA. In contrast, cells seldom responded to acetylcholine or norepinephrine applied by iontophoresis (ACh 1.0;NE .5M) or microperfusion (10^{-3}M for both). These substances respectively excite and inhibit hippocampal neurons in vivo. We shall attempt to modify the transmitter sensitivity of these cells by co-culturing them with explants capable of providing adrenergic or cholinergic innervation.

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105.16 RETINAL GANGLION CELL DEATH DURING NORMAL DEVELOPMENT IN THE SYRIAN HAMSTER. D.R. Sengelaub and B.L. Finlay Department of Psychology, Cornell University, Ithaca, NY 14853

Retinal ganglion cell death during postnatal development in the hamster was studied with light microscopy. Degenerating cell profiles were found throughout the retinal ganglion cell layer in the first 10 postnatal days. On the first postnatal day, degenerating cells were observed at a rate of 1-3 per 1000 live cells. This rate increased rapidly to 7-18 degenerating cells per 1000 live cells by postnatal day 5, and returned to the rate of 1-3 per 1000 by day 10. Although the cell death rates for both central and peripheral retina peaked on the same postnatal day, day 5, higher rates were found centrally for the first 4 postnatal days, and higher rates peripherally for days 5-10, suggesting either a central to peripheral trend in neurogenesis in retina, or a central to peripheral trend in the establishment of central connectivity. Degenerating cells were typically found in clumps rather than evenly distributed thoughout the retina.

The general time course of cell degeneration and its central to peripheral pattern corresponded to the pattern of cell degeneration seen in the superficial gray layer of the superior colliculus. In the superficial gray layer, cell death is also at a maximum on the 5th postnatal day, and the rate of cell degeneration is consistently elevated at the medial, caudal and lateral margins of the superficial gray relative to central superficial gray. The intermediate gray layer, not a target of retinal axons, does not show this central to peripheral disparity. Asynchony in the pattern of genesis of retinal ganglion cells and superficial gray neurons can account for this pattern of differential cell death.

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105.17 ONTOGENY OF ANIONIC HERBICIDE TRANSPORT BY CHOROID PLEXUS AND BRAIN. C. S. Kim*, L. A. O'Tuama and J. R. Pick* (SPON: T. A. Reaves, Jr.) Dept. of Neurology, Pediatrics and Biol. Sci. Res. Ctr., Sch. of Med., Univ. of North Carolina at Chapel Hill 27514.

A distinctive transport system for organic anions has been described in the choroid plexus and the mechanisms of transport appear analogous to those involved in secretion of organic acids by the proximal tubule of the kidney (Pappenheimer et.al., Am. J. Physiol. 200:1-10, 1961). It has also been shown that the anionic herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D) is actively transported on the organic acid system of kidney (Berndt and Koschier, Toxicol. Appl. Pharmacol. 26:559-570, 1973) and choroid plexus (Pritchard, J. Pharmacol. Exp. Ther. 212:354-359, 1980). The present study was undertaken to investigate whether the barrier system to anionic organic acid is functioning at an early stage of development. Albino rabbits of either sex (ages 3 days and 30 days) were sacrificed by decapitation. In each experiment, the brain was rapidly removed from the skull and the choroid plexus (CP) was dissected free. Brain slices were prepared from whole brain. CP and brain slices from both age groups were pre-incubated for 10 min in artificial CSF only and then transferred to experimental medium containing ^{14}C-2,4-D 0.02 μCi/ml (sp. act. 28 mCi/mmol). The initial uptake of ^{14}C-2,4-D at 5 min was reduced 28.55% in lateral ventricular CP (LCP) and 58.75% in fourth ventricular CP (FCP) of 30 day old rabbit incubated with 10 μM probenecid, classical inhibitor ($P < 0.005$) and 58.95% in LCP and 77.35% in FCP with 100 μM probenecid ($P < 0.001$). In the CP of 3 day old rabbit, the inhibition of uptake was 57.79% in LCP and 75.64% in FCP with 100 μM probenecid ($P < 0.001$) and 57.07% with 100 μM ouabain ($P < 0.001$) compared to controls. However, such treatment did not alter ^{14}C-2,4-D uptake or release by brain slices in either age group studied. The finding in this study of inhibition of ^{14}C-2,4-D transport by probenecid and ouabain suggests that the CSF-blood barrier to anionic organic acid transport systems are established at an early stage of development, as early as 3 days old. The active transport of ^{14}C-2,4-D in brain is limited to the CP and possibly similar structures. Early development of facilitated blood-CSF solute translocation is suggested by other studies. Thus the blood-CSF barrier to proteins functions by 18 to 20 days of gestation, the protein concentration of CSF approaches the normal adult value soon after birth (Ramey and Birge, Develop. Biol. 68:292-298, 1979). CP Na^+ -K^+ - ATPase levels remain stable between postnatal and adult rabbit (Mitchell et.al., Proc. Am. Acad. Neurol. 32nd Ann. Mtg., p. 36, 1980).

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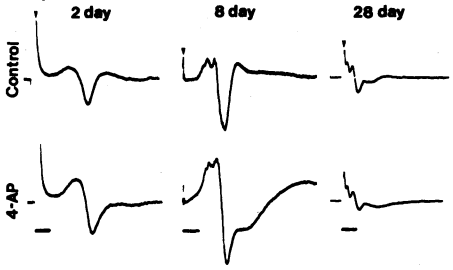
105.18 DEVELOPMENT OF CONDUCTION IN RAT OPTIC NERVE: PHYSIOLOGICAL AND PHARMACOLOGICAL OBSERVATIONS CORRELATED WITH MYELINATION. R. E. Foster, B. W. Connors and S. G. Waxman*. Dept. of Neurology, Stanford Med. Sch. and VA Med. Ctr., Palo Alto, CA 94304.

To study conduction in myelinating CNS axons, we examined the development of the compound action potential in rat optic nerve axons before, during and after myelination using in vitro methods. Myelination of optic nerve begins 6-8 days postnatal and 100% of the axons are myelinated in the adult. Our results indicate that the totally non-myelinated optic nerve (2 day) can conduct (cond. veloc., CV=0.2 m/s). The potential is blocked by application of 0.1 μM tetrodotoxin (TTX) and the amplitude is increased and recovery phase prolonged by application of 0.5 mM 4-aminopyridine (4-AP). These observations suggest that sodium and potassium conductances contribute to the optic nerve action potential prior to myelination. At the beginning of myelination (e.g. 8 days) the action potential exhibits two components (CV=0.4 and 0.6 m/s). Of these only the slower component is sensitive to 4-AP while both components are blocked by TTX. This suggests that, as myelination begins, voltage-dependent potassium conductance disappears. The slower component, presumably non-myelinated axons, retains sodium and potassium conductances. At 28 days (85% myelinated) the action potential has two fast (CV=3.9 and 7.3 m/s) and a slow component (CV=1.8 m/s). All components are TTX sensitive but only the slow component is affected by 4-AP.



(cal = 2 ms; conduction distance and gain not uniform; neg. down)

These observations suggest that during myelination the axon membrane reorganizes from a state where sodium and potassium channels are both present and functional to a state where sodium channels contribute almost the entire conductance which underlies the optic nerve action potential. (Supported in part by the VA, NIH, and NMSS).

105.19 MORPHOLOGICAL STUDIES OF NORMAL AND INDUCED ASTROCYTIC DIFFERENTIATION IN VITRO. P. Trimmer* P. Reier, and T. Oh. Dept. of Anat., Univ. of Md. Sch. of Med., Baltimore, MD 21201

The following study was undertaken 1) to establish the sequence of normal astrocytic maturation in vitro and 2) to compare these maturational events with the induction of process formation by dibutyrl cyclic adenosine monophosphate (dbcAMP) treatment. Tissue cultures consisting of immature glia were prepared from neonatal rat optic nerve as previously described (Trimmer, Reier and Oh, Neurosc. Abst., 5: 760, 1979). Cells at various stages of maturation were observed in vitro and their location was identified with a diamond marker objective. These identified cells were subsequently examined with either electron microscopy or immunocytochemistry. Cultured immature astroblasts are flat, polygonal cells which do not stain with the antibody to glial fibrillar acid protein (GFAP). With further maturation glial filaments gradually accumulated in the perinuclear region. In slightly more mature cells, distinct bundles of GFAP-stained glial filaments were distributed in radiating fascicles throughout the cytoplasm. The ultrastructure of these intermediate stage astroglia was characterized by regions of cytoplasm typical of astroblasts as well as discrete bundles of glial filaments characteristic of the mature cell. As maturation progressed, cytoplasmic vacuoles appeared between the fascicles of filaments. These vacuoles increased in number and coalesed. This results in a cavitation of cytoplasm between pre-existing bundles of glial filaments, giving rise to the mature, multipolar astrocyte. Following treatment with a 1mM concentration of dbcAMP, astroblasts were induced to form processes in a manner similar to the sequence of differentiation just described. A few hours after the addition of dbcAMP, vacuoles appeared in the cytoplasm of astroblasts. These vacuoles increased in number and size and ultimately the cytoplasm regressed leaving behind filament-containing cellular processes. From these observations of normal and chemically-induced astrocytic differentiation in vitro it appears that radial glial filaments play an important role in defining the distribution of astrocytic processes. The shape of the differentiated cell is more directly the result of cytoplasmic erosion around filament-rich areas of the astroblast rather than an active outgrowth of glial processes. (Supported by NIH Grant 13836 and The Paralyzed Veterans of America).

105.20 ULTRASTRUCTURAL CHARACTERISTICS OF MIGRATING MOTONEURONS. I-W. Chu-Wang, R. W. Oppenheim, and P. B. Farel. Neuroembryology Lab., Div. of Research, Dorthea Dix Hospital, Raleigh, NC 27514 and Dept. of Physiology, Univ. North Carolina Sch. Med., Chapel Hill, NC 27514

Migrating motoneurons, presumably destined to join the lumbar lateral motor column (LMC), of the tadpole (R. catesbeiana) can be retrogradely labeled by ventral root application of HRP (Farel and Bemelmans, Neurosci. Let., 1980). These motoneurons can be seen in all regions of the mantle layer as well as in contiguity with the ependymal zone. Migrating motoneurons are not retrogradely labeled when HRP is placed in the limb bud rather than in the ventral root. The present study examines the ultrastructural characteristics of these migrating motoneurons to determine their degree of differentiation compared to motoneurons that have completed their migration and joined the LMC.

Tadpoles of Stages VI-VII (Taylor and Kollros) were used in this study. HRP was applied to the ninth and tenth ventral roots and, three to four days later, the lumbar enlargement was removed and fixed by immersion in buffered 6% glutaraldehyde. Labeled motoneurons were located in 1 μm sections, and adjacent thin sections were examined under the electron microscope.

Motoneurons in the immediate vicinity of the ependymal zone were bipolar and appeared devoid of synapses. They were characterized by relatively sparse cytoplasm and organelles in an early stage of differentiation. Motoneurons midway between the ependyma and the LMC were either bipolar or monopolar, possessed synapses, and appeared cytologically indistinguishable from motoneurons in the LMC of the same animal.

Migrating motoneurons, then are highly differentiated both in terms of their organelles and in that they have synapses. These findings indicate that the mechanisms which specify the particular characteristics of a motoneuron may act before the motoneuron has reached its final location.

105.21 SURFACE SPECIALIZATIONS AT SITES OF CHOLINESTERASE ACTIVITY ON AMPHIBIAN EMBRYONIC MUSCLE CELLS CULTURED WITHOUT NERVE.
P. Weldon*, F. Moody-Corbett* and M.W. Cohen. Dept. Physiology, McGill University, Montreal, Quebec.

Patches of cholinesterase activity form on cultured myotomal muscle cells derived from Xenopus embryos. Such patches develop even though the muscle cells are cultured in the absence of nerve and overlap with sites of high acetylcholine receptor density (F. Moody-Corbett & M.W. Cohen, Proc. Soc. Neurosci. 5 486, 1979). In the present study, we have examined the fine structure of these areas of cholinesterase activity.

Most of the cell surface appeared morphologically unspecialized and was free of cholinesterase activity as indicated by the absence of histochemical reaction product. Discrete regions of reaction product extending for as much as 20 μm were observed on the lower (apposed to the culture dish) and upper cell surfaces and in the intercellular space between closely apposed cells. The majority of these regions were associated with sarcolemmal invaginations which varied considerably in size and depth ranging from shallow infoldings, similar in appearance to postsynaptic folds, to deep and sometimes branched invaginations some of which were similar to the invaginations characteristic of the amphibian myotomal myotendinous junction (T. Nakao, Cell Tiss. Res. 166 241-254, 1976). In cases where the reaction product was not so dense as to obscure other surface specializations, it was found to be associated with the presence of basal lamina and a "thickened" sarcolemma which are also characteristic of both postsynaptic membrane and myotendinous invaginations. These observations, taken together with our previous finding of a high incidence of overlap between cholinesterase patches and acetylcholine receptor patches, suggest that some of the sites of ultrastructural specialization of the cell surface contain high densities of acetylcholine receptors in addition to cholinesterase.

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106.1 QUANTITATIVE ULTRASTRUCTURAL ANALYSIS OF THE GOLDFISH OPTIC TECTUM FOLLOWING ENUCLEATION. Jeanette J. Norden and John A. Freeman, Dept. of Anatomy, Vanderbilt University School of Medicine, Nashville, TN 37232.

The morphological changes which occur in synapses as a function of innervation are of considerable interest in that they may reveal important aspects of underlying mechanisms of synaptogenesis. Of particular interest is whether removal of a major afferent pathway results in sprouting and reorganization of remaining synapses.

We have examined quantitatively the synaptic organization of the neuropil of the optic tectum in goldfish, using a new random sampling method developed for counting synapses (Freeman and Norden, in prep.). The number of synapses in the stratum fibrosum et griseum superficiale (SFGS) (the major target of retinal afferents in goldfish), and the fate of postsynaptic specializations has been followed in normal animals and in goldfish enucleated from 1-280 days. The number of synapses in the SFGS drops dramatically between 5-15 days post-enucleation and by 49 days, the SFGS is significantly reduced in volume. By 280 days post-enucleation, the SFGS is nearly non-existent. The number of synapses in tectal layers not receiving visual afferents, however, remains constant. Few vacated postsynaptic sites can be seen at any time following enucleation, suggesting that the postsynaptic area has been removed along with degenerating retinal afferents. We conclude that no significant sprouting or reorganization of tectal neuropil occurs following enucleation in goldfish.

The present results suggest that the loss of α-BTX binding sites following enucleation (Oswald, Schmidt, Norden and Freeman, Brain Res., 187:113, 1980) is due to the removal of the postsynaptic area containing the ACh receptor (Oswald and Freeman, J. Biol. Chem. 254:3419, 1979) along with degenerating retinal terminals, and that the gradual increase in the number of sites during regeneration (Schechter et al., Brain Res., 166:57, 1979) is due to the de novo synthesis of post-synaptic receptor or reorganization of remaining postsynaptic membrane.

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106.2 IN VITRO SYNTHESIS OF A SPECIFIC PROTEIN IN THE DEAFFERENTED GOLDFISH RETINOTECTAL PATHWAY. Wolfgang Quitschke* and Nisson Schechter. Departments of Biochemistry, Psychiatry and the Long Island Research Institute, SUNY at Stony Brook, New York 11794.

The regenerating goldfish retinotectal pathway is a model system for the study of axonal outgrowth, nerve regeneration and neuronal specificity. A critical aspect of these phenomena is the study of unique proteins associated with the reconnection process. In a previous study utilizing two dimensional gel electrophoresis we identified specific endogenous proteins associated with the retinotectal pathway of goldfish (Brain Res., In press). In this study we report the synthesis of a ^{35}S-methionine labeled protein associated with tectal deafferentation.

Goldfish underwent enucleation of one eye or intraorbital optic nerve crush. Pooled control and experimental tecta were excised and incubated in HEPES minimal salts medium (pH 7.4) for 2 hr at 25°C in the presence of ^{35}S-methionine (50 μci/ml) (J. Neurochem. 22 (1974) 821-830). The tecta were subsequently frozen and lyophilyzed. The dry residue was solubilized in a lysis buffer containing 9.5M urea, 5% β-mercaptoethanol, 20% NP-40, Ampholines 1.6% pH 5-7, 0.4% pH 3.5-10. Protein separation patterns were obtained from experimental and normal tecta at various times after optic nerve crush or enucleation by two dimensional gel electrophoresis according to O'Farrell (J. Biol. Chem. 250, (1975) 4007-4021). Autoradiograms were obtained by exposing the stained and dried gels to x-ray film for 2-3 weeks.

The autoradiograms revealed an intensely labeled acidic protein spot in the 30K molecular weight range which was found in the experimental tectum 2-4 days after optic nerve crush or enucleation. This spot is undetectable in the ^{35}S-methionine labeled normal tectum and it cannot be detected with Coomassie blue staining either in the normal or experimental tectum. This protein is observed for about 12 weeks in the denervated tectum. The decrease in labeling intensity and the gradual return to control levels after optic nerve crush correlates with the time course of axonal regeneration.

These results suggest that the synthesis of at least one specific protein is induced in the optic tectum by disconnection of the optic nerve. The observation that it is highly labeled for a long period of time without ever reaching a level of detectability by Coomassie blue staining suggests a high turnover rate.

Studies are now in progress involving the synthesis of this protein and its detectability during the reconnection process under other conditions of optic nerve disconnection or tectal ablation.

106.3 DEVELOPMENT OF RETINOTECTAL CONNECTIONS. F. Levinthal* and C. Levinthal, Dept. of Biol. Sci., Columbia University, New York, N.Y. 10027.

In order to investigate the mechanism by which retinotectal connections are established we use serial section light and electron microscopy of optic nerves from the eye into the tectum of the Zebrafish. By studying animals from embryonic to adult stages one can observe the initial connection and the sequential growth and development thereafter. Previous reports of Bodick and Levinthal (PNAS 1980) demonstrated that there is in the embryo a well ordered mapping of retinal fibers from the ganglion cells to the optic nerve as it exits the eye. Observation of these fibers suggested that the mechanism by which the order can be established is based on the fact that growth cones of newly forming fibers are in contact with their more mature neighbors and with glia lining the lumen of the optic stalk. The order can be achieved by a simple following mechanism which translates time and position of ganglion cells differentiation into a well defined spatial organization within the optic nerve.

When the optic nerve of the newborn animal is followed from just behind the eye until it reaches the tectum, the growth cones are always found on the ventral portion of the nerve: thus the overall order of fibers everywhere in the nerve remains as it is when the nerve leaves the eye. The fibers in the newborn are not myelinated and they form a compact bundle over their entire path from the eye to the tectum.

In the adult, all of the optic fibers are myelinated and about 5% of them have areas some ten times larger than the majority. The fibers leave the eye as a compact bundle but between the eye and diencephalon they diverge into about 25 separate bundles which merge and rejoin as they proceed. Groups of fibers pass from one bundle to another. On reaching the chiasma the separated bundles come together into a single compact group before the fibers separate as they reach the tectum.

Shortly after hatching the single bundle behind the eye shows what appears to be the beginning of fragmentation into bundles. A large glial cell grows so as to separate the older fibers which lie on the dorsal side of the nerve from those on the ventral side where the fibers which have grown most recently are found. Details of the mapping, the formation of the bundles and the pattern of the nerves as they enter the tectum will be described.

This work was supported by grants from the National Institutes of Health (NS 09821 and RR-00442).

106.4 EFFECT OF THYROXINE ON RETINOTECTAL REORGANIZATION IN GOLDFISH. L.R. Marotte*, R.F. Mark* and J. Wye-Dvorak* (SPON: J.M. Wild). Dept. Behavioural Biol., R.S.B.S., ANU, Canberra, Australia.

After half tectal ablation in goldfish the retinotectal projection may undergo reorganization so that the whole of the visual field is represented on the remaining half tectum. There is an effect of season and lighting conditions on this (Wye-Dvorak, J., Marotte, L.R. and Mark, R.F., Neurosci. 4: 489-802, 1979). Compression of the whole visual field onto the remaining half tectum occurs in summer but not in winter in fish kept in normal light/dark (L/D). Constant light (C/L) conditions prevent compression (Yoon, M., J.Physiol., 246: 673-694, 1975). These results suggest a possible hormonal influence. The effect of thyroxine on reorganization was tested after removal of the caudal half of the left tectum in goldfish 6.5 - 9.0cm in body length. The results are summarised below.

Season of Year & Lighting conditions	Proportion of fish with reduplicated fields or partial compression		Time after operation of electrophysiological mapping
	Operated & Thyroxine	Operated	
L/D winter 4mgm/l thyroxine	6/7	2/8	41-65 days
L/D winter .006 mgm/l thyroxine	8/10	0/9	35-67 days
C/L summer 4mgm/l thyroxine	3/7	0/7	76-163 days

Both high and low doses of thyroxine induced some reorganisation particularly in fish kept in L/D during winter. Ultrastructural examination of tecta showed that this was not related to the amount of optic fiber sprouting. Less sprouting occurred in the L/D group receiving a high dose of thyroxine compared to its controls while no differences were seen in the other two groups. A hormonal influence on reorganization has been demonstrated and this may underlie the effects of season and lighting conditions which are both associated with changes in hormone levels.

106.5 THE ORGANIZATION OF RETINAL PROJECTIONS IN GOLDFISH. S. C. Sharma. Department of Ophthalmology, New York Medical College, Valhalla, New York 10595.

The retinal ganglion cell projections in goldfish were labelled by an injection of HRP solution into the vitreous of the eye. The pattern of optic axons in the optic tract was regular. The optic tract divides into lateral and medial tracts at the level of the anterior pole of the nucleus rotundus. The lateral tract curves around at the lateral and ventral aspect of the nucleus rotundus and divides further into two branches. The fibers in the extreme lateral position innervate the ventral lateral tectum directly, coursing in the SFGS. Fibers innervating the deeper tectal layer of the ventral and lateral tectum course through the tectobulbar tract to reach the tectum. The entries to the SFGS occur at various levels, hence a huge bundle gives the appearance of bending 90^{o} at the rostral pole. Fibers in the medial portion of the lateral tract course around the nucleus rotundus and turn dorsally before innervating the SFGS. Before entering the tectum, a few fascicles disassociate from the tract and innervate diencephalic areas.

Fibers of the medial tract travel medial to the nucleus rotundus, turn dorsally and form a 90^{o} curve from which eminate fascicles which enter the dorsal tectal SFGS. This crossover of fibers at the dorsal rostral pole of the tectum was well pronounced at the level of medial diencephalic target centers. Fibers terminating in the deeper tectal layers in the dorsal tectum separate from the ventral portion of the medial tract. This crossover of medial tract and the medial portion of the lateral tract at the level of the rostral pole of the tectum is similar to that suggested by Scholes in the Cichlid (Nature, 278: 620-624).

In addition, direct retinal projections were seen in the diencephalic centers which include the nucleus dorsolateralis, nucleus dorsomedialis, area pretectalis, nucleus pretectalis, preoptic nucleus, nucleus of the posterior commissure and lateral geniculate nucleus. Direct projection to the ipsilateral diencephalic centers was not observed. However, very few axons in the posterior commissure were labelled, suggesting fiber paths for the ipsilateral diencephalic target centers. In the tectum four different layers were labelled: stratum opticum, stratum fibrosum et griseum superficiale, stratum album centrale and stratum fibrosum periventriculare. In addition, large numbers of radial fibers were seen mostly originating from the deep fiber layer.

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106.6 GOLDFISH OPTIC TECTAL EXPLANT AS A MODEL SYSTEM FOR NEUROPHYSIOLOGICAL AND BIOCHEMICAL STUDIES. T. J. Teyler, D. Lewis, V. E. Shashoua and C. G. Brogna*. Neurobiology Program, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272, and Dept. of Biological Chemistry, Harvard Medical School, Belmont, MA 02178.

We have developed methods for maintaining explants of goldfish optic tecta in a defined medium in vitro. Such cultures offer an excellent model system for studies of the neurophysiological and biochemical properties of an intact neural structure under controlled conditions. Because of the anatomical organization of goldfish tectum as a spheroid structure floating above the ventricular space, it can be removed almost intact by severing its three input and output systems, i.e., optic nerve, connections to dorsal tegmentum and commissural connections. This allows the removal of a 400u thick cortical structure with its laminar organization intact.

Using an incubation medium which approximates the ionic composition of goldfish brain CSF and a 95/5%, O_2/CO_2 atmosphere, we find that such explants can maintain a linear rate of RNA and protein synthesis at 22° C. for at least 17 hours and 24 hours, respectively. Protein synthesis can be inhibited by puromycin but not by chloramphenicol.

Electrophysiological stimulation of the severed optic nerve stump gives rise to short latency responses from widespread regions of the explanted tectum. Population responses to such stimulation at various recording depths display a field potential laminar profile indicative of source-sink relationships corresponding to the anatomical terminations of optic nerve fibers and are in agreement with similar in vivo profiles. A short-latency triphasic potential has been identified as a population fiber volley resistant to a high Mg++, low Ca++ incubation medium. A longer latency negative response centering on the stratum opticum displayed response jitter and variability, was abolished by a high Mg++, low Ca++ medium and followed moderately high (50 Hz) stimulation frequencies. Thus this potential was judged to be a monosynaptic response to optic nerve stimulation. Double-shock stimulation disclosed the nature of facilitation and recurrent inhibitory processes in this circuit. Stimulation at various intensities and locations indicated several additional, longer latency responses apparently mediated by pathways with lower conduction velocities.

These results demonstrate the biochemical and neurophysiological integrity of the system. Such cultures should be suitable for pharmacological and metabolic investigation.

Supported by grants from the McKnight Foundation and NSF.

106.7 ABERRANT RETINAL PROJECTIONS IN EARLY STAGES OF GOLDFISH OPTIC NERVE REGENERATION. A. D. Springer. Dept. of Anatomy, New York Med. Col., Valhalla, NY 10595.

The assumption that optic nerve fibers in goldfish regenerate only to appropriate targets and bypass inappropriate targets was examined by crushing one optic nerve behind the eye. Fish were maintained at 30°C following surgery in order to accelerate regeneration. Separate groups of 2-3 fish were injected intraocularly with 25μc of (^{3}H)proline 24 hr prior to being killed at either 2,4,8,16 or 32 day post-optic nerve crush. Subsequently, the brains were processed for radioautography.

Some fibers had regenerated to the chiasm within 4 days post-crush, but none had reached the diencephalon or tectum. At 8 days post-crush the contralateral tectum was densely labeled medially and laterally, as well as rostro-caudally. Other normal contralateral retinal targets that were labeled at this time included: nucleus dorsolateralis, nucleus dorsomedialis, area pretectalis, nucleus pretectalis, preoptic nucleus, nucleus of the posterior commissure and lateral geniculate nucleus. Several anomalous contralateral targets were labeled 8 days post-crush: nucleus rotundus, nucleus isthmi, torus longitudinalis, nucleus lateralis valvula and corpus cerebelli. In addition, the pituitary gland received fibers from both the contralateral and ipsilateral optic tracts. Furthermore, the ipsilateral optic tectum was extensively labeled. Fibers were observed in several anomalous tracts: ipsilateral optic tract, posterior commissure, intertectal commissure, horizontal commissure, tectobular tract and mesencephalo-cerebellar tract.

Aberrant fibers were only evident in the ipsilateral optic tract and in the stratum opticum of the rostral ipsilateral tectum at 16 days post-crush. By 32 days post-crush all label was confined to the normal contralateral and ipsilateral diencephalic retinal targets, as well as to the contralateral optic tectum.

The present study indicates that regenerating optic nerve fibers grow to extensive regions of the brain. Subsequently, they appear to retract or degenerate from anomalous structures. The occurrence of these aberrant projections was unexpected, as was their transience. An explanation of their transience assumes that the aberrant fibers are collateral branches rather than primary axons. Collateral fibers may fail to successfully compete with existing primary axons and therefore they either retract or degenerate. Previous evidence (Springer, Neurosci. Abstr., 5:684, 1979) suggests that competition between primary axons of different origins leads to fiber segregation, rather than to retraction of foreign fibers.

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106.8 FURTHER RULES FOR ORDER IN THE GOLDFISH OPTIC NERVE. Anne C. Rusoff. University of Michigan, Ann Arbor, Michigan 48109

Axons from annuli of retinal ganglion cells are clustered together in the goldfish optic nerve. This raises the question: are the axons within such a cluster further ordered retinotopically? This question was answered by filling the axons of the ganglion cells from one half of the retina with horseradish peroxidase (HRP) and observing the distribution of axons filled with HRP reaction product in x-sections of the optic nerve and tract. HRP was introduced into the optic axons from half of the retina by cutting one brachium of the optic tract and coating it with HRP. (Axons from dorsal/ventral retina are in the ventral/dorsal brachium of the optic tract.) When the dorsal brachium alone was filled with HRP, a x-section from the optic nerve or distal portion of the optic tract had filled axons only around its outer edges; the central part was mostly free of reaction product. When the other brachium was filled, the converse pattern was observed. Thus, axons from the ventral and dorsal halves of the retina are segregated from each other; therefore, there is at least gross topographic order within the optic nerve.

A second question is: are adjacent clusters of axons in the nerve from adjacent retinal annuli? Since the retina grows by annular addition of ganglion cells, the axons in a cluster are from cells of the same age. A cluster of axons of one age might join the nerve near a slightly older cluster of axons. To test this idea, HRP was injected directly into a small spot on the retina, and filled axons were traced into the nerve. Each injection filled axons from ganglion cells peripheral to the injection site so that a central injection filled axons of both peripheral and central cells. All injections made into ventral retina filled axons around the outer edge of a x-section of the nerve, in agreement with the results above. A peripheral injection filled a short length of the edge, while a more central injection filled a longer length of it, rather than discontinuous clusters of axons. This result suggests that age-related clusters of axons do join the nerve next to slightly older clusters of axons.

In summary, axons in the optic nerve of goldfish appear to be ordered both by the age and position of their cell bodies. These results agree with suggestions of Bunt and of Dawnay and further suggest that axons in the goldfish optic nerve follow the same rules of ordering found in the ribbon-shaped optic nerves of perciform fishes (Scholes. Nature 278: 620, 1979). The "ribbon" is simply less obvious in the goldfish optic nerve. (Supported by PHS Grant # EY05294 to author and EY00168 to S. S. Easter, Jr.)

106.9

Withdrawn by Author

106.10 IN VITRO INTERACTION BETWEEN THE RETINAL TISSUE AND THE CO-CULTURED TECTAL TISSUE, EXPLANTED FROM ADULT GOLDFISH. Myong G. Yoon and Frank A. Baker* Department of Psychology, Dalhousie University, Halifax, Nova Scotia, CANADA, B3H 4J1.

The outgrowths of regenerating neural processes from the retinal tissue into the tectal tissue were studied in vitro under various experimental conditions by co-culturing the retinal and tectal tissues, explanted from the adult goldfish. An elongated rectangular piece of the retina was dissected free along the nasotemporal axis near the equator, and implanted on a culture dish in a predesignated orientation. The regenerating neurites from the retinal explant usually grew in the radial direction towards the phantom optic disc as reported by Johns, Yoon & Agranoff (1978). When the retinal explant showed vigorous outgrowth of neurites (usually 2-5 days after explantation), its topographically matching area of the optic tectum was dissected from the same experimental fish, and co-cultured later with the retinal explant in various geometric configurations. The patterns of neuritic outgrowth from the retinal explant into the co-cultured tectal explant were observed at different intervals in culture. The retinal neurites with bush-like growth cones which contained several philopodia at their advancing tips were firmly attached to the substratum on the culture dish before they grew into the tectal explant. When the retinal neurites successfully invaded the tectal explant, however, they tended to detach from the substratum, and appeared to form cables which linked the retinal and tectal tissues. In one case, the other retinal neurites which failed to invade the tectal explant were withdrawn and disappeared later. Further investigations on the trajectories of the retinal neurites within the co-cultured tectal tissue are in progress.

Johns,P.R., Yoon, M.G.& Agranoff, B.W.(1978). Nature 271, 360.

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106.11 GLUTAMIC ACID BINDING TO GOLDFISH BRAIN:TECTAL ACTIVITY DURING OPTIC NERVE DEGENERATION AND REGENERATION. Andrew Francis*, Wolfgang Quitschke* and Nisson Schechter (SPON: B. Twarog)Cornell Medical School and Departments of Biochemistry, Psychiatry and the Long Island Research Institute, SUNY at Stony Brook, NY 11794

Regeneration of the goldfish optic nerve to optic tectum is a model system for study of axonal outgrowth and nerve reconnection. A critical aspect of reconnection is the analysis of neurotransmitter regulation and types of synapse in the tectum. We have previously shown that about 40% of the tectal nicotinic-cholinergic receptors are lost upon tectal denervation; both the number and concentration of receptors are restored during optic nerve regeneration. In contrast, tectal muscarinic-cholinergic receptors are stable in the denervated tectum (Brain Res 166 57; 185 161). We now report binding of L-Glu to goldfish brain membranes and changes in tectal binding following optic nerve denervation and regeneration. Equilibrium binding (at 21°) of ^{3}H-L-Glu was performed in Tris buffer according to Foster and Roberts (J Neurochem 31 1467). Saturable, reversible, and specific binding occurred to washed membranes from whole goldfish brain to a single population of sites having an apparent Kd of 4 uM and a capacity of 10 pm/mg original tissue. There was a 10-fold regional variation in specific binding (pm/mg original tissue) measured at saturation:cerebellum, 26; diencephalon, 8.4; telencephalon, 7.5; vagal lobe, 6.7; optic tectum, 4.8; spinal cord, 3.3. Specific binding was enriched 1.6 times in membranes derived from a crude synaptosomal (P2) fraction, prepared by differential centrifugation of brain homogenates in 0.32M sucrose. Pharmacological studies were performed by preincubating serial dilutions of drugs with membranes before addition of ^{3}H-L-Glu to a final concentration of 7 nM. The apparent protection constants (Kp) were defined as drug concentrations inhibiting binding by 50%:L-Glu, 4 uM; L-Gln, 30uM; L-Asp, 70 uM; kainic acid, $\gg$ 10mM; GABA, $\gg$ 10mM. Following eye removal, the total number of L-GLU binding sites was stable for 4 days, followed by a rapid loss in activity reaching 40 $\pm$ 12% at 24 days. At 70 days after optic nerve crush, when optic nerve regeneration had occurred, the number and concentration of L-Glu binding sites was not different from control. These observations suggest a delayed loss of L-Glu binding sites in the denervated tectum, which is reversed if optic nerve regeneration occurs. The delayed loss in these sites, compared immediate loss of nicotinic sites and stable muscarinic sites, suggest that these 3 sites are in differing tectal compartments. The L-Glu binding sites may be located on an intrinsic tectal system which is secondarily degraded during tectal denervation.

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106.12 BANDED DISTRIBUTION OF RETINAL GANGLION CELL TERMINALS WITHIN THE STRATUM ZONALE (9A) LAYER OF THE FROG OPTIC TECTUM. Margaret I. Law* and Martha Constantine-Paton (Spon. D. Kelley) Princeton University, Princeton, New Jersey 08544

The existence of bands of retinal ganglion cell terminals within the stratum zonale (Potter's layer 9A) of the optic tectum were revealed through labelling with horseradish peroxidase (HRP) histochemistry. In normal postmetamorphic animals optic nerves were cut and HRP placed against the distal stumps. After two days survival the presence of HRP was detected through a $CoCl_2$-diaminobenzidine reaction. Layers 9B-F of the contralateral tectum contained the typical, uniformly labelled optic projection. Within 9A, however, 80-100μ wide bands of labelled neuropil alternated with 100-200μ wide terminal free areas.

This striped input appeared to develop in late tadpole life since no bands were observed in similarly labelled tadpoles. Serial reconstruction of postmetamorphic optic projections demonstrated that the bands extended in a rostral-caudal direction over the anterior 3/4ths of the tectal surface.

The interband regions are thought to be occupied by fibers originating in the nucleus isthmi. This nucleus mediates intertectal connections such that visual information from one tectal lobe is retinotopically mapped over the other lobe.[1,2] Thus, by way of a direct contralateral retinal projection and a poly-synaptic ipsilateral pathway both retinas are mapped within each tectum. Recent physiological and anatomical evidence located the isthmi's intertectal projection to be just below the pial surface, in layer 9A.[1,2]

We have previously reported the induction of periodic bands throughout layers 9B-E of the dually innervated tecta of 3-eyed and single tecta frogs.[3,4] The bands observed in normal animal's layer 9A are only half as wide and are not as regular as the induced bands of dually innervated tecta. Nevertheless, in both cases retinal ganglion cell axons terminate within similarly oriented bands of tectal neuropil receiving retinotopically organized visual information from two eyes.

1. P. Grobstein et al. Brain Res. 156:117-123 (1978)
2. E. Gruberg and S. Udin J. Comp. Neurol. 179:487-500 (1978)
3. M. Constantine-Paton and M. Law Science 202:639-641 (1978)
4. M. Law and M. Constantine-Paton PNAS 77(4):2314-2318 (1980)

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106.13 CENTRIPETAL CELL LOSS IN THE RETINA OF RANA PIPIENS TADPOLES. Ricardo G. Cigarroa* and M. Constantine-Paton, Department of Biology, Princeton University, Princeton, New Jersey 08544

We have studied cell proliferation and cell loss in the retina of the larval leopard frog Rana pipiens. Frog embryos and tadpoles were multiply pulsed with ^{3}H-thymidine (NEN, specific activity 6.7Ci/mmole; 1μCi/gm) at Shumway[1] st. 21, Taylor and Kollros[2] st. 4,6,8,12,18 and 25. Two days after each pulse some animals were sacrificed to compare the initial location of labeled cells with their position at subsequent stages of development.

Our results confirm earlier observations on retinal histogenesis in this species in that new retinal cells appear to be generated only at the ciliary margin[3]. After a ^{3}H-thymidine pulse and a short survival all labeled retinal cells are located at the retinal perimeter. This labeled zone has an arrowhead shape with labeled cell bodies in the inner nuclear layer extending further toward the fundus of the eye than equally labeled cell bodies in the outer nuclear or retinal ganglion cell layers. The pattern of label suggests that for a given segment of retina, ganglion cells and receptors become post-mitotic before the inner nuclear layer.

Animals surviving for long periods of time showed a progressive and previously unsuspected absolute movement of the labeled cellular annulus toward the optic disc. For example, the labeled band of cells resulting from a pulse of thymidine at T & K st.IV will be completely absent from the ventral rim of the optic disc at T & K st. 18 and will be just discernable as a small band of labeled cells on the longer dorsal rim. By T & K st. 25 (total 131,000 ganglion cells) none of the retina generated before T & K st. VI (total 22,000 ganglion cells) is present. These cells have moved to the optic disc where they appear to die or migrate out of the eye.

This pattern of growth implies that none of the cells in the neuroepithelium that initially makes contact with the brain are present in the mature retina and that the position of each retinal ganglion cell relative to the visual world must change progressively during tadpole life.

Our analysis suggests that theories of retinotectal connectivity should be re-evaluated so that they take into account the fact that this anuran amphibian can generate an ordered topographic visual projection with a presynaptic cell population that is in a continual state of flux.

1. W. Shumway. Anat. Rec. 78:139.
2. A.C. Taylor and J.J. Kollros. Anat. Rec. 95:7
3. J. Hollyfield. Dev. Biol. 18:163

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106.14 ORDERLY FASCICULATION IN THE EARLY OPTIC NERVE OF XENOPUS LAEVIS. Jacob A. Sapiro*, Jerry Silver and Marcus Singer. Dept. Anat., Sch. Med., Case Western Reserve U., Cleveland, OH 44106.

In an effort to elucidate the mechanisms which may be responsible for establishing the point to point specificity of retino-tectal projections, eyes and optic stalks of Xenopus laevis embryos at stages 31 to 39 were examined with transmission and scanning electron microscopy. Throughout early development, optic axons grew along the vitreal margin of the retina and the dorsal rim of the optic fissure. In the stalk, optic axons were grouped in a compact bundle, which was always located ventrally. Growth cones were observed with both transmission and scanning electron microscopy. They appeared as large bulbous neurite processes containing numerous vesicles, mitochondria, and other organelles. The growth cones were located primarily in the ventral part of the early nerve, usually in association with the glia limitans. The glia limitans was formed by processes of ventral stalk neuroepithelial cells that enwrapped the fascicle. Older axons, from more ventral regions of the posterior pole of the early retina, occupied a more dorsal position in the nerve. Scanning electron microscopy showed that optic axons tended to maintain their positions relative to their neighbors. It is therefore likely that the constant ventral accumulation of growth cones displaces older axons dorsally, thereby creating retinotopic order in the dorsoventral axis of the early nerve. This spatio-temporal sequence of orderly fasciculation onto the early optic nerve may, in turn, play a role in the establishment of ordered synapses in the tectum.

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106.15 **ANOMALOUS IPSILATERAL RETINOTECTAL RESPONSE, FOLLOWING THE SELECTIVE ABLATION OF ONE CLASS OF RETINAL GANGLION FIBER IN THE CONTRALATERAL TECTUM OF THE FROG,** **S. Glasser and S.C. Sharma. Dept. Opthal., N.Y. Med. Col., Valhalla, N.Y. 10595**

The rostral tectum of the frog receives input from both contralateral and ipsilateral retina.

Fibers from the contralateral retina project directly through the optic chiasm to the tectum, whereas ipsilateral retinotectal input is relayed first through the contralateral tectum (Keating and Gaze, 1970).

We previously reported plasticity in the contralateral frog retinotectal projection following a partial ablation of only one class of retinal ganglion fiber. Class 2 fibers found just beneath the pia respond with a sustained discharge when a small black spot is brought in and stopped within their excitatory receptive field (ERF). Small, superficial, mediolaterally oriented cautery lesions in the rostral tectum were found to eliminate class 2 fiber activity in a region directly caudal to the burn. Such lesions, however, spare deeper class 3 fibers, which respond with a brief burst when an object is moved into their ERF. Three weeks following the lesion, in the region previously devoid of class 2 activity, class 3 responses were found at both class 2 and class 3 loci. The ERF's of the aberrant, superficial class 3 terminals completely overlap those of the normal, deeper class 3 terminals (Glasser, Sharma and Ingle: ARVO, 1979).

We now describe ipsilateral visuotectal response following superficial cautery lesions in the contralateral tectum.

Ipsilateral visually driven units in rostral frog tectum are characterized as class 2 or class 3 using the same criteria as for contralateral units. Superficial cautery lesions selectively abolish class 2 ipsilateral response in the tectum contralateral to the burn. Such lesions, however, have no effect on the density or response properties of the deeper, class 3 ipsilateral fibers. The class 2 scotoma in the ipsilateral tectum corresponds to that found in the contralateral tectum.

Three weeks postlesion, class 3 ipsilateral responses exactly mirror class 3 activity in the operated tectum. In the region of ipsilateral class 2 scotoma, ipsilateral class 3 units are found at both ipsilateral class 2 and ipsilateral class 3 loci. The ERF's of the aberrant, superficial class 3 terminals completely overlap those of the normal, deeper class 3 terminals.

The implications of these findings will be discussed

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106.16 GROWTH AND DIFFERENTIATION OF THE OPTIC TECTUM OF PETROMYZON MARINUS. K. Rubinson, M.C. Kennedy and H. Cain*. Dept. of Physiol. Biophys., New York Univ. Med. Ctr., New York, NY 10016 and Dept. of Biology, New York Univ., New York, NY 10003.

The visual system of the sea lamprey, Petromyzon marinus develops slowly over the larval and transformation periods which encompass approximately five years of the seven-eight year life span of the animal. We have previously reported the presence of retinotectal projections throughout this period (Kennedy and Rubinson, 1977) although retinal differentiation and function are not accomplished until the middle of transformation (Rubinson, et al., 1977). Histological study shows that the differentiation of the laminae of the optic tectum reaches its adult character early in transformation (stage II). However, Golgi impregnations reveal that the tectal cells of the early transformer exhibit dendritic patterns comparable to those of the larva.

Planimetric analysis of the tectum from 10μ epoxy sections reveals a progressive increase in tectal surface area from early larval through young adult specimens. This growth pattern parallels the growth of the retinal surface and of the whole brain. From measurements of total tectal tissue volume and from surface measurements, an averaged index of tectal thickness was derived. This index shows an abrupt increase between stages II and V of transformation. The cellular laminae primarily responsible for this increase are the stratum cellulare externum and stratum marginale which are the site of retinal efferent fibers. This superficial zone grows during transformation to occupy nearly three-fourths of the tectal thickness. Thus, the increase in tectal thickness is contemporaneous with dendritic differentiation in the superficial laminae of the tectum and with the functional maturation of the retina.

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106.17 REAGGREGATION OF CHICK RETINAL OR TECTAL CELLS CULTURED IN INTRACEREBRALLY IMPLANTED CAPSULES. E. Hibbard and B.L. Ruskin*. Dept. of Biol., Penn State Univ., Univ. Park, PA 16802.

A technique which has been developed to provide a functional artificial pituitary in rats (Hymer et al., Endocr. Soc. 61st-meeting [#231] P. 130, 1979) has been adapted to allow long term culture of embryonic neural cells as implants in the brains of chicks. Dissociated cells of neural retina and/or tectum of 7 day chick embryos were encapsulated in hollow H5P10 dialysis fibers (Amicon Corp.) and were inserted into the neostriatum of newly hatched chicks. Survival of the hosts was excellent and there was no apparent behavioral impairment. After varying survival times up to one month, the capsules were removed, fixed, critical point dried, opened and gold-coated for SEM. Cells cultured by this method reaggregated rapidly, developed neural processes, underwent a high degree of morphological differentiation, and showed apparent selective neuron to glia associations. Different developmental patterns were noted between retinal and tectal reaggregates. Retinal cells tended to aggregate into large masses and their well-developed processes appeared to preferentially associate with flattened epithelial-like cells which we interpret to be Mueller cells. Tectal cells form small aggregates which became interconnected by single neurites or fascicles. These fascicles of nerve fibers increased in complexity in older cultures. This method provides several advantages over conventional culture systems. The technique is simple and reliable and it provides the reaggregating cells with a constant, nearly normal cell environment at constant temperature. The 10,000 M.W. cut off of the fiber precludes invasion of the culture by phagocytes while permitting nutrient and gaseous exchange with the vascular or cerebrospinal fluid compartments of the host. There is little chance of contamination provided the capsules are prepared and implanted under aseptic conditions. The cultures are self-maintaining and when removed from the host are easily handled because the capsule need not be opened until processing has been completed. All cells are fixed in situ, even those which do not adhere to the substrate. The system provides a better environment than either plate or rotating suspension cultures because it provides a stable substrate while at the same time allowing the culture to develop three-dimensionally.

106.18 OBSERVATIONS ON THE EFFECTS OF MONOCULAR AND BINOCULAR EYE REMOVAL ON THE DEVELOPMENT OF THE CHICK VISUAL SYSTEM. D.D.M. O'Leary and W.M. Cowan. Dept. of Anatomy and Neurobiology, Washington Univ. Sch. Med., St. Louis, MO. 63110.

The effects of monocular and binocular eye removal, before and after the stage at which the retina is known to be specified with respect to its projection upon the optic tectum (i.e. stage 12/13 of the Hamburger and Hamilton series) have been studied in a series of chick embryos which were subjected to intraocular or tectal injections of ^{3}H-proline shortly before hatching (stages 43-45). We have confirmed that following the removal of one eye before stage 12, the surviving retina develops a substantial projection to the ipsilateral diencephalon and midbrain; in some cases the "aberrant" ipsilateral retino-tectal projection appears to be almost as substantial as the projection to the contralateral tectum, and it clearly persists throughout embryonic development. When the optic cup was removed after stage 12 (usually at stages 15-17) an "aberrant" ipsilateral retinotectal projection was usually present, but this was invariably less substantial than in those cases in which the eye removal had been done before stage 12.

In order to determine whether the topographically ordered projections of the optic tectum upon other brainstem structures can develop normally in the absence of retinal input, bilateral eye removals were carried out both before and after stage 12. Following bilateral eye removal, before stage 12, more than 90% of the embryos showed a fusion of the two tectal lobes, so that the dome-shaped single tectum closely resembled that seen after early mesencephalic alar plate ablations. Comparable tectal fusions were not seen when the optic cup removals were carried out after stage 12. By injecting ^{3}H-proline into different parts of the optic tectum in chick embryos which had earlier bilateral eye removals, we have been able to show that the projection of the optic tectum upon the nucleus isthmi pars parvocellularis and on the nucleus semilunaris is organized in a manner essentially similar to that seen in normal animals. This observation argues strongly for the view that the topographic specification of the connections of the optic tectum is not determined by its retinal input.

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107.1 DIFFERENT CHOLINERGIC SYNAPSES CONVERGING ONTO NEURONS IN APLYSIA PRODUCE THE SAME SYNAPTIC ACTION. Michael Segal* and John Koester. Div. of Neurobiol. & Behavior, Dept. of Physiology, College of Physicians & Surgeons, Columbia University, New York, N. Y. 10032

If a neuron receives synapses from two different cells that use the same transmitter, can one cell depolarize and the other hyperpolarize by virtue of activating different types of postsynaptic receptors? In other words, when a postsynaptic neuron concentrates receptors at synapses, does it concentrate one type of receptor to a particular transmitter at one synapse and a different type of receptor to the same transmitter at another synapse?

To approach this question we have investigated neurons in the abdominal ganglion of Aplysia californica that receive inputs from more than one cholinergic interneuron. Two of the interneurons (L10 and Int XIII) have been shown previously to be cholinergic. Using both biochemical and pharmacological tests we have shown that a third interneuron, L24 (Int XI), is also cholinergic. We have compared the sign of the connection to seven postsynaptic cells (L10, L11, LD_{HI}, $L9_C$, R15, RB, L7). Some of these connections were previously known, and others we have recently identified.

For any given cell, we found that different cholinergic inputs converging onto the cell all produce the same sign of synaptic action. This finding holds even for L7, a cell known to have both depolarizing (D) and hyperpolarizing (H) ACh receptors; all three interneurons produce conjoint (D-H) PSPs in L7. These data are consistent with the hypothesis that a single postsynaptic cell does not segregate one type of ACh receptor to one type of synapse and a different type to other synapses.

If generally true, this lack of segregation may provide insights into some of the organizational principles of nervous systems. If all cholinergic inputs to a particular cell produce excitation, then inhibitory inputs to that cell must use a different transmitter. In addition, if two cells use the same transmitter and influence a particular cell in different ways, one would expect that at least one of the connections will be polysynaptic, involving an interneuron with a different transmitter. Thus, a lack of segregation of postsynaptic receptors for a particular neurotransmitter may help to explain why a nervous system uses many neurotransmitters as well as explain a need for certain interneurons. Supported by NIH Grant NS 14385 and NIH Medical Scientist Training Grant GM 0737-04.

107.2 TWO RELAXATION COMPONENTS ASSOCIATED WITH THE ENDPLATE CURRENT DECAY AND WITH THE CARBAMYLCHOLINE-INDUCED CURRENTS FOLLOWING A VOLTAGE JUMP. D.A.Farquharson*(SPON:F. C.G.Hoskin).Dept.Biol.,Ill. Inst. Tech.,Chicago,IL.

Some insight into the functioning of the acetylcholine receptor(AChR),has been gained by applying the methodology of chemical relaxation to chemically excitable membranes. This has usually involved either observing the decay of the endplate current(EPC)or observing the relaxation of the agonist-induced currents following a voltage jump. For either kind of relaxation experiment, results have shown that some component of AChR function is voltage sensitive. The relaxations themselves have been described as single exponential. There are occasional observations of a second relaxation component(e.g.Magleby & Stevens,J. Physiol. 223:173-197,1972) Also a wide range of endplate treatments have been reported to change single component relaxations of the AChR into relaxations with 2 or more components. This evidence suggests that under certain conditions more than one molecular process can be revealed(or produced) in an AChR relaxation. I find thatin the eel electroplaque,at temperatures above 20°C,AChR relaxations usually can be described as having 2 exponential components. This result was obtained both in voltage jump experiments with carbamylcholine(carb)as the agonist and in experiments involving the decay of the neurally evoked EPC. The faster component rate constant increases with membrane positivity. The slower component is less sensitive to membrane voltage and its rate constant increases with negativity when measured in voltage jump experiments. For EPC decay experiments, the voltage-sensitivity of the slow component is less clear. Ca^{++} has a differential affect on the 2 components of relaxation described here. With Ca^{++} in the bathing medium the slow component rate constant declines with time in carb. No statistically significant,similar decline is observed for the fast component.Also,when Mg^{++} is substituted for Ca^{++},no decline in the slow component rate constant occurs. When Ca^{++} is increased from 2mM to 4mM, the fast component rate constant increases, but the slow component rate constant is unaffected. Ca^{++} also has a separate affect on the relative current amplitudes of the slow and fast components. (Research supported by ARO grant DAAG29-78-G-0090)

107.3 QUANTITATIVE ANALYSIS OF THE ALTERATION OF FAST EXCITATORY POSTSYNAPTIC CURRENT DECAY BY ATROPINE. Elizabeth A. Connor*, Steven M. Levy*, and Rodney L. Parsons. Depts. of Physiol. and Biophys. and Anat. and Neurobiol., Univ. of Vermont, Burlington, VT. 05405.

Atropine decreases the amplitude of the fast excitatory postsynaptic current (EPSC) recorded at sympathetic ganglia and splits the current decay into at least two components; one faster and one slower than control (MacDermott et al., J. Gen. Physiol. 75:29, 1980). We have quantitated the concentration and voltage dependence of this action of atropine. Experiments were done on B cells in the IX and X sympathetic ganglion of the bullfrog, Rana catesbeiana, maintained in a HEPES-buffered solution at 21-23°C. In 18 control cells, the EPSC rose to a peak value of -7.4±4.0 nA (m±S.D.) within ∿2ms then decayed with a single time constant of 5.0±0.7ms. The concentration dependent reduction of the EPSC size and alteration of the decay time course by atropine in cells voltage clamped to -50mV is summarized below:

Atropine Conc.	Peak EPSC	EPSC Decay τ_2	EPSC Decay τ_1	#of Cells
($\times 10^{-5}$M)	(nA)	(ms)	(ms)	
1.0	-6.8±2.6	3.3±0.8	17.0±11.1	10
2.5	-4.1±1.8	2.3±0.6	16.3± 6.2	7
5.0	-4.0±1.7	1.7±0.7	21.0± 2.7	8
7.5	-1.7±1.0	1.7±0.4	16.4± 4.8	8

At a given atropine concentration, hyperpolarization markedly increases, τ_1 with no consistant effect on τ_2. The current-voltage relationship in the range of -20 to -100mV is linear in both control and atropine-treated cells. The concentration and voltage dependent alteration in the EPSC could be described by the following sequential scheme of channel blockade:

$$nA + R \underset{\text{closed}}{\overset{K_d}{\rightleftharpoons}} AnR \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} \underset{\text{open}}{AnR^*} + Q \underset{F}{\overset{G}{\rightleftharpoons}} \underset{\text{blocked}}{AnR^*\cdot Q}$$

For simplification we have assumed that only the open state conducts ions, and that channels open instantaneously at the peak of the EPSC. For each concentration of atropine, the two decay rates and the ratio of their initial amplitudes uniquely determine the reaction rates α, G, and F. Our analysis indicates that α remains constant with increasing atropine concentration [α(-50mV, 10μM)= 211 sec; α(-50mV, 75μM)=202 sec] whereas, the equilibrium blocking constant G/F appears to decline with increasing concentration [G/F (-50mV, 10μM)=70833/M; G/F(-50mV, 75μM)=30609/M]. At a given concentration G/F is exponentially dependent upon voltage, changing e-fold for each 49.7±8 mV, which implies that ∿50% of the voltage is applied between the external solution and the atropine binding site. The results of this kinetic analysis are consistent with the view that atropine binds in the synaptic channel, blocking the passage of the ions. Supported by NIH Grant NS 14552.

107.4 INFLUENCE OF CALCIUM ON LIGAND BINDING TO THE ION CHANNEL ASSOCIATED WITH THE NICOTINIC ACETYLCHOLINE RECEPTOR. Dennis B. McKay*, Gerald O. Carrier and Robert S. Aronstam. Department of Pharmacology, Medical College of Georgia, Augusta, GA 30912.

Several conformations of the nicotinic acetylcholine (ACh) ion channel complex of Torpedo membranes can be detected by monitoring the binding of radiolabeled probes to the ion channel. The binding of two ion channel probes, tritiated phencyclidine ([^{3}H]PCP) and perhydrohistrionicotoxin ([^{3}H]H_{12}-HTX) is increased several fold in the presence of nicotinic agonists. This increase can be lessened in a time- and concentration-dependent manner by preincubating the membranes with a receptor agonist. The influence of calcium and other divalent cations on the binding kinetics characteristic of resting, "stimulated" and "desensitized" forms of the complex was determined.

Calcium inhibited the specific binding of [^{3}H]PCP and [^{3}H]H_{12}-HTX to the resting ion channel with ED50 values of 11 and 10 mM, respectively. However, the corresponding values for inhibition of binding to the stimulated receptor-ion channel complex (measured in the presence of 10^{-6} M ACh) were reduced to 0.7 and 1 mM, respectively. This increased inhibition was not due to inhibition of ACh binding to the receptor since [^{3}H]ACh binding was not decreased by calcium (10^{-4}-10^{-1} M). The sensitivity of [^{3}H]PCP and [^{3}H]H_{12}-HTX binding to "desensitized" receptor-channel complexes (measured after preincubation of the membranes with 10^{-5} M ACh) was the same as that of the stimulated binding. The chloride salts of several Group IIA cations inhibited ion channel binding. In terms of their inhibition of either [^{3}H]PCP or [^{3}H]H_{12}-HTX to the stimulated ion channel, Ba > Ca > Mg ≥ Sr. However, no ion selectivity was apparent with binding to sites on the resting ion channel (i.e., in terms of their ability to inhibit ligand binding, Ba=Ca=Mg=Sr). Double reciprocal plots of [^{3}H]PCP ion channel binding measured in the presence of various concentrations of calcium indicated a competitive inhibitory interaction. Removal of intrinsic stores of membrane calcium through prolonged incubation with EGTA decreased the affinity of both [^{3}H]PCP and [^{3}H]H_{12}-HTX for the ion channel, as well as the ability of ACh to stimulate channel binding. The amount of the inhibition was related to the calcium content of the membranes. (Supported in part by BRS-2S07-RR-05365-19).

107.5 PHENOBARBITAL ANTAGONIZES PENTOBARBITAL-INDUCED REDUCTION IN LIFETIME OF ACETYLCHOLINE-ACTIVATED SODIUM CHANNELS IN APLYSIA. Ruth E. Wachtel and W. A. Wilson, Dept. of Pharmacology, Duke Univ. Medical Center and Epilepsy Center, V.A. Medical Center, Durham, N. C. 27705.

In Aplysia, both pentobarbital and phenobarbital depress sodium-dependent excitatory responses to short iontophoretic pulses of ACh and GABA. Peak responses to both transmitters are reduced 50% by 75μM pentobarbital or 125μM phenobarbital (Cote and Wilson, JPET, in press).

The average lifetime of ACh-activated sodium channels has been determined from current relaxations in response to voltage jumps. During steady state current responses to ACh, hyperpolarizing voltage clamp commands produce an increase in current that consists of two components. After subtraction of capacitive transients, the current first appears to increase instantaneously due to a change in driving force on the ions, then relaxes to a new steady state level. This relaxation occurs because the channels remain open longer at hyperpolarized potentials. According to theory, the time constant of this relaxation reflects the average lifetime of an open channel.

We have previously shown that pentobarbital shortens the average open time of ACh sodium channels in Aplysia. In control, the current relaxations are a single exponential, with a time constant of ∿40msec at 90mv. In the presence of pentobarbital, however, current relaxations show an initial fast component which becomes faster at higher drug concentrations. At 75μM pentobarbital, the time constant of this fast component is ∿5-10msec.

In contrast, phenobarbital does not alter current relaxations at concentrations as high as 1mM. After exposure to phenobarbital, concommitant application of pentobarbital is then ineffective in reducing channel lifetime. Phenobarbital thus appears to be an antagonist of pentobarbital depression of ACh sodium channel lifetime in Aplysia.

107.6 CHLORISONDAMINE BLOCKS CRUSTACEAN MUSCLE GLUTAMATE AND SYNAPTICALLY ACTIVATED CHANNELS. E. Marder, C. Lingle, and J. Eisen. Biology, Brandeis University, Waltham, MA. 02254.

The excitatory innervation of the striated gm6 muscles of the decapod foregut is probably mediated by glutamate (Glu). We have studied the effects of chlorisondamine(CHL), a presumed nicotinic ganglionic antagonist, on neuromuscular transmission to gm6 muscle fibers from the crab, Cancer borealis, and the lobster, Panulirus interruptus. CHL reduced the peak amplitudes of intracellularly recorded excitatory junctional potentials and iontophoretic Glu responses in a dose-dependent manner; 50% block was seen at $2x10^{-4}$M CHL. Additionally, the time courses of both potentials were prolonged by CHL. Similar effects were seen on iontophoretic quisqualate responses. When synaptic transmission was blocked by 20 mM Mn^{++} saline, the effectiveness of the CHL block was unchanged. Dose-response curves done with iontophoretic Glu application suggested that the CHL block was non-competi tive. CHL produced no effect on muscle membrane potential or resistance. The effects of CHL on focally recorded extracellular excitatory junctional currents(f.ejcs) were examined. CHL reduced the amplitude of the peak of the f.ejcs. The block was more effective at hyperpolarized levels; 10^{-4}M CHL reduced peak f.ejcs about 50% at -120mV and about 25% at -60mV. The rate of decay of the f.ejcs approached a single exponential. Time constants were calculated from regressions over about 80 to 15% of peak amplitude. The f.ejc decay at resting potential (-60mv to -70mv) and at 14°C was substantially shortened from about 7 ms in normal saline to about 2 ms in 10^{-4}M CHL. Frequently the f.ejc decay in CHL was best fit by a double exponential where the slow decay was about 15-30 ms. The slow component was more evident at hyperpolarized levels than at rest. The data support the idea that at least part of the effect of CHL is to block open channels activated by either Glu or the natural excitatory transmitter. In contrast to these results, Glu-elicited Cl^- and K^+ conductance increases that are found on cells of the stomatogastric ganglion were unaffected by 10^{-4}M CHL. In addition to its effects on a glutamatergic excitatory process, CHL blocks excitatory ACh responses and f.ejcs at two orders of magnitude lower concentrations on acetylcholine-sensitive foregut muscles. Other pharmacological agents including many ganglionic nicotinic antagonists block foregut cholinergic responses, but not gm6 f.ejcs or Glu responses. Thus, CHL may help us directly compare the characteristic of ACh-gated and glutamate-gated excitatory conductances on similar muscles. CL is a postdoctoral fellow of the Muscular Dystrophy Assoc. Supported by a McKnight Foundation Scholars Award and NSF BNS 78-15399.

107.7 USE OF ARSENAZO III AS A MONITOR FOR CHANGES OF INTRACELLULAR FREE Ca^{++} AND pH IN ARCHIDORIS NEURONS DURING IONTOPHORETIC AND BATH APPLICATIONS OF NEUROTRANSMITTERS. P.E. Hockberger and J.A. Connor. Dept. of Physiology and Biophysics, and the Neural & Behavioral Biology Program, Univ. of Illinois, Urbana, IL 61801.

Recently, the dye Arsenazo III has been used for simultaneous measurements of intracellular changes in free Ca^{++} and pH under voltage clamping conditions in giant molluscan neurons (Z. Ahmed and J.A. Connor, J.Gen.Physiol., 75:403, 1980). This technique is very sensitive, has a time resolution in the millisecond range, and exhibits a steady baseline which can be monitored for hours. We therefore decided to examine the somata of some of the identifiable neurons in Archidoris *monteryensis* for possible changes in intracellular Ca^{++} and pH in the presence of particular neurotransmitters: acetylcholine (ACh), dopamine (DA), and serotonin (5-HT). In the course of these studies we also watched for changes induced by spontaneous synaptic activity.

Our results can be summarized as follows: (1) iontophoretic responses to DA and ACh which resulted in depolarization of the neuron did not yield a detectable change of either free Ca^{++} nor pH. No change was seen during or for many seconds after transmitter application even in high Ca (40mM) saline and at desensitizing levels of transmitter. (2) Spontaneous synaptic potentials exhibiting a wide variety of shapes were examined in many neurons. Again no correlation was found between the synaptic events and intracellular free Ca^{++} and pH levels. (3) Bath application of micromolar amounts of 5-HT, DA, or ACh did, however, result in an intracellular pH decrease in specific cells, although a corresponding change in free Ca^{++} has not been convincingly demonstrated. This pH decrease required exposure times in excess of two minutes, and it progressed throughout the exposure period (10-15 minutes). The response was usually reversible, although this required wash-out times greater than 20 min.

One possible source of this pH change may be the degradation of induced cyclic nucleotides, since bath applied 5-HT and DA have been shown to increase cyclic AMP levels in Aplysia ganglia (H. Cedar and J.H. Schwartz, J.Gen.Physiol., 60:570, 1972). This possibility is supported by two additional lines of evidence. We have injected cyclic AMP intracellularly and applied bath perfusion of 8-parachlorophenylthio-cyclic AMP (10^{-4}M) to our cells and observed a pH decrease using the dye technique. Both procedures resulted in larger and more rapid decreases than those evoked by the transmitters. Again these responses were reversible, with the injected response returning to baseline more quickly (i.e., within one or two minutes after termination of a 5 minute injection).

107.8 DISSOCIATION OF EXTRACELLULAR EPSP AND POPULATION SPIKE LONG TERM POTENTIATION IN THE RAT DENTATE GYRUS. R.C. Wilson, W. B. Levy and O. Steward. Depts. of Physiology and Neurological Surgery, Univ. of VA Sch. of Med., Charlottesville, VA 22908

A dramatic example of synaptic potentiation has been noted in the dentate gyrus of the hippocampal formation (Bliss, T.V.P. & Lømo, T., J. Physiol., 232: 331, 1973). Following conditioning stimulation of the dentate's perforant path input with several short (20 msec) bursts of high frequency (400 Hz) pulses, test stimuli generate an increased excitatory synaptic potential recorded extracellularly which is accompanied by a large increase in dentate granule cell discharge as evidenced by the population spike. The increased responsiveness persists for hours and in some cases days, hence its designation as long term potentiation (LTP). Previous investigators have, in certain instances, observed a disjunction in the occurrence of synaptic and population spike LTP. Since the results of synaptic processing in the dentate can only be expressed in terms of granule cell discharge, such a dissociation of synaptic change and population spike change might be of significance to dentate function as well as providing information concerning LTP mechanisms. We therefore analyzed the relationship between the extracellular population EPSP and the accompanying population spike before and after induction of LTP.

This relationship was illustrated by plotting population spike slope as a function of the slope of the EPSP which evoked it. Prior to potentiation, successive increments in the EPSP produced greater than linear increases in the population spike. Following LTP induction this relationship remained an increasing non-linear function but the rate of population spike increase over any range of EPSP values was greater than before potentiation. The net effect of this change was that an EPSP of a given size evoked a larger population spike following conditioning than a comparable EPSP did before. This result suggests that two processes may be involved in LTP; an increased synaptic drive and a separate factor or process which results in an additional increase in the population spike. Such factors as alterations in granule cell threshold or changes in inhibitory tone are considered.

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107.9 A PICROTOXIN-RESISTANT HYPERPOLARIZING RESPONSE IS ELICITED BY ORTHODROMIC STIMULATION OF HIPPOCAMPAL NEURONS. R. H. THALMANN AND G.F. AYALA, Departments of Cell Biology and Neurology, and the Program in Neuroscience, Baylor Coll. Med., Houston, TX 77030.

When orthodromic pathways of the rat hippocampal slice were stimulated, a two-component hyperpolarizing response often followed the EPSP. The second component, or 'late hyperpolarizing response' typically became evident between 40-140mSec following the onset of the first hyperpolarizing component, the 'early IPSP', and was usually detectable for an additional 150-1,000mSec. The late hyperpolarizing response was accompanied by an increased conductance, and its reversal potential was negative to that of the 'early IPSP'. We have observed this response in CA1 and CA3 neurones following stimulation of Schaffer collaterals and mossy fibers, respectively; and in granule neurons of the dentate gyrus in response to stimulation of a) the perforant pathway, b) the molecular layer of the dentate g., and occasionally, c) in response to stimulation of mixed orthodromic and antidromic circuits in CA3. Although more intense stimulation increased the amplitude and duration of the late hyperpolarization, an action potential (AP) was not a necessary antecedent; the response occurred when the EPSP was below the threshold for elicitation of AP, and has been observed in granule neurons when Na-dependent AP were blocked by intracellular iontophoresis of a local anesthetic. We have not been able to establish whether the late hyperpolarization can be elicited in the complete absence of an EPSP since each of our attempts to elicit a pure recurrent IPSP has thus far elicited a small 'EPSP' in the initial 2-7mSec following the stimulus. Therefore this response may or may not correspond to one of similar appearance which follows an apparently pure recurrent IPSP in CA1 (Dingledine, 1980). It probably is equivalent to Fujita's (1979) 'dendritic' IPSP of CA1-CA3 neurons. Picrotoxin (PX) was applied either by pressure injection of $5X10^{-4}M$ PX near the observed neuron through a 10μ pipette tip, or by perfusion of $2X10^{-5}M$ PX. In each case the 'early IPSP' was reduced or blocked, but the late hyperpolarization was spared and often increased in duration. Intracellular injection of Cl failed to reverse the late hyperpolarization. In contrast, when K concentration in the perfusion medium was increased from 3.5 to 10.5mM (N=2 granule neurons), the reversal potential of the response became less negative. Thus far, then, neither Cl injection, nor the prelimary manipulations of K have been consistent with the existence of a major Cl component in the conductance increase of the late hyperpolarization. In addition, since it is thought that PX may act at or near a Cl ionophore, the failure of PX to block the response is also suggestive of an ionic basis other than Cl. Finally, AP are inhibited during the late hyperpolarization, even when PX has blocked the early IPSP which may overlap it. Supported by USPHS grants RR0425 and NS11535

107.11 MODULATION OF RHYTHMIC ELECTRICAL ACTIVITY IN AN ANTERIOR PITUITARY CELL LINE. M. Adler, N. Busis, H. Higashida*, S. Sabol*, A. Rotter*, and M. Nirenberg. Lab. of Biochemical Genetics, Natl. Heart, Lung and Blood Institute, NIH, Bethesda, MD 20205.

The electrophysiological properties of the clonal mouse anterior pituitary cell line AtT-20/D16-16, known to synthesize, store, and secrete ACTH and β-endorphin were studied by intracellular recording techniques at 37°C. Cells grown in Dulbecco's Modified Eagle's Medium and 5% fetal bovine serum had low resting potentials (-33 ± 2 mV, n=24). In cells held at -70 to -100 mV, depolarizing pulses elicited one or two brief spikes followed by a slower depolarization lasting 6-30 sec. The early spike had a threshold of ∿ -50 mV, varied with external $[Na^+]$ and was blocked by 1 μM tetrodotoxin (TTX). The slow component had a threshold of ∿ -30 mV, varied with external $[Ca^{2+}]$, and was blocked by the Ca^{2+} antagonists Ni^{2+}, Co^{2+}, and La^{3+}. A small proportion (13%) of the cells had irregularly occurring spontaneous depolarizations that resembled the evoked Ca^{2+} action potential. The voltage-sensitive Ca^{2+} permeability of AtT-20 cells was demonstrated also by $^{45}Ca^{2+}$ flux measurements. Depolarization of cells by 80 mM K^+ ions resulted in a 3.5-fold increase in cellular Ca^{2+} levels in 15 sec. Growth of the cells in medium supplemented with 1 mM dibutyryl-cyclic AMP for 9-12 days resulted in: 1) a 20 mV increase in resting potential; 2) an increase in rate-of-rise and overshoot of the Na^+ action potential; 3) an increase in rate-of-rise and shortening in the duration of the Ca^{2+} action potential; and 4) induction of rhythmic spontaneous action potentials in over 90% of the cells. The spontaneous responses were very regular and usually persisted for the duration of the impalement. A single spontaneous event consisted of several brief Na^+ spikes superimposed on the rising phase of a 50-200 msec Ca^{2+} spike that terminated in a 10-15 mV hyperpolarization. The frequency of events was sensitive to membrane potential, varying from 2 Hz at -50 mV to 8 Hz at -25 mV. Spontaneous activity was absent at potentials negative to -60 mV and was reduced to subthreshold oscillations at potentials positive to -15 mV. A similar rhythmic pattern could be evoked by long depolarizing pulses from cells held at -70 to -100 mV. Spontaneous responses generally persisted in the presence of TTX, although at a lower frequency, but disappeared after addition of 5-10 mM Co^{2+}. Brief focal application of norepinephrine (10 μM) by pressure ejection from micropipettes broadened spontaneous action potentials and induced spontaneous activity in quiescent cells. Since extracellular Ca^{2+} is required for the depolarization-induced release of ACTH and β-endorphin from these cells, changes in rhythmic Ca^{2+} influx may be important in modulating secretory activity.

107.10 DENDRODENDRITIC INHIBITION AND THE ACTIONS OF PUTATIVE CENTRIFUGAL TRANSMITTERS IN THE OLFACTORY BULB. C.E. Jahr and R.A. Nicoll, Depts. of Pharmacology and Physiology, UCSF, San Francisco, CA 94143.

Dendrodendritic reciprocal synapses have been demonstrated anatomically between dendrites of mitral cells (olfactory bulb relay neurons) and the gemmules of dendrites of the inhibitory interneurons, granule cells. Recent evidence obtained with intracellular recording indicates that synaptic inhibition of mitral cells is largely dependent on activation of this reciprocal pair of synapses (C.E. Jahr and R.A. Nicoll, Science 207: 1473, 1980). Mitral cell action potentials, whether spontaneous or evoked by injection of depolarizing current through the recording electrode, are followed by all-or-none bicuculline-sensitive chloride-dependent IPSPs.

Centrifugal fibers constitute an additional component of the reciprocal synapse since many of these fibers directly terminate onto the dendritic gemmules of granule cells. Mitral cells do not receive direct input from centrifugal fibers (J.L. Price and T.P.S. Powell, J. Cell Sci. 7:157, 1970). This centrifugal input can, therefore, only modify mitral cell excitability indirectly. We have examined the effects of norepinephrine (NE) and glutamate, two putative centrifugal transmitters, on mitral cell excitability. NE (10-50 μM) markedly decreases the size of the mitral cell IPSP. This decrease is due to an action on granule cells since NE has little effect on mitral cell V_m, R_m or Ca^{2+} spikes. Furthermore, the iontophoretic response to GABA was unaltered by NE. As a result of the IPSP attenuation, the frequency of spontaneous firing of mitral cells, which is governed in part by the size of the obligatory IPSP, increases. Thus, although the occurrence of the IPSP that follows individual spikes is all-or-none, its size can be altered in a graded manner by NE. Conversely, glutamate blocks spontaneous firing by hyperpolarizing mitral cells. Since Co^{2+} converts this response to a depolarization, we presume that glutamate is acting indirectly by depolarizing granule cells and releasing GABA. In summary, both NE and glutamate alter mitral cell excitability primarily by indirect actions; NE blocks GABA release from granule cells whereas glutamate induces GABA release. The granule cell, then, is the final common pathway on which the centrifugal fibers and their transmitter candidates exert presynaptic control and thereby modulate the flow of information through the olfactory bulb.

108.1 THE INTERACTION OF THE INSECTICIDE TETRAMETHRIN WITH THE NERVE MEMBRANE SODIUM CHANNEL. Albert E. Lund* and Toshio Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

We recently found that pyrethroid insecticide, tetramethrin, modifies a fraction of the sodium channels in crayfish giant axons in such a way as to drastically alter the channel gating kinetics. The modified channels activate with a time course of several hundred milliseconds and inactivate with a time course of several seconds. We now propose a kinetic scheme for the interaction of tetramethrin with the sodium channel. Crayfish and squid giant axons were internally perfused and bathed in K^+-free media at 10° C. Cs^+ and tetramethylammonium were substituted for K^+ in internal and external media, respectively. Internal application of 1-200 µM tetramethrin to the squid axons had little effect on the rising phase of the sodium current but dramatically slowed the falling phase and caused a large tail current to flow following repolarization, indicating that a second, persistent open state was produced by the insecticide. The falling phase of the sodium current and the decay of the tail current were second order. The time constants for the fast phase of inactivation and the tail current in the poisoned axon were identical to those of the normal inactivation and the tail current suggesting that only some of the channels were modified. The time course of the arrival of channels in this modified open state is clearly second order having a quickly developing phase with a time constant of about 8 msec and a slowly developing one with a time constant of about 80-100 msec. Thus there appears to be at lease two kinetic steps or pathways which lead to the modified open state of channels. It appears that some channels are modified in the resting state and then open and close in response to changes in membrane potential while others are modified only after they have been opened by the normal process. This second modification pathway is necessarily fast since it must compete for the normal state with inactivation. This model predicts that any treatment which interferes with the h process should potentiate the tetramethrin modification of channels. This has been shown to be the case: following pretreatment with pronase or N-bromoacetamide, the dose-response relation for the tail current amplitude was shifted in the direction of low dose and the maximum response was more than doubled. Supported by NIH grant NS 14143.

108.2 GENETIC ALTERATION OF TTX SENSITIVITY AND NERVE REFRACTORY PERIOD IN A MUTANT OF DROSOPHILA MELANOGASTER. C.-F. Wu, B. Ganetzky* and R. W. Joyner* (SPON: J. L. Denburg). Dept. of Zoology and Physiology, Univ. of Iowa, Iowa City, IA 52242 and Lab. of Genetics, Univ. of Wisconsin, Madison, WI 53706.

Single-gene mutations that disrupt axonal conduction provide one means to study the components and mechanisms that underlie membrane excitation. The recessive mutant nap^{ts} (no action potential, temperature-sensitive) is rapidly paralyzed at high temperatures (≥ 34°C) and recovers rapidly when temperature is lowered. It has been demonstrated in the larval preparation that nap^{ts} blocks the sodium action potential in nerves at high temperatures but synaptic transmission or the calcium action potential of muscle are unimpaired (Wu et al., PNAS 75, 4047, 1978). We have examined the tetrodotoxin (TTX) sensitivity of the nerve action potential in nap^{ts} and in normal individuals. The excitatory junction potential was recorded from larval muscle at different doses of TTX as a means of monitoring the activity of the motor axon at 23°C, a temperature at which nap^{ts} individuals behave normally. The concentration of TTX required to block axonal conduction is 9.8 ± 4.4 nM ($\bar{X}$ ± S.D.) in nap^{ts} and 40.3 ± 11.1 nM in normal larvae. This increase in TTX sensitivity is not restricted to the motor axon because similar results were obtained by monitoring the compound action potentials of larval segmental nerves.

A possible alteration in the density and/or kinetics of ionic channels in nap^{ts} is also suggested by an increased refractory period of the compound action potential. Twin pulses were used to stimulate the segmental nerve at one end and a suction electrode was used to record propagated responses. We defined $\tau_{\frac{1}{2}}$ as the interval between stimuli needed to obtain a second response with amplitude 50% of the first. At every relative stimulus strength, the mutant requires a longer interval for recovery than does the control. The minimum $\tau_{\frac{1}{2}}$ is about 4 msec in control larvae but is 10 msec in the mutant.

Computer simulations of active conduction in a Hodgkin-Huxley model axon of the size found in Drosophila showed that the observed increase in refractory period and the conduction block at high temperatures can be best accounted for by a decrease in Na conductance. Alteration of either K or leakage conductance can not satisfactorily explain the observed differences.

The above results are consistent with the idea that nap^{ts} alters the function and/or density of Na channels.

108.3 INTRACELLULAR RECORDING FROM VERTEBRATE MYELINATED AXONS: MECHANISM OF THE DEPOLARIZING AFTERPOTENTIAL. Ellen F. Barrett and John N. Barrett. Dept. Physiology and Biophysics, Univ. of Miami Med. Sch., Miami, FLA. 33101

Action potentials and afterpotentials were recorded intracellularly from peripheral myelinated axons (mainly motor) of grass frogs and lizards. Axons selected for study had resting potentials of at least -60 mV, and action potentials with peak amplitudes of at least 60 mV. All axons showed a prominent depolarizing afterpotential which decayed with a time constant of 50-150 msec. The peak amplitude of the depolarizing afterpotential was voltage-sensitive, increasing to up to 20 mV in axons more hyperpolarized than -80 mV, and virtually disappearing (but not reversing) in axons more depolarized than -55 mV. The amplitude of the depolarizing afterpotential was not affected by substituting a divalent calcium antagonist (2-10 mM nickel, cobalt or manganese) for bath calcium. The depolarizing afterpotential persisted during and after exposure to zero chloride (sulfate substituted) solutions. Reduction of bath sodium (to one-third normal, tetramethylammonium substituted) or addition of 1-10 uM tetrodotoxin decreased the amplitudes of both the action potential and the depolarizing afterpotential. The amplitude of the depolarizing afterpotential did not vary with bath potassium (range 0 - 7.5 mM), provided that the membrane potential was held constant, suggesting that the depolarizing afterpotential is not due to extracellular potassium accumulation. The time course of the depolarizing afterpotential was not slowed by reducing bath temperature from 20 to 10° C. The passive electrical response to subthreshold depolarizing and hyperpolarizing current pulses shows a prominent slow component kinetically similar to the depolarizing afterpotential. These results suggest that the depolarizing afterpotential is a passive capacitative discharge of the myelinated axon. We suggest that this heretofore unreported, long time constant current pathway results from discharge of the internodal membrane capacitance through an approximately 20 megohm resistance under or through the myelin sheath. (The voltage-sensitivity of the depolarizing afterpotential probably reflects the voltage-sensitivity of the nodal input conductance.)

Frog peripheral myelinated axons have a prolonged post-activation superexcitable period whose time course resembles that of the depolarizing afterpotential (see Raymond, J. Physiol. 290: 273, 1979). We suggest that the depolarizing afterpotential produces this superexcitability. Supported by NIH grants NS-12404 and NS-12207.

108.4 DEPRESSION OF EXCITABILITY FOLLOWING ACTIVITY SHOWS A WIDE RANGE IN FROG SCIATIC AXONS. Stephen A. Raymond and David A. Cohen*. Research Laboratory of Electronics, MIT, Cambridge, Massachusetts 02139

Depression of excitability follows impulse activity in peripheral nerve fibers, becoming quite pronounced after long tetani or high frequency bursts (Raymond, J. Physiol. 290:273-303,1979). Depression governs the periodicity of intermittent responsiveness observed in frog sciatic nerve fibers given long, regular trains of near threshold stimuli, and is implicated in the strongly periodic bursts associated with paresthesia recorded from single units in human median and ulnar nerves (Torebjörk, H.E. et. al. Acta Physiol. Scand. 105:518-520,1979)

Single fibers teased from frog sciatic nerve were recorded using flexible plastic suction electrodes. Conditioning trains were produced in the whole nerve by current pulses delivered 7 cm from the recording electrodes, and the threshold of the individual fibers was tracked by a threshold hunter.

Fibers depress to very different degrees. Some axons show less than a 5% change in threshold diminishing to control levels within two minutes after a burst of 20 impulses/sec fiven for one minute. Other fibers are so "depressible" that their threshold is raised dramatically by single pulses (200% following short bursts). Depressibility correlates with rapidity of recovery from depression, ie following a given level of activity axons that showed higher maximum thresholds also showed faster recovery to the control level.

Depression is completely inhibited by Ouabain, and is quite temperature sensitive. The process involved in depression and recovery is unknown in detail, but measures of threshold appear to provide an indication of the rate of metabolic ion pumping as it is altered by impulse conduction. Any one fiber shows consistent levels of depressibility over several days, and differences between fibers are much greater than the differences in conduction velocity or other parameters used to characterize peripheral nerve fibers.

These differences between fibers seem to reflect a wide range of pumping capability in axons of the same nerve.

108.5 FLUORESCENT DERIVATIVES AND A PHOTOACTIVATED FLUORESCENT DERIVATIVE OF TETRODOTOXIN TO LOCATE THE FUNCTIONAL SITES OF THE SODIUM CHANNEL OF RAT AXONAL MEMBRANES. Kimon J. Angelides* (SPON: P.E. Braun). Department of Biochemistry, McGill University, McIntyre Med. Sci. Bldg., 3655, Drummond Street, Montreal, Quebec CANADA, H3G 1Y6.

The permeability of excitable nerve cell membranes to specific ions is controlled by intrinsic membrane proteins which provide a channel through which these ions can pass. Over the years, considerable work has been directed towards a biochemical and electrophysiological description of their mode of action and regulation. The neurotoxin tetrodotoxin (TTX) has been used with considerable success to probe the extracellular face of the membrane and identify the sodium selectivity filter. The toxin eliminates the sodium current (the transient flow of Na^+ ions) while not affecting the gating current. We have synthesized three fluorescent and one photoactivated fluorescent derivative of tetrodotoxin. The three fluorescent compounds anthranilamide-glycine hydrazide (AG), N-methylanthranilamide-glycine hydrazide (NMAG) and anthraniloyl hydrazine (AH) were coupled to a ketone function at C_6 of TTX to form stable fluorescent hydrazones of TTX. Competitive binding with [^{3}H]-TTX for the TTX receptor on rat axolemma vesicles or from binding isotherms obtained by the fluorescence enhancement or polarization indicate a K_D=6.2 nM for AG-TTX; K_D=7.1 nM for NMAG-TTX; and a K_D=6.09 nM for AH-TTX, only about 5 times less active than TTX itself. The photoactivated fluorescent derivative of TTX, 2-azido-benzoyl-glycine hydrazone-TTX binds to the fast sodium channel of rat axolemma vesicles only 7 times less tightly than tetrodotoxin (K_D=16.8 nM) in the absence of light. Upon photolysis the association becomes irreversible and gives rise to a large fluorescent signal. These site-specific probes have been used to investigate the conformational dynamics of the toxin site, characterized by nanosecond lifetime and time-resolved anisotropy measurements as well as to locate the functional sites of the sodium channel by Förster singlet-singlet energy transfer. Supported by a grant from the Research Corporation.

108.6 SODIUM CURRENTS IN RABBIT SKELETAL MUSCLE FIBERS. G. E. Kirsch* and C. H. Wu. Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Our progress in understanding the pathogenesis of membrane electrical events in human myotonic disorders has been frustratingly slow. Despite the availability of various voltage clamp techniques, it has not been easy to obtain adequate voltage clamp results in mammalian muscles. This is mainly because of the difficulties in dissecting single fibers and their relatively small sizes. Recent reports indicate the feasibility of obtaining satisfactory spatial voltage control and adequate temporal resolution of fast sodium kinetics in mammalian muscle fibers (Pappone, Biophys. J. 1977; Duval and Léoty, J. Physiol. 1978; DeCoursey and Bryant, Biophys. J. 1979). We now report our observations of the sodium currents in rabbit skeletal muscle fibers using the vaseline-gap technique of Hille and Campbell (J. Gen. Physiol. 1976).

Single fibers were dissected from the extensor digitorum longus muscle in the relaxing solution and the ends cut in 160 mM CsF. With the holding potential set at -90 mV, the peak sodium conductance reached 140 mS/cm^2. An occasional secondary current peak, probably originating from the currents in transverse tubule system, could be eliminated by maintaining the fiber at temperature below 16°C or applying 2 nM tetrodotoxin or bathing the fiber in the external solution containing 50% Na^+. At 14°C, half of the channel population was inactivated by conditioning depolarization to -80 mV (±7 mV S.D.). The time constant of fast sodium inactivation was 1.4-2.0 ms at E_m between 20-110 mV (5.5°C).

We have examined the effects of two aromatic monocarboxylic acids which are capable of inducing myotonia in mammalian muscles. 9-Anthroic acid at high concentration (0.1 mM) had no effects on the fast sodium kinetics other than shifting the current-voltage curve by 20 mV toward depolarization, probably resulting from changes in the surface potential. 2,4-Dichlorophenoxyacetic acid (1 mM) blocked the sodium conductance by 19%. We conclude that the repetitive discharges induced by these two drugs are not due to retardation of the fast sodium inactivation.
(Supported by NIH grant AM 25454 and a research grant-in-aid from the Muscular Dystrophy Association).

108.7 KINETICS OF Ca^{2+} CURRENT IN TWITCH MUSCLE FIBERS OF THE FROG. J.A. Sánchez and E. Stefani. Dept. Physiology and Biophysics, CIEA-IPN, A.P. 14-740, México 14, D.F.

Calcium current (I_{Ca}) kinetics in sartorius muscle of Rana temporaria was studied using the three microelectrode voltage clamp technique. Signals were digitalized and stored for analysis in a microcomputing system. Linear capacity and leakage currents were substracted. The saline contained (mM): 117 tetraethylammonium (TEA), 2.5 K^+, 10 Ca^{2+}, 350 sucrose and methanesulphonate as the anion. The temperature was 20°C. The membrane potential was clamped to -90 mV (E_h) and pulses to different potentials (E) were delivered. Remaining outward K^+ currents were blocked with 5 mM 3-4 diaminopyridine. In chloride saline, Ca currents could be recorded, but they were modified during depolarizing pulses by outward Cl^- currents. For a pulse to -10 mV, I_{Ca} decayed following a single exponential with a time constant of 540 msec. The time constant of decay of the amplitude of tail currents recorded at the end of pulses with different durations had a similar value of 590 msec. This indicates that I_{Ca} inactivates, since E_h=E_k. The steady state inactivation curve (9 sec. prepulses) was fitted to $h_\infty=|1+\exp(E-V_h)/k|^{-1}$ with k=5.9 mV and V_h=-31.7 mV.

I_{Ca} recorded with different pulse amplitudes were analyzed according to the Hodgkin & Huxley model. The records were fitted to the function: $A|1-\exp(-t/\tau_m)|^n|h_\infty-(h_\infty-h_o)\exp(-t/\tau_h)|$, where A is an amplitude factor. The best fitting for n was 3, therefore the values of A, τ_m and τ_h were computed. A, at potentials of -10 to +20 mV, and tail current amplitude had a linear relation with the voltage and subsequently m_∞ was calculated as a function of membrane potential from the expression: $A=\bar{g}_{Ca}(E-E_{Ca})m_\infty^3$. With the values of τ_m, τ_h, h_∞ and m_∞ the rate constants α_m, β_m, α_h and β_h were calculated for each potential value (E), and were fitted to the expressions: $\alpha_m(seg^{-1})=\bar{\alpha}_m(E-\bar{V}_m)/(1-\exp(-(E-\bar{V}_m)/V_{\alpha m}))$, $\beta_m(seg^{-1})=\bar{\beta}_m\exp(-(E-\bar{V}_m)/V_{\beta m})$, $\alpha_h(seg^{-1})=\alpha_h\exp(-(E-\bar{V}_h)/V_{\alpha h})$, $\beta_h(seg^{-})=\bar{\beta}_h/(1+\exp(-(E-\bar{V}_h)/V_{\beta h}))$. The values of the constants were: $\bar{\alpha}_m$=.29 sec^{-1} mV^{-1}, $\bar{V}_m$=-40.4 mV, $V_{\alpha m}$=1.4 mV, $\bar{\alpha}_h$=.34 sec^{-1}, $\bar{V}_h$=-29.2mV, $V_{\alpha h}$=13.3 mV, $\bar{\beta}_m$=2.2 sec^{-1}, $V_{\beta m}$=19.0 mV, $\bar{\beta}_h$=1.36 sec^{-1}, $V_{\beta h}$=3.2 mV. The calcium spike reconstructed with these values agreed reasonably well with the experimental ones.

This work has been supported by CONACyT, grant PCCBNAL 790022.

108.8 LOSS OF MYOTONIA FOLLOWING DENERVATION OF GASTROCNEMIUS FIBERS IN THE MYOTONIC GOAT. K. Owenburg* and S.H.Bryant. Dept. of Pharmacol. & Cell Biophysics., Univ. of Cincinnati Col of Medicine, Cincinnati, OH 45267.

In the hereditary myotonia of goats Brown and Harvey (Brain 62:341,1939) reported that skeletal muscles continued to show myotonia through the eighth day following surgical denervation. Recently it has been reported by several laboratories that the abnormal repetitive firing of myotonia induced by chemical agents does not occur in chronically denervated fibers. We have re-examined the effect of denervation on myotonic goat fibers using microelectrode stimulating and recording from gastrocnemius fibers in vitro. We transected the tibial branch of the sciatic nerve in the thigh on one leg and after periods of 10, 14, 21 and 28 days the gastrocnemius muscle from both legs were removed, bundles of a few hundred fibers were cleaned from each biopsy and placed in normal goat ringer at 37°C. Electromyographic EMG recordings were also made from the exposed control and denervated muscles during biopsy. Excitability was monitored by passing constant current pulses into a fiber through one electrode while observing threshold for the action potential and ability to fire repetitively to super threshold pulses. Confirming Brown and Harvey we observed little change in the EMG and the abnormal excitability of myotonic fibers at 10 days of denervation. From 14 to 28 days of denervation marked changes occured in both myotonic and normal fiber excitability. Current threshold increased in myotonic fibers with decreasing number of action potentials in a repetitive train and absence of post stimulus discharge or myotonic EMG by the end of the period. In fibers from normal goats after 14 days, anthracene-9-carboxylic acid was unable to induce myotonic behavior, and myotonic and normal fibers had TTX resistant action potentials. Fibrillation was present in myotonic and normal denervated fibers after 14 days. In the myotonic fibers at 14 days fibrillation was present with diminished myotonia and could be distinguished from it. By 28 days only fibrillation activity was seen suggesting that fibrillation is not a consequent of increased excitability. By the end of 28 days of denervation, resting chloride conductance is low and potassium conductance is high for both myotonic and normal fibers. Their excitabilities have diminished to similar levels. Thus, these data suggest that loss of myotonia in congenitally myotonic fibers and the resistance of normal fibers to induction of myotonia by drugs after chronic denervation results from alterations in voltage dependent sodium and potassium conductances.

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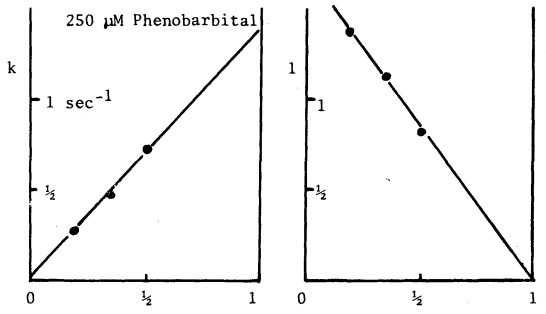
108.9 ABNORMAL ELECTRICAL PROPERTIES OF MYOTONIC MUSCULAR DYSTROPHY IN CULTURE OBSERVED WITH VOLTAGE CLAMP. Michael Merickel, Richard Gray, Priscilla Chauvin* and Stanley Appel. Dept. of Neurology, Baylor College of Medicine, Houston, Texas 77030.

We have approached the study of MyD by utilizing electrophysiological techniques to investigate the membrane properties of muscle fibers from normal and MyD patient biopsies grown in a primary tissue culture system. We have previously demonstrated that MyD myotubes in culture have abnormalities in some of their basic electrical properties compared to control myotubes. These include: 1) Decreased resting potential of approx. 10 mV; 2) Hyperexcitability, manifested as an increased tendency to fire multiple action potential (A.P.'s) in response to a single stimulus (i.e., myotonia); and 3) Decreased A.P. afterhyperpolarization (Merickel, M., Gray, R., Chauvin, P. and Appel, S., (1979), Soc. for Neuroscience Abstracts, 5, 378).

Further comparison of control and MyD myotube firing characteristics and membrane currents with voltage clamp has provided a clearer picture of the membrane electrical events underlying myotonia, the hallmark of MyD. The A.P. of at least 50% of the MyD myotubes examined exhibited a depolarizing afterpotential (DAP) in contrast to the typically hyperpolarizing afterpotential of control myotubes. DAP's have been observed in a number of invertebrate and vertebrate nerve and muscle systems which exhibit repetitive firing. The occurrence of DAP's in MyD myotubes accounts for their decreased average A.P. afterhyperpolarization amplitude. The voltage clamp technique is required to examine the ionic basis of the repetitive firing, DAP's and other electrical abnormalities observed in MyD myotubes. Comparison of the slow membrane currents of control and MyD myotubes with the single electrode voltage clamp has demonstrated that the outward-going current of MyD myotubes, particularly in MyD myotubes exhibiting DAP's, activates much more slowly than in control myotubes. The steady-state outward-going current, however, has been found to be only slightly decreased in MyD myotubes. The slower activation time course of the MyD outward-going current is believed to underlie their DAP's and repetitive firing behavior. Experiments are in progress to identify and examine the shorter time course currents responsible for the delayed activation. These experiments demonstrate that the observed electrophysiological abnormalities in MyD myotubes can be detected and studied with voltage clamp and may provide important insight into the ionic basis of the MyD abnormalities in tissue culture. Support for this work is acknowledged from the Muscular Dystrophy Association, Jerry Lewis Neuromuscular Disease Research Center and the Kleberg Foundation.

108.10 APPLICATION OF THE MODULATED RECEPTOR HYPOTHESIS TO ANTICONVULSANT DRUG ACTIONS. K. R. Courtney . Palo Alto Medical Research Foundation, Palo Alto, CA 94301

Anticonvulsant drugs such as phenytoin and phenobarbital have been shown to block nerve sodium channels in a frequency-dependent manner. Such a drug action, which leads to greater blocking efficacy at higher nerve discharge rates, can be modeled using Hille's modulated receptor hypothesis first described for local anesthetics. In this study two anticonvulsants, phenobarbital and mexiletine, have been applied to voltage-clamped semitendinosis muscle fibers (of bullfrog) in order to more precisely determine the sodium channel states involved in closed channel blocking and unblocking reactions. The blocking (k) and unblocking (l) rate constants were determined as a function of channel inactivation by measuring the amounts of non-frequency-dependent block and the block relaxation rates at several different holding potentials.



fraction of inactivated channels, 1-h

Observed increases in k and decreases in l with small depolarizations lead to the conclusions that (a) primarily resting (non-inactive) channels are involved in the unbinding step and that (b) primarily inactive channels are involved in the binding step. Thus these two anticonvulsants may manifest frequency-dependent excitability block primarily via their selective affinity for inactive sodium channels.

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109.1 THE RESPONSE LATENCY OF ELECTRICAL STIMULATION OF THE TONGUE OF THE RAT. Mohsen S. Nejad* (SPON : Fereshteh Motamedi). Lab. of Neurophysiology, National University of Iran, School of Medicine, Tehran - Iran.

The rat chorda tympani and lingual nerves offer excellent conditions for studying the sensitivity of the tongue to different kinds of stimuli. The rat's tongue may be stimulated with chemical solutions or electrical currents and afferent impulses may be recorded from the chorda tympani or the lingual nerve.

The purpose of this study was to obtain qualitative and quantitative information concerning the response and response latency of gustatory receptors and nerve terminals of the tongue of the rat due to electrical stimulation.

Integrated neural activities of the chorda tympani nerve when the tongue (10 rats and 5 runs on each animal) was stimulated electrically (0.035-0.68 milliamps/unit area of the tongue) were very similar to those occuring when the tongue was stimulated chemically (0.025-1.0 M NaCl). The threshold for the lingual nerve was greater (0.225 milliamps/unit area of the tongue) than that of the chorda tympani.

When the tongue was pulled in between the stimulating electrodes both anodal and cathodal currents elicited activities in the chorda tympani and the lingual nerves. However, when the indifferent electrode was placed somewhere on the jaw of the rat, anodal polarization of the tongue surface caused a discharge and cathodal current inhibited the existing spontaneous discharges in the chorda tympani.

The response latency of chorda tympani at the threshold (0.035 milliamps/unit area of the tongue) was 16 ± 1 milliseconds. The plot of latency versus stimulus intensity in the chorda tympani showed a sudden drop in latency at a current approximately 0.4 ± 0.05 milliamps at which the latency became about 4 ± 1 milliseconds. This value was close to the latency of the lingual nerve when the tongue was stimulated at the threshold with anodal current. The existance of a two-phase-mechanism may be suggested. The first phase has a long latency and a rather small threshold (taste receptors), and the second phase has a short latency and a relatively large stimulus threshold (the nerve endings).

109.2 SELECTIVE INHIBITION OF BINDING TO A TASTE RECEPTOR SITE. Robert H. Cagan and John H. Teeter. Veterans Administration Medical Center and Monell Chemical Senses Center, Philadelphia, PA 19104.

The cutaneous taste system of the channel catfish *Ictalurus punctatus* is sensitive to a number of amino acids. Previous work demonstrated that the initial binding interaction at the plasma membrane underlies recognition of taste stimuli (1-3). An electrophysiological study of single nerve fibers in the catfish barbel (4) suggested that at least two populations of taste receptors exist, one that is relatively specific for L-arginine and another that is broadly sensitive to L-alanine and several other amino acids. Because our studies (1) had shown significant levels of binding of L-alanine and L-arginine to the receptor preparation, they were studied as prototypical ligands. Studies with additional amino acid ligands are in progress.

In the present studies, β-chloro-L-alanine markedly inhibits binding of L-[^{3}H]alanine but is without effect on binding of L-[^{3}H]arginine. For example, 4 μM β-chloro-L-alanine inhibits binding of 0.4 μM L-[^{3}H]alanine by 58% but shows no inhibition of 0.4 μM L-[^{3}H]arginine binding. The inhibition is concentration-dependent, and its effect is reversible. Furthermore, it is stereospecific, as β-chloro-D-alanine shows no inhibition of binding of L-[^{3}H]alanine.

Summated electrophysiological recordings from multi-fiber nerve preparations in the maxillary barbel provide corroborative evidence. The summated response elicited by a mixture of 10^{-4} M β-chloro-L-alanine + 10^{-5} M L-alanine was decreased by 40-60% compared with L-alanine alone. Although 10^{-4} M β-chloro-L-alanine evoked a small response in the nerve, it appeared similar in magnitude to the response with a control pulse of water. The response to a mixture of 10^{-4} M β-chloro-L-alanine + 10^{-5} M L-arginine was the same or slightly larger than with 10^{-5} M L-arginine alone.

The selective inhibition by β-chloro-L-alanine therefore shows it to be an antagonist for L-alanine taste receptor sites but not for L-arginine sites. As previously suggested (4), the present studies demonstrate biochemically and physiologically that *at least* two populations of taste receptor sites exist in the catfish. β-Chloro-L-alanine should be extremely useful in further defining the specificity of the catfish receptor sites. [Supported in part by NIH grant NS-08775/NS-15740 from NINCDS].

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109.3 A MODEL FOR ACTION OF THE GYMNEMIC ACIDS AND ZIZIPHINS ON TASTE RECEPTOR CELLS. L.M. Kennedy and B.P. Halpern*. Worcester Fndn. Exptl. Biol., Shrewsbury, MA 01545 and Dept. Psych. and Sect. Neurobiol. & Behav., Bio. Sci., Cornell Univ., Ithaca, NY 14853

The gymnemic acids (GA) and ziziphins (Z) (from G. sylvestre and Z. jujuba) selectively suppress human sweetness perception (Meiselman et al., Physiol. Behav., 17: 313, 1976; Warren and Pfaffmann, J. Appl. Physiol., 14: 40, 1959), inhibit fly behavioral responses to sucrose, and inhibit and stimulate fly taste receptor neural responses (Kennedy et al., Physiol. Behav., 14: 755, 1975; Kennedy and Halpern, Soc. Neurosci. Abstr., 5: 412, 1979; Physiol. Behav., 24: 135, 1980). GA and Z are saponins, amphipathic molecules with surface active properties, and data support the notion of a role for surface activity in their taste-modifying action (Kennedy and Halpern, Chem. Senses, 1980, in press).

We propose a biphasic membrane penetration process for the action of GA and Z on taste receptor cells. In our model, the penetrating GA and Z molecules interact first, with the receptor cell plasma membrane surface and second, with the lipid membrane interior. The initial interaction is postulated to involve a selective effect on processes functional in the transduction and quality specification of a sweet stimulus and the second interaction to involve a general membrane disruption and nonselective effects on taste perception. This hypothesis is consistent with, and/or can account for (a) data on GA and Z chemistry, (b) psychophysical and neurophysiological data on the duration and selectivity of the taste-modifying action of GA and Z, (c) neurophysiological effects of GA and Z on taste receptor cells, (d) thermodynamic and kinetic theory for cell membrane penetration and (e) knowledge about the action of saponins on cell membranes. It offers an explanation for the puzzling fact that amphipathic saponin molecules, which probably penetrate into and generally disrupt the receptor cell membrane, have a selective effect on taste receptor cell neurophysiology and on taste perception. In addition, the model makes explicit predictions which can be tested in future experiments.

If the model holds, it has important implications for the design and interpretation of psychophysical, neurophysiological and chemical experiments that use GA and Z as tools to study taste perception. Moreover, if the model holds, it places at least one process involved in the specification of a sweet stimulus at the surface of the receptor cell membrane.

(Included in Kennedy, L.M., Ph.D. Thesis, 1979, Harvard Univ. Supported by NSF BNS 77-09925 to B.P.H. and NSF BNS 78-22149 to L.M.K. and B.P.H. L.M.K. was supported by a Danforth GFW Fellowship).

109.4 **SINGLE UNIT TASTE RESPONSES FROM THE PETROSAL GANGLION OF THE MONGOLIAN GERBIL.** **M.A. Hosley* and B. Oakley, Div. Biol. Sci., Neuroscience Lab. Bldg., Univ. of Mich., Ann Arbor, Mich. 48109.**

Microelectrode recordings were obtained from single petrosal ganglion (C.N.IX) gustatory units of the Mongolian gerbil (Meriones unguiculatus) in response to gustatory stimulation by 0.3M NH_4Cl, 0.3M NaCl, 0.5M sucrose, 0.01M HCl, 0.01M quinine-HCl and a "white taste" solution consisting of all of the above solutions in their listed concentrations. Single units were resolved by the criterion of constant spike height using an electronic window discriminator. Responses were measured in impulses per second for the five second interval of maximal activity during taste stimulation.

Gustatory units were, in general, responsive to all taste stimuli and thus lacked specificity for individual taste solutions. Analysis of units categorized according to their most effective "basic taste" stimulus (NaCl, sucrose, HCl or quinine-HCl) provided no clear evidence for types or classes of units. The high correlation between responses to 0.3M NH_4Cl and 0.3M NaCl suggests that these chemicals have shared or co-occurring receptor sites. Complex neural interactions are indicated by the responses to the "white taste" solution, whose effectiveness varied among neurons from the least effective solution to the most effective solution of the six taste solutions employed. The present results are consistent with an across fiber pattern theory of neural coding for taste qualities among gustatory units in the gerbilline petrosal ganglion. **Supported in part by NIH grant NS-07072.**

109.5 ACCESS OF LOW-VOLATILE STIMULI TO THE RODENT VOMERONASAL ORGAN DURING SOCIAL AND FEEDING BEHAVIORS. C. J. Wysocki, G. K. Beauchamp*, J. L. Wellington* and S. Erisman*. Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104

Access of low-volatile chemical stimuli to the vomeronasal organ of mice, rats, pine voles and meadow voles was assessed during one of the following behavioral situations: investigation of a social stimulus (urine); social grooming, stimulated by the application of a foreign substance (carboxymethylcellulose, CMC) to the fur of a stimulus animal; self grooming, stimulated by the application of CMC; drinking of a novel solution (saccharin) or a familiar solution; or eating. In all situations the stimulus was mixed with a nonvolatile fluorescent dye, rhodamine B, prior to its presentation or application. The animal was allowed to contact the stimulus ad libitum for a predetermined amount of time. Immediately thereafter the animal was killed and the vomeronasal organ was removed, sectioned and surveyed with epi-fluorescence microscopy for the presence of rhodamine specific fluorescence.

The results were clear cut. In the majority of animals, the nonvolatile fluorescent dye was observed in the vomeronasal organ subsequent to each of the behaviors. In the absence of rhodamine no fluorescence could be detected from the vomeronasal organ.

Thus, at least some low-volatile substances encountered in urine, a cellulose-based gum, novel or familiar solutions, or food have access to the vomeronasal organ. That this access occurs in a variety of contexts suggests that this receptor organ could be involved in the regulation of social behavior, ingestion and in "self-smelling", a much broader array of behaviors than has generally been presumed.

We interpret these results to indicate that, as is the case for olfaction, animals sample their chemical environment with the vomeronasal organ during a variety of behaviors. However, unlike the olfactory system, the vomeronasal system may provide the organism with access to the wealth of information which can be stored in large molecules.

Specific experiments designed to test these hypotheses are required. Furthermore, although stimulus access to a chemo-receptor is necessary for stimulation to occur, it is not sufficient. At present, we do not know the properties of molecules which may activate the vomeronasal organ.

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109.6 ANATOMICAL FEATURES OF THE BOVINE VOMERONASAL COMPLEX. V.L. Jacobs, R.F. Sis,* P.J. Chenoweth,* W.R. Klemm, C.J. Sherry Depts. of Vet. Anat., Med. and Surg., and Biol. Texas A&M Univ. College Station, Tex. 77843.

The vomeronasal organ (VNO) has been considered an important sensory apparatus related to sexual behavior in animals. Our studies have shown that Brahman bulls produce rapid tongue compression strokes (TCSs) of the hard palate while investigating the vulva of females. We believed that TCSs compressed the incisive and vomeronasal ducts to produce movements of fluid into the vomeronasal organ for estrus detection.

The hard palates of ten animals were removed following head perfusion with 10% buffered formalin. Gross observations on 6 animals were made on 1 cm sections cut with a band saw. Four animals were used to examine 6 µm sections taken from each cm segment of the incisive duct and vomeronasal organ. Alternate histologic sections were stained with H and E, Verhoeffs, PAS, Masson or Van Giesson. One fresh head was used to produce a latex model of the vomeronasal duct and organ.

The gross and microscopic specimens demonstrated a slit-like incisive duct 1 cm above the palatal mucosa. A comma shaped cartilage protects the incisive duct laterally and dorsally. The vomeronasal duct leaves the dorsal part of the incisive duct 1.5 cm caudal to the incisive pit. An expanded mucosal swelling bulges into the incisive duct at this juncture. The vomeronasal cartilage is remodeled further caudally so that the vomeronasal duct is protected medially and ventrally by it. The incisive duct lies 1.3 cm above the palatal roof and beneath the cartilage.

The position of the ducts above the palate near the midline may be easily compressed and manipulated by the tongue during TCS. Urine or vaginal secretion samples may be drawn into the incisive duct by TCSs where estrus pheromones could gain access to the VNO.

Supported in part by USDA/Sea/Cr. Project AH-6393.

109.7 AN EXAMINATION OF THE COLLATERALIZATION OF MITRAL CELL AXONS BY RETROGRADE DOUBLE LABELING TECHNIQUES. M.B. Luskin* and J.L. Price. Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110

The axonal projection from the olfactory bulb (OB) to the olfactory cortex is diffuse; i.e., small areas within the OB project to almost the entire olfactory cortex, and small areas of the olfactory cortex receive fibers from all parts of the OB. Mitral cell axons leaving the OB in the lateral olfactory tract have been observed to give off a number of collaterals (Cajal, 1911), suggesting that individual mitral cells also have a diffuse projection to separate areas of the olfactory cortex. In the present study we have used retrograde double labeling techniques in the rat in order to test this hypothesis.

Three pairs of tracers have been used: HRP and tritiated HRP (^{3}H-HRP), HRP and ^{125}I-wheat germ agglutinin (I-WGA) and the fluorescent dyes, bisbenzimide and true blue. The fluorescent dyes have been the most useful because of their sensitivity and their differential distribution to the nucleus (bisbenzimide) and cytoplasm (true blue), which facilitates the identification of double- and single-labeled cells. Non-overlapping injections were made into anterior and posterior parts of the piriform cortex at several dorsal-ventral levels. The survival times used were: 1-2 days for HRP, ^{3}H-HRP and I-WGA, 2-4 days for true blue, and 6-8 hours for bisbenzimide. Following fixation and sectioning, the tracers were demonstrated using the DAB P-cresol procedure for HRP, autoradiography for ^{3}H-HRP and I-WGA, and epi-illumination fluorescence microscopy for bisbenzimide and true blue.

With each pair of tracers, a substantial number of double labeled mitral cells were observed, as well as cells labeled singly with either tracer. There was considerable overlap in the distribution of single and double labeled cells throughout the OB, and there were no regions of the OB which possessed only one class of labeled cells. The concentration of labeled cells was variable in different areas of the OB, and in some cases clusters of single and double labeled cells were encountered. Usually the occurrence of double labeled cells was a function of the density of single labeled cells, such that an increase in the number of single labeled cells tended to coincide with an increase in the number of double labeled cells.

These experiments indicate that individual mitral cells have branching axons which terminate in widely spaced areas of the olfactory cortex.

(We are grateful to Drs. Larry Swanson and Paul Sawchenko for gifts of the fluorescent dyes and for helpful advice. Supported by NIH Grants NS-09518 and T32-NS-07057.)

109.8 AFFERENT AND EFFERENT CONNECTIONS OF THE OLFACTORY BULB IN THE SOFT-SHELL TURTLE (Trionyx spinifer spinifer). Ronda R. Rolon and Leslie C. Skeen. Department of Psychology and Institute for Neuroscience, University of Delaware, Newark, Delaware, 19711.

We investigated the afferent and efferent connections of the olfactory bulb in the soft-shell turtle. Horseradish peroxidase (Sigma Type VI + Boehringer, 1:1) was injected unilaterally into the olfactory bulb using either pressure (3.0-0.3 µl, 40%) or iontophoresis (3 µA, 10-15 min., pulsed) to obtain injection sites of varying sizes. After appropriate survival times (6-13d.), the animals were perfused and frozen sections (30-40 µm) from their brains were processed with a variation of the tetramethyl benzidine procedure (Mesulam, '78).

Three efferent pathways can be traced from the injected olfactory bulbs. One is a component of labeled fibers that courses medially from the injection site to cross the midline and ramify in all layers of the contralateral bulb deep to the glomerular layer. The remaining two are composed by labeled fibers which course caudally through the internal plexiform layer to form the medial and lateral olfactory tracts. The former spreads a component of fine fibers and sparse terminals superficially across the medial aspect of the anterior olfactory nucleus and olfactory tubercle, and more caudally, across the rostral most portion of the hippocampus and medial parolfactory nucleus. The lateral olfactory tract forms the largest component of labeled efferents. Some of these fibers course laterally and caudally through the plexiform layer of the dorsal pyriform cortex distributing, more superficially, a pattern of dense terminals. Additional fibers course laterally and caudally through the cell layers of the dorsal and ventral pyriform cortex to spread a sheet of coarse fibers most superficially across the ventral pyriform cortex. The terminations of these fibers are also dense, but in this area, are distributed immediately subjacent to the fibers. Some of these fibers continue their caudal course superficial to the olfactory tubercle and amygdala with no obvious terminations. At the level of the amygdala these fibers turn medially and then dorsally into the stria terminalis for a short distance before joining the stria medullaris. These fibers then cross in the habenular commissure and distribute in the same fashion to the contralateral pyriform cortex.

Afferent connections of the olfactory bulb were found to originate from a number of central structures. Retrogradely labeled neurons are present in the contralateral olfactory bulb and, ipsilaterally, in the anterior olfactory nucleus, in layers II and III of the dorsal and ventral pyriform cortex, and in the vertical and horizontal limbs of the diagonal band of Broca.

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109.9 ZINC SULFATE TREATMENT AND 2-DEOXYGLUCOSE UPTAKE PATTERNS IN THE RAT OLFACTORY BULB. William B. Stewart and Charles A. Greer. Sections of Neurosurgery, Neuroanatomy & Gross Anatomy, Yale Univ. Sch. Med. New Haven, Conn. 06510

We have employed the 2-deoxyglucose (2DG) method of Sokoloff to examine the functional activity in the rat olfactory bulb following intranasal zinc sulfate treatment, which destroys olfactory receptor cells (Smith, 1938). As a part of a larger study examining the potential for return of olfactory function following regeneration of a new complement of olfactory receptor cells, we have examined the functional activity remaining one week after injury. Since this time period is too short for new connections to be formed in the olfactory bulb, activity present at this time should be due to an incomplete destruction of the mature olfactory receptors.

Thirteen twenty day old rats, anesthetized with ether, underwent intranasal irrigation, either retropharyngeally or in the external naris with one percent zinc sulfate. Several rats in the latter group were also pretreated with atropine sulfate. Following a week recovery the rats were injected with C^{14}-2-deoxyglucose (200 uCi/kg) and exposed in an odor chamber to a 10^{-1} flow dilution of amyl acetate. Prior to the 2DG experiment some rats were tested for their olfactory function by determining their latency to discovery of a hidden food reward.

There was considerable variability in the amount of odor-evoked 2DG uptake in the olfactory bulbs. In two rats there was no evidence of 2DG foci in the glomerular layer. In some rats there were a few small foci present while in others there were large regions of increased 2DG uptake. In general the medial portions of the olfactory bulb were the most likely to show activity following zinc sulfate treatment. The behavioral data correlated well with the 2DG data. Rats with no discernible 2DG foci were unable to find the hidden chocolate, while rats with only a few foci had latencies clearly longer than control. Those rats with substantial regions of 2DG uptake did not exhibit latencies different from control.

We conclude that intranasal zinc sulfate irrigation may produce highly variable amounts of olfactory receptor cell damage, as inferred from both the 2DG and behavioral assessments. The correspondence between the 2DG and behavioral data suggests that the 2DG technique is an extremely sensitive method for assaying the extent of neuronal damage and recovery following trauma in the peripheral olfactory system.

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109.10 CORRELATION OF HISTOLOGY AND 2-DEOXYGLUCOSE UPTAKE IN THE DEVELOPING RAT OLFACTORY BULB. C.A. Greer, W.B. Stewart, M.H. Teicher, J.S.Kauer and G. Shepherd. Sections of Neuroanatomy, Neurosurgery and Gross Anatomy, Yale Univ. Sch. Med., New Haven, CT. 06510

Studies employing the Sokoloff 2-deoxyglucose (2DG) technique have been directed toward describing the sequence of appearance of odor-induced activity in the olfactory bulbs of developing rats (Teicher et. al., Neurosci. Abst. 5:134, 1979). Of particular interest has been the identification of a modified glomerular region located in the dorsal posteromedial region of the main bulb near its border with the accessory olfactory bulb, which appeared to be a site of increased 2DG uptake during suckling, a behavior which may be directed, in part, by pheromonal cues. To further our understanding of the developmental sequence of odor processing in the olfactory bulb we are conducting a parallel analysis of 2DG uptake in response to an odorant, amyl acetate (AA), and correlating these findings with the histological ontogeny of the olfactory bulb. Beginning within 12 hrs. of birth rat litter mates were either injected I.P. with 20uC/100 g body wt. of C^{14}-2DG, followed by a 1 hr. exposure to a 10^{-1} flow dilution of AA and conventional processing for autoradiography, or perfused with Bouin's solution for paraffin embedding and histological study.

Increased 2DG uptake following AA exposure was apparent in even the youngest (12 hr. postnatal) rats. The patterns of uptake in the glomerular layer, however, differed somewhat from those typical of the adult. Punctate foci were present but they were widely dispersed. In addition, relative to the adult, the number of foci was smaller. Finally, the foci, although discrete, did not exhibit the same clarity or definition usually found in the adult. In the course of development these observations became less applicable as the adult characteristics emerged clearly around 12-15 days postnatally. Our observations on the histological development of the olfactory bulb are generally consistent with previous investigations in rodents (J. Hinds, J.C.N. 134: 267 & 305, 1968). At 12 hr. postnatal the rats exhibited a very poorly defined glomerular layer. Indeed, the incidence of identifiable glomeruli was very low and they tended to be scattered. During ensuing development the glomerular layer increased in definition; this increase appeared to be approximately correlated with the increased frequency of 2DG foci in odor exposed rats.

In summary, these results demonstrate an ontogenetic correlation between odor-induced physiological activity and histological differentiation in the olfactory bulb. Further studies designed to clarify the interdependence of these variables are currently in progress.

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109.11

Withdrawn by Author

109.12 INDUCTION OF NEURONAL DEGENERATION IN SECONDARY OLFACTORY AREAS BY OLFACTORY BULB LESIONS AND ITS USE IN THE TRACING OF TERTIARY OLFACTORY CONNECTIONS IN THE GUINEA PIG. J. de Olmos, J. Carlsen and L. Heimer. Inst. Invest. Medica "M.y.M. Ferreyra", Cordoba, Argentina, and Inst. of Anatomy, Univ. of Aarhus, Aarhus, Denmark.

In 22 young guinea pigs weighing 150-300 g "indirect Wallerian degeneration" was induced in the olfactory system by means of complete surgical transection or chemical destruction of one olfactory bulb. After postoperative survival times of 1-6 days the animals were anesthetized, perfused and the tissue processed according to an allantoin-cupric silver procedure developed recently in our laboratory. In order to verify the validity of the hodological information obtained by the study of "indirect Wallerian degeneration", an additional group of animals was prepared in which HRP or fluorescent compounds (Nuclear Yellow or Granular Blue) were injected either by hydraulic pressure or by iontophoresis in some of the apparent targets of axons emanating from the degenerating neurons.

Like in the rat, strongly argyrophilic degenerating neurons were observed in the primary olfactory cortex, the ventral part of the lateral entorhinal area and in the postero-lateral cortical amygdaloid nucleus. Degenerating neurons appeared already after 24 hours following the operation, and the number of heavily argyrophilic neurons reached a peak in animals with 3-4 days survival time.

At this time axonal and terminal degeneration could be identified throughout most parts of the anterior olfactory nucleus, the olfactory tubercle, the nucleus of the lateral olfactory tract and the anterior amygdaloid area. The distribution pattern and morphological characteristics of this type of degeneration could easily be distinguished from that related to the lateral olfactory tract. In addition, terminal degeneration was observed in part of the basolateral amygdaloid complex, in the basomedial amygdaloid nucleus, and the temporal parts of the subiculum and dentate gyrus. Control experiments by the aid of the HRP technique and the retrograde fluorescent method seem to support the presence of tertiary olfactory projections to the areas of terminal degeneration revealed by the indirect Wallerian degeneration. (Supported by NIH and the Danish Medical Research Council).

109.13 PHYSIOLOGICAL AND MORPHOLOGICAL ANALYSIS OF PYRAMIDAL CELLS IN THE PIRIFORM CORTEX WITH INTRACELLULAR RECORDING AND INJECTION TECHNIQUES. L.B.Haberly and J.M.Bower. Depts. of Anatomy and Neurophysiology, Univ. of Wisconsin, Madison, WI 53706.

Intracellular responses of pyramidal cells in the opossum piriform cortex to shock stimuli applied to the lateral olfactory tract (LOT) and association axons have been analyzed with the following methods: 1) analysis of responses to paired shocks, 2) application of steady de- and hyperpolarizing currents to study properties of synaptic potentials, 3) current pulses to measure threshold and impedance changes during synaptic potentials, 4) iontophoresis of Cl^- to invert IPSPs. After physiological study neurons are injected with HRP for morphological study.

Experiments thus far have concentrated on an analysis of events during period I of the response to LOT stimulation. Results are consistent with the hypothesis (Haberly & Shepherd, J. Neurophysiol., 36: 789, '73; Haberly & Price, JCN, 178: 711, '78) that pyramidal cells are monosynaptically excited during the A_1 time period by LOT axons, then re-excited via association axons from other pyramidal cells during the B_1 time period. Evidence from the present experiments supporting this hypothesis includes: 1) Analysis of the time course and other characteristics of the EPSP evoked by LOT sitmulation indicates that it is generated by membrane currents during time periods A_1 and B_1. 2) A direct demonstration that pyramidal cells give rise to association axons by intracellular injection with HRP. 3) Demonstration of antidromic activation and apparent monosynaptically evoked EPSPs in pyramidal cells from direct stimulation of association fibers through electrodes in layer III up to 1.4cm distant from recording sites. 4) In addition, preliminary results obtained with the EM-autoradiographic technique indicate that terminals of association fibers are morphologically similar to those thought to mediate excitatory effects in other cortical systems: round vesicles and asymmetrical contacts onto dendritic spines. An unexpected finding is that an IPSP resulting from LOT stimulation begins during the B_1 time period, thus overlapping the EPSP generated through the association fiber system.

Analysis of the morphology of intracellularly injected pyramidal cells in layer II has revealed several new features: 1) These cells give rise to multiple rostrally & caudally, dorsally & ventrally directed association axon branches, most of which are very small in diameter ($\bar{x}$ 0.3μm). 2) These branches generate a widespread matrix of varicosities and boutons (presumed terminals) in layer III. No evidence for a restricted, columnar organization has been obtained. 3) There is a great diversity in spine morphology including very long, small diameter, varicose spines on apical and basal dendrites. Supported by NIH Grant # NS 15004.

109.14 OLFACTORY NEOCORTICAL AREAS IN THE RAT. S.J. Wiegand and J.L. Price. Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110

Previous studies have shown that cells deep to the olfactory cortex project to the central segment of the mediodorsal thalamic nucleus (MDc), which in turn projects to two prefrontal cortical areas in the dorsal bank of the rhinal sulcus, the ventral agranular insular area (AIv) and the lateral orbital area (LO). AIv, at least, also receives a direct input from the piriform cortex (PC).

In the present experiments Holtzman rats were anesthetized with chloral hydrate and fixed in a stereotaxic apparatus. The right olfactory bulb (OB) was stimulated with bipolar tungsten electrodes 1-2 mm deep to the surface. Following single or short trains of electrical shocks (5-10 V, 100 usec) evoked field potentials and unit activity were recorded in the PC, AIv and LO with tungsten microelectrodes (3-5 MΩ). In the PC a large increase in unit activity was observed 4-14 msec after OB stimulation followed by a longer period (ca. 50-100 msec) of inhibition. In both the AIv and LO a less marked increase in unit activity was usually observed 8-20 msec after OB stimulation followed by a period (ca. 30-100 msec) of decreased unit activity. This suppression of unit activity was sometimes observed without an initial increase in unit activity.

In these same experiments small injections (10-50 nl) of HRP (10-20%) or HRP conjugated to wheat germ agglutinin (HRP*WGA) (0.05-0.1%) were placed in the AIv or LO to determine their connections with olfactory related structures. In both cases, retrograde cell labeling was observed in the MDc and in the PC. Anterograde axonal labeling was also observed in the MDc and, especially when the AIv was involved, in the olfactory tubercle, anterior cortical amygdaloid nucleus and, to a lesser extent in the PC. These experiments also suggest that there may be differential inputs to the AIv and LO. For example, the cells which project to the LO are concentrated in the anteromedial part of the PC, while those which project to the AIv are more widely dispersed in the PC and adjacent olfactory areas. Autoradiographic experiments also support this distinction; injections of ^{3}H-amino acids into the posterior PC, label axons in the AIv only, while injections which involve the anteromedial PC also label axons in the LO.

Therefore, on the basis of these and previous experiments, we suggest that the AIv and LO are specific neocortical olfactory areas which receive olfactory-related inputs directly from the PC and indirectly from the MDc. (Supported by NIH grants NS09518 and T-32-NS07057)

109.15 THERMAL SENSITIVITY OF PARABRACHIAL TASTE NEURONS IN THE HAMSTER. Susan E. Plock and David V. Smith. Dept. Psychol., Univ. Wyoming, Laramie, WY 82071.

Hamster chorda tympani (CT) fibers are responsive to both gustatory and thermal stimuli (Ogawa, Sato & Yamashita, 1968). As part of an investigation of the gustatory sensitivities of neurons in the hamster parabrachial nucleus (PbN), we have examined the responsiveness of 13 cells to thermal stimulation. The gustatory stimuli were: 0.1 M sucrose, 0.03 M NaCl, 0.003 M HCl and 0.001 M quinine hydrochloride (QHCl), presented at room temperature (24° C). These stimuli and the thermal stimuli followed preadaptation of the tongue to distilled water at 24° C. Thermal stimuli ranged from a mean of 16° C (cool) to a mean of 39° C (warm). Twelve of these neurons responded to at least one of the thermal stimuli, 5 excited by both warm and cool stimuli, albeit differentially, 5 excited by either warm or cool stimuli, and 2 excited by cool stimuli and inhibited by warm. The mean firing rates to these stimuli were: 64.7 impulses/5 sec to sucrose, 71.2 to NaCl, 19.2 to HCl, 41.9 to QHCl, 37.7 to cooling, and 50.2 to warming. The across-neuron correlations between the gustatory and thermal stimuli were positive between responses to sucrose and warming ($\underline{r}$ = +0.86) and QHCl and warming ($\underline{r}$ = +0.49), but there was no relationship between responses to NaCl ($\underline{r}$ = +0.20) or HCl ($\underline{r}$ = +0.01) and warming. Cooling the tongue produced responses that were correlated across neurons with those to NaCl ($\underline{r}$ = +0.45), HCl ($\underline{r}$ = +0.62), and QHCl ($\underline{r}$ = +0.47), but not to sucrose ($\underline{r}$ = -0.07). The spontaneous rate of discharge in these neurons was correlated with the response to cooling ($\underline{r}$ = +0.63), but not to warming ($\underline{r}$ = +0.20). These relationships are similar to those reported for peripheral taste fibers in the hamster (Ogawa et al., 1968), except for the positive correlation between the response to QHCl and warming, which was negative in peripheral fibers. The mean responses to these stimuli in sucrose-best cells ($\underline{n}$ = 4) in PbN were 113.3 impulses/5 sec to sucrose, 49.5 to NaCl, 12.8 to HCl, 19.0 to QHCl, 8.5 to cooling, and 102.3 to warming. In NaCl-best neurons ($\underline{n}$ = 6), these responses were: 36.8 impulses/5 sec to sucrose, 88.2 to NaCl, 3.3 to HCl, 11.3 to QHCl, 45.2 to cooling, and 0.5 to warming. In the rat PbN, excitatory responses to thermal stimuli occur mainly to cooling (Norgren & Pfaffmann, 1975; Perrotto & Scott, 1976), which reflects the sensitivity of peripheral taste fibers in this species (Ogawa et al., 1968). The thermal sensitivity of hamster CT neurons is maintained in third-order PbN cells, adding further to their already broad responsiveness to gustatory stimuli. Whether this sensitivity to temperature is greater or less than that of peripheral fibers is not certain without further parametric investigation.

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109.16 ASCENDING GUSTATORY AND VISCERAL AFFERENT PATHWAYS IN THE MONKEY. J.R. Morse, R.M. Beckstead, T. Pritchard and R. Norgren. The Rockefeller Univ., New York, NY 10021.

The ascending efferent projections from the parabrachial area (PBA) and the parvicellular portion of the ventral posteromedial nucleus of the thalamus (VPMpc) were studied in cynomolgus monkeys using the autoradiographic anterograde fiber-tracing method. Previous investigations in the same species established that the nucleus of the solitary tract (NST) projects directly to both these areas (Beckstead, Morse & Norgren, J. Comp. Neurol., 1980, 190, 259-282). The predominantly viscerosensory, caudal third of NST sends axons to the PBA, while fibers arising from the predominantly gustatory, rostral third of NST bypass the PBA and ascend to the ipsilateral VPMpc. Neurons in the middle third of NST project to both the dorsal pons and thalamus. Current experiments involve characterizing the sensory input to the PBA and VPMpc by electrically stimulating the vagus and glossopharyngeal nerves and by applying mechanical stimuli to the intra- and perioral fields, as well as thermal and sapid stimulation to the tongue. When an appropriate response is obtained, the recording locus is infiltrated with tritiated amino acids applied microelectrophoretically. To date, data from three parabrachial and four thalamic cases have been analyzed. Deposits in the lateral PBA at sites responsive to vagal and glossopharyngeal nerve stimulation yield labeled fibers that can be traced to the amygdala and bed nucleus of the stria terminalis (BNST). These fibers ascend in the central midbrain tegmental reticular formation and through the medial subthalamic area, partly in Field H of Forel and partly intermingled in the dorsal part of the medial forebrain bundle. Some fibers diverge laterally dorsal to the rostral part of the substantia nigra and curve around the lateral edge of the cerebral peduncle to terminate in the central nucleus of the amygdala. Distinct axon labeling is also present in the supracommissural part of the BNST and probably arises from axons that take a dorsalward course parallel to the internal capsule. A few fibers can be traced into the supraoptic commissures, but cannot be followed to a contralateral target with certainty. Axons have been traced from the rat PBA to ventrobasal thalamus and far lateral hypothalamus (Norgren, J. Comp. Neurol., 1976, 166, 17-30), but these connections are not evident in our primate material. Isotope deposits in the monkey's VPMpc, where neurons respond to taste stimuli, label fibers that enter the thalamic radiations to distribute in the dorsal part of the rostral insular cortex ipsilaterally. In contrast, axons emanating from sites in VPM, where neurons respond to lingual tactile stimulation, project to the lateral convexity of the cortex. Supp. by BNS76-81408 and NS06301-01.

109.17 CHEMOSENSORY CONTROL OF FOOD-RELATED BEHAVIORS IN THE HAMSTER. Robert D. Sweazey* and David V. Smith. Dept. Psychol., Univ. Wyoming, Laramie, WY 82071.

Hamsters were placed for six days in a semi-natural environment, consisting of a sand-filled chamber maintained on a 12-hr light/dark cycle, from which a series of plastic tunnels projected "underground", in constant red light. Water was available ad lib in the sand-filled chamber and 5 gm each of 10 novel foods were placed in this area at the beginning of the six-day period. Twenty-four hours after injection of saline or apomorphine (30 mg/kg), either paired with a distinct gustatory cue or given alone, the hamster was introduced into the tunnel system and its nest location and food distribution were noted daily. At the end of the session, the animal was removed and the location and amount remaining of each food was measured.

The saline-injected control animals typically hoarded all the foods within the first day, storing them consistently near the nesting site, always at the lowest points in the tunnel system. Preference among the foods was remarkably consistent, peanuts being the most favored, followed by sunflower seeds, oats, rye, wheat, rice, soybeans, barley, corn and Great Northern beans. Eighty percent of the peanuts were consumed, but only sixteen percent of the Great Northern beans.

Animals injected with apomorphine prior to the six-day session did not show normal patterns of food consumption or hoarding behavior. If the animal was poisoned in the absence of a distinct gustatory cue, the consumption of all foods was reduced by 60-100 percent, the foods were not hoarded immediately, and were scattered throughout the tunnel system over the entire six-day period. Additional animals were given apomorphine paired with a distinct gustatory stimulus (sucrose, NaCl, HCl or quinine). Rather than a widespread aversion to all novel foods, these animals demonstrated consistent and unique patterns of food consumption, depending upon which taste quality was paired with the illness. For example, after sucrose aversion the most preferred foods (peanuts and sunflower seeds) were less avidly consumed, with concomitant increases in the consumption of other foods. Hoarding behavior tended to be even more consistent in animals with specific taste aversions than in saline controls. Thus, the gustatory (and possibly olfactory) cues provided by these taste stimuli have a very direct influence on food-related behaviors. In the absence of these cues, the animal develops a strong and persistent avoidance of all novel foods, even though these are all that is available over the six-day period. If the animal can associate the poison-induced illness with distinct oral cues, then its behavior toward novel food substances comes under oral control.

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109.18 THE EFFECTS OF TEMPERATURE ON THE PERCEIVED SWEETNESS OF SUCROSE. L. M. Bartoshuk, K. Rennart*, J. Rodin*, and J. C. Stevens. J.B.Pierce Fndn. Lab and Yale Univ., New Haven, CT 06519.

Early studies of the effects of temperature on taste were done almost exclusively with threshold measures. Only one previous study (Moskowitz, 1973) evaluated a range of concentrations of a sweet substance (glucose) in order to assess possible effects of temperature on the perceived intensity of sweetness. Moskowitz reported that the exponents for the psychophysical functions (which were power functions) for glucose were invariant but that when the stimuli were at approximately the same temperature as the tongue (35°C), they all tended to be sweeter.

Three experiments presented here show different results for sucrose. All three experiments had these features in common: the sweetness judgments were obtained with the method of magnitude estimation, and each stimulus was tasted after a rinse with deionized water warmed to approximately mouth temperature (34°C).

In the first experiment, solutions were tasted with the "sip and spit" method. Solutions at room temperature (22°C) and body temperature (34°C) were compared. The room temperature solutions were less sweet at concentrations less than .3-.5 molar, but were equally sweet at higher concentrations.

In the second experiment, similar temperatures were compared but two tasting methods were used: "sip and spit" and "sip and swallow." The results for both tasting methods agreed with those in the first experiment. Lower concentrations were less sweet at the lower temperature.

In the third experiment, stimuli were tasted with the "sip and spit" method and the temperature range was expanded to include: 4, 12, 20, 28, 36, and 44°C. The results confirmed the first two experiments and showed even greater effects of temperature on sweetness with the more extreme temperatures. That is, around .3-.5 molar, solutions were perceived to be about equally sweet. For lower concentrations, the warmer stimuli were sweeter and for higher concentrations, the cooler stimuli were sweeter.

It should be noted that there may be chemical sources for effects of temperature on the perceived sweetness of sugars since temperature is known to affect molecular conformation.

110.1 EFFECT OF MORPHINE ON PREFERRED DURATION OF ELECTRICAL BRAIN STIMULATION IN THE MOUSE. Hugh E. Criswell and Debi M. Starnes*
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When rats or mice are allowed to control delivery of electrical brain stimulation(EBS) to the lateral hypothalamic area, in a shuttle-box paradigm, they rapidly acquire shuttling behavior which results in precise control of both the duration of the stimulation and the time between stimuli (on time and off time). Systemic morphine has been shown to have a selective effect on the preferred duration of EBS in the rat. Moderate doses(5-10mg/kg) increase on times while higher doses produce catatonia which is associated with an increase in both on and off times(Levitt, et al, Psychopharmacol., 1977, 54,307-311). Some strains of mouse do not become catatonic at high doses of morphine. Instead, as dose is increased, their activity level increases. To determine the relation between morphine effects on EBS and activity level, we examined shuttle-box behavior for EBS in the mouse.

Swiss Webster(ICR) mice were implanted with 36 gauge nicrome electrodes using standard stereotaxic procedures. Electrodes were aimed at the perifornical lateral hypothalamus. Following recovery, animals were tested for shuttling to obtain EBS. Only animals which attained stable shuttling behavior with on times under 5 sec. and off times under 10 sec., with less than 50uA. stimulation were retained. Each animal then received control, 10 and 20 mg/kg I.P. injections of morphine in counterbalanced order, ½ hour prior to daily 15 min. testing sessions. Each drug day was separated by one day of non drugged testing. Mean on and off times were recorded for each condition. On times averaged 2.8 sec. for control days, 2.2 sec. for 10mg/kg and 2.6 for 20mg/kg. These times were not statistically different(P 0.10). Off time was 5.5 sec. for controls, 10.0 sec. at 10mg/kg and 12.1 at 20mg/kg. Both drug tests differed from controls (P 0.01) but did not differ from each other (P 0.10).

Mice respond to morphine by increasing the time between stimuli while rats respond by increasing the duration of the stimulus. The fact that mice and rats differ in the effect of morphine on their shuttling behavior for EBS suggests that the effect of morphine on EBS is more closely linked to its effect on activity level(where rats and mice also differ) than to its analgesic effect (where mice and rats respond similarly). The selective effect on off times in the present study rules out nonspecific toxic effects of the morphine as the reason for the increased off times.

110.2 PHARMACOGENETICS OF INITIAL TOLERANCE TO ETHANOL. STUDY OF PHARMACOKINETIC AND PHARMACODYNAMIC TOLERANCE USING DROSOPHILA AS ANIMAL MODEL. F. Garcin[1], S. Chawla[1]*, J. M. Perron[2]* and C. Radouco-Thomas[1]. Unit for Research on Drug and Alcohol Abuse, Hospital St.François d'Assise, Dept. of Pharmacol., Fac. of Med.[1], and Dept. of Biology, Fac. of Sciences[2], Laval Univ., Quebec, Canada.

It is well known that the two Drosophila sibling species, D. melanogaster and D. simulans, differ in their initial tolerance to ethanol (ETOH). D. melanogaster possess a much higher tolerance level to ETOH than D. simulans. Initial tolerance is known to be the resultant of two components, the metabolic or pharmacokinetic tolerance related to disposition of ethanol and the functional or pharmacodynamic tolerance related to the sensitivity of the main target tissue, the central nervous system.

Biochemical studies on alcohol dehydrogenase (ADH) activity have shown that differences in initial tolerance to ethanol in the two species are, at least partially, due to metabolic factors. As a matter of fact, ADH activity in D. melanogaster is twofold higher than in D. simulans (Garcin et al. 1979, 1980). No study has been devoted to the functional tolerance to ethanol. This is due to the lack of valid biochemical studies.

In our laboratory we have developed a specific behavioral test which allows, on the one hand, to differentiate the two components of the initial tolerance and on the other hand, to assess them quantitatively. Individual flies are introduced into a air tight tube of 155 ml containing ethanol vapors at a concentration of 245 mg/l. The "knock down" is recorded for each individual fly. Time elapsing from introduction until knock down is referred to as "knock down induction time" (Ki) and that elapsing from the beginning to the end of knock down as "knock down duration" (Kd). Ki which is related to the immediate response of the fly is proposed as a measure of functional tolerance, whereas Kd would be an indicator of the rate of ethanol metabolism.

Our results show that Ki is shorter in D. simulans (4,62 min) than in D. melanogaster (7,5 min). On the contrary, Kd is higher in D. simulans (15,5 min) than in D. melanogaster (7,4 min). These data suggest that the higher initial tolerance found in D. melanogaster would be related to both pharmacokinetic and pharmacodynamic factors.

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110.3 INTRACRANIAL SELF-ADMINISTRATION OF MORPHINE INTO VARIOUS BRAIN REGIONS IN RATS. M. A. Bozarth and R. A. Wise. Center for Research on Drug Dependence, Department of Psychology, Concordia University, Montreal, Quebec, H3G 1M8.

In an attempt to specify the opiate receptor population at which morphine acts as a reward, we have begun studies of intracranial drug self-administration (ICSA). To minimize the problem of poorly controlled drug delivery, infusions were given by a method that did not require flexible tubing or a fluid swivel to maintain unrestricted movement of the animals during testing (M. A. Bozarth & R. A. Wise, J. Neurosci. Methods, in press).

Experimentally naive rats quickly learned a lever-pressing response which produced 100 ng infusions of morphine sulfate (in 100 nl of Ringer's solution) into the ventral tegmental area (VTA). Mean response rates were relatively stable across 4 hours of testing and they markedly exceeded lever-pressing rates of rats passively receiving the same number and pattern of infusions (yoked control condition) or of rats working for Ringer's solution alone. ICSA was blocked by naloxone (10 mg/kg, i.p.) indicating that ICSA was due to an action at opiate receptors and not due to nonspecific changes in local pH, osmolarity, or calcium flux. Additional behavioral controls and manipulations of unit dose and volume are in progress.

These results indicate that truly rewarding (not merely behaviorally activating) effects of morphine can be demonstrated with central injections. Although injections of morphine into the VTA produced increased general activity, this effect did not result in increased accidental lever contacts as evidenced by the nearly identical response rates of the yoked and Ringer's control groups. The use of experimentally naive animals eliminated the possibility, inherent in other paradigms, that responding was due to reinstatement of habits previously learned for other rewards.

Preliminary results suggest that rats may learn to self-administer drug into the lateral hypothalamic area (LHA), caudate nucleus (CAUD), and central gray, but ICSA into the LHA and CAUD has been much more variable than ICSA into the VTA. In addition, morphine injections into the LHA has thus far been accompanied by increased lever-press counts in yoked control rats who receive their drug passively. Additional testing is necessary before ICSA at these sites can be meaningfully compared to that from the VTA. Confirmation of an anatomically restricted target for the rewarding effects of opiates will require demonstration that responding is contingent on drug delivery and that the drug is not acting by diffusion to other sites.

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110.4 REPEATED NIALAMIDE TREATMENT BLOCKS THE BEHAVIORAL SYNDROME PRODUCED BY 5-METHOXY-N, N-DIMETHYLTRYPTAMINE I. Lucki and A. Frazer. Depts. of Psychiat. and Pharmacol., Univ. of Penna. and Vet. Adm. Hospital, Phila., Penna. 19104.

We have shown (J. Pharmacol. Exp. Therap. 212:259, 1980) that repeated, but not acute, administration of monoamine oxidase inhibitors (MAOIs) to rats reduces binding sites for ^{3}H-serotonin in brain. It was of interest to determine whether the decrease in brain serotonin receptors induced by repeated administration of MAOIs had any behavioral effects. To do this, the behavioral syndrome produced in rats given intraperitoneal injections of the serotonin agonist 5-methoxy-N, N-dimethyltryptamine (5-MDMT) was studied. This behavioral syndrome is characterized by repetitive forepaw treading, hindlimb abduction, tremor, lateral head weaving, and Straub tail (J. Pharmacol. Exp. Therap. 206:339, 1978). Rats were given either a single intraperitoneal injection of nialamide (40 mg/kg) or 13 injections of the drug over a period of seven days. Control rats received injections of 0.9% NaCl. Twenty-four hours after the final saline or nialamide injection, rats were administered 5-MDMT, at a dose of either 3 mg/kg or 9 mg/kg. In saline-treated rats, both doses of 5-MDMT produced the behavioral syndrome (in seven of eight rats). In rats given a single injection of nialamide, 5-MDMT produced the same behavioral syndrome that it did in control rats. By contrast, 5-MDMT failed to produce the behavioral changes described above in all ten rats given nialamide repeatedly. This difference between the effect produced by 5-MDMT in control rats and in animals treated repeatedly with nialamide is significant ($\chi^2 = 10.9$; $p < 0.005$). Thus, the decrease in ^{3}H-serotonin binding sites produced by repeated administration of MAOIs may be correlated with a reduced behavioral response elicited by activation of serotonin receptors in brain. (Supported by Research Funds from the Vet. Adm., USPHS Grants MH 29094 and MH 14654).

110.5 MODIFICATION OF AMYGDALOID-KINDLED SEIZURES IN RATS BY OPIATES. William S. Stone*, Cynthia E. Eggleton*, and Robert F. Berman (SPON: David Asdourian). Dept. of Psychology, Wayne State University, Detroit, MI 48202.

A variety of experimental data indicate that endorphins may be involved in the development of amygdaloid kindling. Morphine and the enkephalins have both analgesic and epileptic properties when injected intraventricularly in the rat (Urca, Frenk, & Liebeskind, Science, 197: 83, 1977). Furthermore, kindling develops most rapidly following electrical stimulation of the amygdala; a region extremely high in opiate receptors (Kuhar, Pert, & Snyder, Nature, 245: 447, 1973) and enkephalinergic terminals (Uhl, Kuhar, & Snyder, Brain Res., 149: 223, 1978). Previous attempts to alter amygdaloid-kindled seizures with the opiate agonist morphine or the antagonist naloxone have either failed or have reported only slight effects (Post, Davenport, Pert, & Squillace, Comm. Psychopharm., 3: 185, 1979; Corcoran & Wade, Life Sci., 24: 791, 1979), while results of the present studies, however, indicate that these agents can significantly alter amygdaloid kindled seizures. Male Long-Evans rats (300 g) were stereotaxically implanted bilaterally with bipolar electrodes in the medial amygdala. After recovery from surgery, the after-discharge (AD) threshold was determined for each rat by stimulating through one of the implanted electrodes (1 sec, 1.0 msec pulse duration, 100 Hz, biphasic, symmetrical, square waves) with increasing current intensities until an AD was recorded. Rats were then kindled once daily for 1 sec at the AD threshold. Kindling was continued until Stage V seizures (Racine, EEG & Clin. Neurophysiol., 32: 281, 1972) were elicited on two consecutive days. On the following day, animals were injected (ip) with either saline, naloxone (10 mg/kg), naltrexone (10 mg/kg) or morphine sulfate (10 mg/kg) and were stimulated at the AD threshold 20 minutes later. Saline injected animals showed long bilateral AD's (27.5± 3.5 sec ipsilateral, 21.0± 4.0 sec contralateral) and behavior typical of Stage V kindled animals. In contrast, rats injected with 10 mg/kg naloxone showed significantly reduced AD activity (11.5± 1.9 sec ipsi., 6.6± 2.1 sec contra.) with almost complete absence of behavioral seizure activity. Naltrexone (10 mg/kg) similarly reduced AD's and behavioral seizures. In contrast, rats injected with morphine sulfate (10 mg/kg) showed increased AD's and more severe behavioral seizures than controls. Morphine potentiation of kindling was more pronounced when given during an intermediate stage of kindling (Stage III) suggesting that previous failures to observe potentiation by morphine may have been due to a relative ceiling effect present at Stage V.

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110.6 Generality of Selective Breeding for Sensitivity to Alcohol in Mice. Michael E. Abbott* and Bruce C. Dudek. Dept. of Psychology SUNY at Albany, Albany, NY 12222

The selective breeding program of McClearn and Kakihana (Behav. Genet. 3: 490-410, 1973) produced lines of mice which differ markedly in sensitivity to the hypnotic effects of ethanol. The Long Sleep (LS) mice lose the righting reflex for about four hours and the Short Sleep (SS) mice for less than 30 min after a 4.2 g/kg, i.p., dose. This has been shown to reflect differential neural sensitivity to ethanol. These lines thus are a tool for investigation of genetic effects on the nervous system and behavior. For example, LS mice are more sensitive to the actions of gamma-butyrolactone on brain dopamine levels.

Behaviorally, the LS and SS mice also show differential response to low doses of ethanol. SS mice are highly activated while LS mice are depressed and severely uncoordinated. There exists controversy as to whether these low dose phenotypes are genetically independent of the original selection phenotype (sleep-time) or whether they are also a direct result of the selective breeding. The present studies addressed this question with a genetic analysis. Reciprocal F_1 hybrids were tested for loss of righting reflex at 3.8 or 4.2 g/kg of ethanol. Their loss of righting reflex durations were intermediate of the two parental lines at both doses. No differences between the reciprocal hybrids were found, suggesting that maternal effects were not important for the selection. Small but reliable sex differences showed male mice to have longer loss of righting reflex times. A second experiment investigated coordination with the grid test. Locomotor activity was concurrently measured by photocell beam interuption for 20 min following treatment. Mice were treated with ethanol at 0.0, 1.5, 2.0 or 2.5 g/kg, i.p. SS mice were more active at all doses of ethanol than controls. LS mice showed depressed locomotor activity at all doses. These effects were dose dependent in both lines. Hybrid mice were activated relative to controls when treated with 1.5 g/kg, but were depressed at the other two doses. The two reciprocal hybrids did not differ from each other. Ethanol had marked disruptive effects on coordination (normalized to activity) in all genotypes, but LS mice were most severely affected and SS mice were least affected. Clear dose response effects were apparent in all groups. Reciprocal hybrids did not differ from each other, but were again intermediate of the two parental lines. In sum these studies suggest a shift of the ethanol dose response curve to the left for LS mice and to the right for SS mice. The intermediacy of F_1 mice is consistent with a common polygenic interpretation of the genetic influence on both low and high dose responses.

110.7 EFFECTS OF COCAINE ON THE DETECTION OF FOCAL BRAIN STIMULATION. Henry Lesse and Jeremiah P. Collins. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, Calif. 90024

It has been found that cocaine reduces the electrical current necessary to evoke epileptiform afterdischarges (AD) in the amygdala and hippocampus (Lesse, Collins & Denea 1977; Lesse & Collins 1980). However moderately high doses are required to alter these thresholds. Repeated evocation of AD results in progressive electrophysiological changes, and this method is limited to brain structures in which AD are readily induced. The present study provides a more sensitive indicator of regional excitability changes. The method is applicable to most cerebral structures, and tests can be repeated over prolonged periods of drug administration without inducing AD or kindled seizures.

Cats prepared with arrays of indwelling electrodes were trained to use low intensity electrical stimulation (3Hz, 200-350 ma, 0.5 msec. pulses) of selected subcortical sites as a discriminative stimulus (cue) for a milk-reinforced bar pressing response. Stimulus detection thresholds (the minimal current required to elicit the discriminative response) were determined using a modified method of limits. After stable threshold values were obtained, the effects of alternate administrations of saline and cocaine were examined. Repeated testing over periods extending to 9 months resulted in neither electroencephalographic abnormalities nor evidence of seizure development. Results indicate that low doses of cocaine (0.1-0.4 mg/kg, i.m.) reduce detection thresholds for both amygdalar and hippocampal stimulation. These dose levels, an order of magnitude lower than those required to change AD thresholds, result in few, if any, observable aberrations in behavior. The stimulus detection threshold reductions were not correlated with non-specific changes in discriminative behavior (e.g. changes in responses rates, response latencies or error responses). These results suggest that cocaine alters the sensitivity of limbic structures functionally important in the regulation of emotion. The excitatory effects following administration of low doses are manifest in the absence of induced afterdischarges and appear independent of the convulsant properties of cocaine.

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110.8 THE ESCALATING EFFECT OF COCAINE ON AGGRESSION IN ISOLATED FIGHTING MICE. M.G. Hadfield, D.E.W. Mott* and E.A. Nugent*, Med. Coll. Va., Va. Commonwealth U., Richmond, VA 23298 Neuropathology Laboratory

Illicit cocaine use is currently increasing at a rapid rate, partly because it has been promoted as a relatively "safe" drug. Yet Jaffe warns us that violence may accompany cocaine use because the stimulant properties may drive the toxic abuser to act out on his persecutory delusions (in Goodman and Gilman, 1965). The results of the present study in isolated fighting mice also indicate that cocaine enhances aggression. Thus cocaine may not indeed be a "safe" drug.

ICR male mice, isolated for six weeks, were separated into 12 groups of four. Each group was tested twice under each of four different conditions: no injection, physiological saline medium injection (1cc/100 gm. body weight), 10 mg/kg cocaine hydrochloride and 35 mg/kg cocaine hydrochloride. Injections were given, i.p., approximately 10 minutes before the animals were placed together. The no-injection controls were not handled at all during the ten minute interval. The order of treatments was different for each group, except that drug conditions alternated with no-drug conditions. The mice were tested on four consecutive weekdays, two weeks in succession. Minute-by-minute fight durations as well as behavioral observations were recorded for fifteen minute sessions, after which the animals were returned to their home cages. A total of 24 fights were observed under each condition. The fighting arena had a floor approximately 13 x 13 cm.

Cocaine produced a dose dependent increase in fight duration. The total fight duration was approximately 110 seconds in the saline injected animals, 175 seconds in animals receiving 10 mg/kg. cocaine and 240 seconds in animals receiving 35 mg/kg. cocaine. The fight duration in the non-injected resting controls was approximately 140 seconds. By graphical methods, the ED_{50} for cocaine on the fighting response (over a saline baseline) was determined to be about 15 mg/kg. assuring a logarithmic dose-response characteristic. As regards fighting over the time course, cocaine produced its greatest effect in the last five minute time block. Again, this effect appears to be strongly dose dependent. Without cocaine, there was no significant difference in fight duration between the 2nd and 3rd 5 minute time block. The first 5 minute time block displayed shorter fight durations than later time blocks for all conditions, but again cocaine elevated fighting in this block. The cocaine treated animals also showed qualitative differences. They were more agitated and initially tried to jump over the steep walls of the fighting arena. The initial response was one of irritative--avoidance but this soon gave way to intense fighting.

110.9 EFFECTS OF ATROPINE ON PHENCYCLIDINE (PCP)-INDUCED RESPIRATORY TOXICITY AND LETHALITY IN RATS. M. P. Holsapple*, D. J. Ritter*, W. R. Pfister*, and G. K. W. Yim (SPON: R. Babington). Dept. of Pharmacology and Toxicology, Purdue University., W. Lafayette, IN 47906.

To further evaluate the potential role of the anticholinesterase actions of PCP (Kloog et al., Eur. J. Pharmacol. 45: 221, 1977), the effects of atropine were determined. In rats anesthetized with urethane (1.2 g/kg ip), PCP infusion (0.25 mg/min into the jugular vein) induced apneusis and gasping, followed by progressive depression of respiratory rate and diaphragmatic contractions, and eventual respiratory arrest (within 40-60 min). After stopping the PCP infusion at the apneustic stage (2mg/kg), approximately 30 min was required for recovery to pre-drug respiratory rates and phrenic discharge patterns. Intravenous atropine (0.8 mg/kg) immediately converted the irregular apneustic-gasping respiration to a more regular pattern (rate and amplitude). Atropine pretreatment also prevented the development of the apneustic PCP pattern. However, apneusis could not be induced by infusion of the centrally active anticholinesterase agent, physostigmine. In addition, atropine had an erratic effect on lethality. Possible protection, suggested in initial experiments, could not be consistently duplicated in subsequent experiments. Moreover, pretreatment with atropine (1 mg/kg ip) failed to protect unanesthetized rats (and mice) from convulsions and lethality induced by 100 mg/kg PCP ip.

These results thus indicate that atropine is not an effective antagonist of PCP-induced respiratory toxicity and lethality, and that PCP-induced apneusis is not solely due to the anticholinesterase actions of PCP. (Supported, in part, by a Purdue Research Foundation Fellowship to M.P.H., N.S.F. Grant #SPL-7826649 and U.S.P.H.S. Grant DA/DA-02327-01).

110.10 COCAINE-INDUCED SPINDLES ARE GENERATED IN THE PREPYRIFORM CORTEX IN THE RAT. Jeffrey S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

In large doses, cocaine produces a characteristic form of extracellular field potential in the olfactory forebrain (cocaine spindles) which consists of bursts of high-amplitude sinusoidal electrical activity in the range of 20 - 50 Hz. This phenomenon was first reported by Eidelberg, Lesse, and Gault (1963), who recorded it from electrodes implanted in the amygdala of the cat. Subsequent experiments indicated that other local anesthetics such as lidocaine produce a similar effect. Because this form of drug-induced electrical activity can be recorded by electrodes in the amygdala, it has sometimes been assumed that it is generated by neuronal activity within that structure. However, cocaine spindles closely resemble naturally occurring activity generated in the olfactory bulb and prepyriform cortex in the presence of olfactory input (olfactory spindles). The purpose of the present experiment was to determine if the drug-induced spindle activity which can be recorded in the amygdala is in fact generated in the adjacent portion of the prepyriform cortex by the same circuits which generate naturally occurring olfactory spindles. Rats with chronically implanted electrodes in the prepyriform cortex were injected with doses of cocaine or lidocaine sufficient to produce spindle activity (5 mg/kg i.v. or 40 mg/kg i.p.). Both drug-induced and naturally-occurring spindles showed the same amplitude profile, with the lowest amplitude in the posterior prepyriform cortex (nearest the amygdala). Multipolar recordings indicated that the amplitude of naturally-occurring or drug-induced spindles was highest within the prepyriform cortex and lower at sites 1 - 2 mm dorsal to the prepyriform cortex (e.g., in the amygdala). Furthermore, both naturally-occurring and drug-induced spindles exhibited a polarity reversal near the superficial pyramidal cell layer of the prepyriform cortex. Finally, neither form of spindle occurred when nasal air flow was blocked (absence of olfactory input). These observations support the conclusion that cocaine and lidocaine-induced spindle activity which can be recorded in the amygdala is generated by circuits in the adjacent prepyriform cortex which also produce naturally occurring olfactory spindles.

110.11 EFFECTS OF DOPAMINERGIC AND CHOLINERGIC AGENTS ON PHENCYCLIDINE-INDUCED BEHAVIORS IN RATS. S. Castellani and P.M. Adams. Department of Psychiatry and Behavioral Sciences, University of Texas Medical Branch, Galveston, Texas 77550.

Previous studies have shown that many biochemical and behavioral effects of phencyclidine (PCP) are affected by dopaminergic and cholinergic neurotransmission. Thus, PCP-induced dopaminergic effects include reuptake blockage and potentiation of presynaptic dopamine action in the corpus striatum, and dopamine mediation of stereotyped behaviors. Also, cholinergic effects of PCP indicate competitive inhibition of CNS and peripheral acetylcholinesterase activity in vitro, and anticholinergic potentiation of locomotor activity. This study was undertaken to examine the effects of dopaminergic and cholinergic agents on concurrent PCP-induced locomotor activity, stereotypy and ataxia in rats.

Fischer 344 male rats were rated for PCP-stereotypy and ataxia in 1 minute observation periods at 10, 20, 30 and 40 minutes post-injection (0.0,2.0,4.0, and 6.0mg/kg) and assessed for locomotor activity by means of automated counters in three 10 minute post injection intervals through 70 minutes. The rating scales used in this experiment were developed by the authors for quantification of PCP-induced stereotypy and ataxia, and were validated in previous dose-response studies. Dopaminergic drugs used were haloperidol (.5mg/kg) and apomorphine (.2mg/kg); and the cholinergic drugs used were atropine (1.0mg/kg) and physostigmine (.2mg/kg).

Both stereotypy ratings and locomotor activity counts were decreased by haloperidol and increased by apomorphine, and also showed decreases and increases following injection of physostigmine, and atropine, respectively. However, these effects were PCP dose dependent for dopaminergic and cholinergic agents. In contrast to higher PCP dosages, apomorphine decreased PCP locomotor activity at the 2mg/kg dose level, which is in agreement with a recent study of Garey et.al. (Life Sci. 26:277-287, 1980) who reported an inhibition of PCP motor behaviors with low doses of apomorphine. Ataxia ratings were increased following injections of haloperidol at 2 and 6mg/kg dosage levels. These data indicate that dopaminergic and cholinergic mechanisms are important in the mediation of PCP-induced stereotypy and locomotor activity across a wide range of PCP dosage levels. The inhibitory effects of apomorphine on locomotor activity may be due to presynaptic effects of this drug, as suggested by Garey et.al. (1980). In addition, the enhancing actions of haloperidol on PCP-induced ataxia could be the result of extrapyramidal effects (akinesia) of the drug.

110.12 ALCOHOL EFFECTS ON NEUROELECTRICAL ACTIVITY AND BEHAVIOR OF MONKEYS IN A COGNITIVE TASK. J. M. Fuster, T. J. Willey, D. M. Riley* and J. W. Ashford*. Dept. Psychiatry and Brain Res. Inst., Sch. of Med., Univ. California, Los Angeles, CA 90024.

This study is designed to elucidate effects of ethyl alcohol on electrophysiology of brain structures involved in visual perception and short-term memory. Monkeys were trained in a delayed color-matching task. A trial consists of the following sequence: (1) a white (strobe) flash as an alerting signal; (2) a colored light--the sample, red or green--in a translucid button (which the animal turns off by pressing); (3) a delay of 10 sec; (4) simultaneous appearance of both colors, red and green, in two separate buttons; (5) animal's choice of one color. If the chosen color matches the sample, juice reward is given. The color of the sample and its position in the choice buttons are changed randomly. Thus the animal must perceive and retain through the delay the sample-color. Measures of performance are: (a) number of correct choices; (b) sample reaction time; (c) choice reaction time. Electrodes were implanted for recording EEG from lateral geniculate body, visual cortex, inferotemporal cortex, amygdala, and reticular formation; Ag/AgCl electrodes for recording eye-position. Control saline or ethanol solution (0.25, 0.5, 1.0, and 2.0 g/Kg) was injected intravenously to performing animals with remote Sage pump. Blood alcohol levels were determined. All records were analyzed by PDP-12 computer.

Alcohol induced a performance deficit: increase of matching errors and generally longer and more variable reaction times. Following alcohol injection, a transient lowering of spectral intensity was observed in EEG (Fourier analysis). It was followed by long-lasting increase of spectral intensity to higher levels than before injection. This increase was dose-dependent and affected most markedly frequencies in 0.5-3.5 and 13-24 cps ranges. It was accompanied, especially at highest dose, by cyclical fluctuations of intensity in the four canonical frequency bands. Visual evoked responses (VER's) to the alerting flash were scarcely modified by alcohol, even at high doses. In some locations, notably reticular formation, the amplitude of these responses was increased. By contrast, VER's to sample were diminished in dose-related manner everywhere, in correlation with effects on performance. However, the effect on sample VER's could be seen even at alcohol levels insufficient for performance deficit. In conclusion, alcohol has a general effect on electrical excitability of the neural substrate of vision. Remarkably susceptible to alcohol are central reactions to discrete stimuli that contain critical information for short-term memory.

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110.13 EFFECTS OF PRENATAL EXPOSURE TO METHADONE ON REACTION TO METHADONE AND ENVIRONMENTAL STIMULI. L.D. Middaugh, L.W. Simpson*, T.N. Thomas and J.W. Zemp. Depts. of Biochem. and of Psychiat., Med. Univ. So. Car., Chas., S.C. 29402.

Methadone injected into pregnant rats alters behavior of both developing and mature offspring. The behavioral changes suggest that the offspring are more reactive to environmental stimuli. Other studies have demonstrated that prenatal exposure to narcotics alters the analgesic properties of the drugs after maturity. The present experiments were completed to test the hypothesis that prenatal exposure to methadone alters reactivity to stimulus presentation and to acute injections of the drug.

C57BL/6J mice were injected for the last third of pregnancy, with saline or methadone hydrochloride in doses of 2.5 mg, 5.0 mg or 10 mg/kg. Control dams received equivalent volumes of saline (.01/ml/g). At birth litters were reduced to 6 and reared by their biological mothers until weaning at 24 days of age. The 10 mg/kg dose reduced weight gains during pregnancy by 28% and delayed parturition by 12-24 hours. The two lower doses did not influence these parameters. Litter size and body weight of 21-day-old offspring were unaffected by maternal drug exposure.

Reaction to environmental stimuli was assessed by recording activity prior to, during, and following presentation of a one minute pulsating tone-light complex stimulus. One male and female animal per dam injected with either saline or methadone at 5 mg/kg was tested. Male offspring of both treatment groups had similar patterns of activity with an initial reduction following stimulus presentation and a gradual return to pre-stimulus levels. Female offspring of the two groups, however, differed extensively. Activity of saline offspring changed very little across the entire test having only a slight reduction immediately following termination of the stimulus. Female offspring of methadone dams, however, reduced their activity during the entire period of stimulus presentation and for a short period of time following termination of the stimulus.

Behavioral reaction to methadone (.75 mg or 1.5 mg/kg) of male and female offspring of dams injected with saline or methadone (5 mg or 10 mg/kg) was assessed by determining drug effect on 120 day-old mice lever pressing for food delivered according to a fixed-ratio 20 schedule of reinforcement. Methadone, as previously reported for offspring of untreated dams, disrupted the lever response. Prenatal exposure to methadone, however, did not influence the degree of disruption for either male or female offspring. In summary, maternal injections of methadone at high doses decreased weight gains of pregnant mice and delayed parturition. The treatment did not alter the behavioral response to the drug in mature offspring, however, did increase the reactivity of female offspring to environmental stimuli. (Supported by Grant #01750).

110.14 SPECIFIC INHIBITION OF D-AMPHETAMINE BUT NOT APOMORPHINE-INDUCED STEREOTYPY BY MORPHINE. T.F. Seeger* and K.R. Carlson. Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605.

It is a long-standing hypothesis that morphine can act as a post-synaptic dopamine receptor blocking agent in the striatum, and thereby inhibits the stereotyped behaviors elicited by dopamine (DA) agonists (Puri and Lal, 1973).

However, recent evidence suggests that there are opiate receptors located on the pre-synaptic dopamine axon terminals, suggesting that antagonism of dopamine-mediated behaviors may come about via inhibition of transmitter release (Pollard et. al., 1977).

We have tested the relative inhibitory effect of morphine on the actions of an agonist which releases DA (D-amphetamine), as opposed to a direct DA receptor agonist (apomorphine). In addition, we have tested the effect of the opiate antagonist naloxone on DA agonist-elicited behaviors, in order to determine whether the pre-synaptic opiate system may exert a tonic modulatory influence on dopaminergic activity.

Experimental subjects were male, Sprague-Dawley rats, weighing between 400 and 500 grams at the time of testing. Groups of twelve were given i.p. injections of apomorphine (0.25, 0.50 or 2.0 gm/kg) or D-amphetamine (2.0 or 5.0 mg/kg). Beginning 5 min. after apomorphine injection and 15 min. after D-amphetamine injection, the durations of stereotypic chewing and sniffing behaviors were individually quantified and recorded, during three 2-min. rating periods spaced 10 min. apart. Three days later, the testing was repeated 45 min. after pretreatment with morphine (5 mg/kg i.p.). Three days after this test, the procedure was repeated 15 min. after pretreatment with naloxone (2 mg/kg i.p.)

The most prominent stereotypy at 2 mg/kg D-amphetamine, sniffing, was 45% inhibited by morphine ($p < .001$). At 5 mg/kg, a considerable amount of chewing behavior was seen, which was 72% inhibited by morphine ($p < .002$). In contrast, morphine did not inhibit apomorphine-induced chewing or sniffing at any dose tested. Naloxone had no effect on the stereotypic behaviors induced by either drug.

The results thus provide behavioral evidence for a pre-synaptic interaction of morphine with dopamine neurons in the striatum. In addition, the lack of effect of naloxone argues against a tonic modulatory action by the endogenous opiate peptide system.

110.15 KETAMINE: CONVULSANT OR ANTI-CONVULSANT? Michael S. Myslobodsky, Vladimir Golovchinsky * and Matti Mintz *, Psychobiology Research Unit, Dept. of Psychology, Tel-Aviv University and Dept. of Anaesthesiology, UCLA School of Medicine, Los Angeles, LA 90024

Ketamine hydrochloride has fallen from favor due to seizure phenomena reportedly time-related to its action. However, the ability of ketamine to inhibit the high affinity transport system responsible for the uptake of norepinephrine (Pharmacol. 16:325, 1974) and to accelerate dopamine turnover (Acta Anesth. Scand. 20:216, 1976) may suggest that is has antiepileptic properties.

In experiments with Wistar and Sprague-Dawley rats ketamine alone (doses from 5 to 100 mg kg^{-1}, I.P.) never produced electrographic or clinical epileptiform manifestations.

In doses producing narcotic-cataleptic effects in rats (50-100 mg kg^{-1} I.P.), ketamine reduced the intensity of picrotoxin-induced convulsions and eliminated seizures caused by metrazol (40 mg kg^{-1}) administration. Subcataleptic doses (5-20 mg kg^{-1}) increased the duration of mitigated convulsive symptoms (abortive grand mal fits, jerks) especially those evoked by picrotoxin (4 mg kg^{-1}). Narcotic-cataleptic doses of ketamine considerably increased the duration of the period of single and multiple jerks produced by picrotoxin administration. Animals pretreated with GABA-transaminase inhibitor, γ-acetylenic GABA (100 mg kg^{-1} I.P.) and given ketamine 4-5 hr later, responded to challenging doses of metrazol with lethal status epilepticus. In a dose of 50 mg kg^{-1} ketamine completely antagonized grand mal seizures while myoclonic phenomena were not controlled by this dose of ketamine.

Both convulsants transformed 1-2 Hz "Ketamine complexes" into 2-4 Hz Wave-spike discharges which appeared in a quasi-periodic fashion alternating with periods of relatively suppressed electrocortical activity. Electroencephalographic grand mal patterns were typically dissociated from behavioral manifestations under 50-100 mg kg^{-1} of ketamine, followed by a short period of postictal depression and a rapid recovery of preseizure electrographic patterns. The findings are interpreted as suggesting that mechanisms involved in seizure alleviation may be responsible for sustaining mitigated convulsive phenomena.

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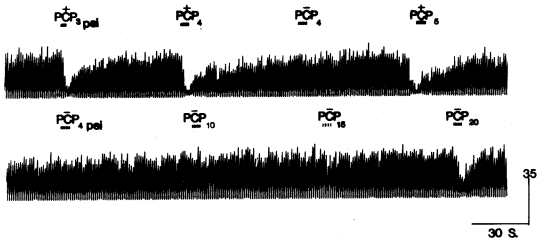
110.16 THE INTERACTIONS OF PHENCYCLIDINE WITH CENTRAL NORADRENERGIC SYNAPSES. M.R. Palmer, J. Marwaha, K. Rice†*, S. Paul†*, P. Skolnick†*, B.J. Hoffer* and R. Freedman*. Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262, and †NIAMDD, Bethesda, MD 20205.

The interactions of locally administered phencyclidine (PCP) with noradrenergic (NE) synapses was studied. The PCP-induced depression of noradrenoceptive cerebellar rat Purkinje (P) cells is reversibly antagonized by agents which block NE input, such as phenothiazine and butyrophenone antipsychotics, and lithium. PCP is ineffective when administered to animals in which central NE fibers were destroyed by 6-hydroxydopamine or in which transmitter release was reduced by local application of Mg^{++}. The dextrorotatory isomer of 3-methyl PCP is 5-10 fold more potent than the levorotatory enantiomer in slowing P cell discharge.



A similar difference in potency is found behaviorally using the rotorod test. After parenteral administration of (+)-3-methyl PCP, the time course of slowing of P cell discharge is similar to the disruption of rotorod behavior. Taken together, these results suggest that the behavioral effects of PCP are, in part, mediated by presynaptic noradrenergic mechanisms. (Supported by USPHS Grant #DA 02429, DA 07043, and MH 00289-01. M.P. has a fellowship from the PMA Foundation and a scholarship from the Reiger Educational Trust.)

110.17 AMPHETAMINE-INDUCED GROWTH IMPAIRMENT IN THE RAT. Wm. J. Pizzi and June E. Barnhart*. Dept. of Psychology, Northeastern Illinois Univ., Chicago, IL 60625.

In recent years a scientific controversy has developed around the use of stimulant drugs in the treatment of hyperactive children. The primary cause for concern is that stimulant drugs have been implicated in the production of growth deficits in these patients. The original studies based on clinical populations have been severely criticized for their methodological inadequacies, especially those concerning sampling. Recent studies on clinical populations have failed to demonstrate growth impairments in children treated with stimulants.

This study was carried out to determine if amphetamine administered to young animals is capable of producing growth impairment. Subjects were 51 Long Evans hooded male rats born in the laboratory and randomly assigned to a control group or one of four drug groups. The drug groups included 0.75, 1.5, 7.5, or 15 mg/kg b.w. amphetamine. These doses were calculated to range from a moderate therapeutic dose in humans to an extremely toxic dose in the rodent. The drug was administered s.c. daily from day 20 to 40 after birth. All animals were autopsied between days 41 and 43, and femur lengths were determined to the nearest .025 inch. An ANOVA of femur lengths resulted in a significant F ($p < 0.01$). A post hoc analysis showed all amphetamine treated groups to have shorter femurs than controls ($p < 0.006$). An analysis of body weights at day 40 failed to show any significant differences when amphetamine-treated animals were compared to their respective control groups. The lack of a significant change in the body weights of amphetamine-treated animals indicates that the growth impairment seen in this study resulted independently of the anorexic effects of the stimulants which might have led to a nutritional deficit. Our tentative working hypothesis is that amphetamine interferes with the normal release of growth hormone in these animals.

The results of this study suggest an immediate need for further study of the growth impairing ability of various stimulant drugs. If future studies verify the results of this research it will also be important to determine if any other neurobehavioral toxicological effects occur following the administration of stimulant drugs to immature organisms.

110.18 EFFECTS OF HIPPOCAMPAL LESIONS ON ALCOHOL DISCRIMINATION IN RATS. H. E. Modrow*, F. A. Holloway and L. D. Devenport (SPON: J. A. Holloway) Dept. Psych. Behav. Sci., Oklahoma University Health Sciences Center, Oklahoma City, OK 73190.

Hippocampal lesions have been shown to produce a wide variety of behavioral impairments (Kimble, J. COMP. PHYSIOL. PSYHCOL. 56:277, 1963). One of us (Devenport, SCIENCE 2-5:712, 1979) has suggested that hippocampal lesions produce a state of behavioral invariance in which the animal has a diminished capacity to change or shift its response rate or topography. Unlike shams, hippocampals do not display response shifts with the introduction of a novel stimulus (Wickelgren and Isaacson, NATURE 200:48, 1963). In our lab, hippocampals have been shown not to exhibit alcohol-induced state-dependency in a runway task (Devenport, Devenport & Holloway, SWPA, 1980). Perhaps the hippocampals were either unable to detect the cue properties of alcohol or were unable to exhibit a behavioral shift in response to the novel state change. To decide between these possibilities, we attempted to train hippocampals to discriminate alcohol from a saline state utilizing a standard operant discrimination paradigm.

Twelve male food-deprived rats (6 shams, 6 hippocampals) were shaped to bar-press on one lever in a two-lever operant chamber, while in the saline state. Operant training in the saline state continued until a stable performance was obtained under a terminal FR30 schedule of food reinforcement. Animals then were placed in the alcohol drug state (1.5 g/kg, 10% w/v, i.p.) for two days and given further training with food reinforcement on the opposite lever. Alcohol-drug discrimination training continued (2 days alcohol, 2 days saline, etc.) until an alcohol discrimination criterion was met. The animals then were given generalization tests with various doses of alcohol every third day in order to obtain an alcohol generalization curve.

The results of this study indicate that although the hippocampal animals learned to bar press slightly more rapidly than the shams in the saline state, they required significantly more sessions to meet the alcohol discrimination criterion. The alcohol generalization curves indicated that hippocampals required a higher dose of alcohol (i.e., nearer the training dose) before they responded to the drug lever. The hippocampals may have been less sensitive to the discriminative properties of alcohol. However, they eventually were able to meet the alcohol discrimination criterion. Thus, the hippocampals' difficulty in learning the discrimination as well as the differences in their alcohol generalization curve also might reflect an inability to shift responding with a change in state.

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113.1 RELATIONSHIP BETWEEN DISCHARGE FREQUENCY AND GLUCOSE UTILIZATION IN VISUAL CORTEX OF CAT AND KITTEN. A. Schoppmann* and M. P. Stryker. Department of Physiology, University of California, San Francisco, CA 94143.

It has been clear for several years that, at least in a general way, increased neuronal firing frequency is associated with increased cerebral glucose utilization as determined by the 2-deoxyglucose autoradiographic technique (Sokoloff et al., J. Neurochem. 28: 897-916, 1977). We have attempted to measure this relationship for cells in several layers of the visual cortex and for animals of several ages.

Cats were prepared for visual physiology in the conventional manner, and were then flaxedilized and artificially ventilated using a mixture of 75% nitrous oxide and 25% oxygen. Horizontal microelectrode penetrations were made through the medial bank of the visual cortex in order to sample the activity of a long sequence of cells within a single cortical layer. Fine electrodes (maximum OD 50 μm) were used in order to minimize disruption of the brain. We isolated single units at intervals of approximately 100 μm and measured both the spontaneous discharge frequency and that evoked by a moving pattern of vertical stripes. Under the conditions of this experiment, these discharge frequencies were fairly stable. Six to eight electrode positions along the course of the 6-8 mm penetration were marked with microlesions. On completing the electrode penetration, we injected 100 μCi/kg 14-C-2-deoxyglucose in order to measure the rates of glucose utilization evoked by the same pattern used for measurement of impulse discharge frequency. Brains were sectioned in the plane of the microelectrode penetration, and the sections, along with radioactive standards, were used to expose X-ray films. Densitometric scans of the course of the microelectrode penetrations on the autoradiographs allowed us to relate discharge frequency to local glucose utilization. The relation is quite steep in adult cats and less so in 5-week kittens.

We have also attempted to measure the loss of resolution that results from our histological and autoradiographic procedures. Brains were labelled using different doses of 14-C-2-deoxyglucose and cryostat sections of known thickness cut from each of these brains. Alternate high- and low-dose sections, still frozen, were assembled into sandwiches. These sandwiches were then cut in a plane perpendicular to the plane of their component sections, picked up on cover glasses, dried conventionally on a 70 degree hot plate, and used to expose X-ray films. Periodic variations in density visible in autoradiographs of sandwiches composed of 20 μm sections suggests that the loss of resolution is no worse than 20 μm. [Supported by NIH and DFG.]

113.2 RETINOTOPIC ORGANIZATION OF THE MACAQUE VISUAL SYSTEM AS DEMONSTRATED BY THE ^{14}C-DEOXYGLUCOSE TECHNIQUE. S.J.Herdman, S.Juliano P.Hand and L.A.Palmer. Depts. of Anat. and Animal Biol., Schools of Med. and Vet. Med. and Inst. of Neurol. Sciences, Univ. of Pa. Phila., Pa. 19104.

The ^{14}C-deoxyglucose technique has an advantage over electrophysiological mapping in that multiple cortical and subcortical visual areas can be studied simultaneously. Four Macaques were used in this study - two blindfolded control animals and two experimental animals; all animals were prepared according to the protocal established by Sokoloff et al ('77). The experimental animals were studied in room light with both eyes open. A moving stimulus of high contrast black and white lines of all orientations was used to activate a specific portion of the visual field.

In the blindfolded control animals, area 17 was faintly labeled only in layers IVc and IVb. By contrast, the portion of the striate cortex which was activated by the background lighting of the room was labeled more heavily in layers IVc, IVb and VI; the other layers were less densely labeled. These findings correspond well with those of Kennedy et al ('76). As one moves into the portion of area 17 driven by the moving stimulus, the labeling becomes markedly denser and, in addition, layer IVa is noticeable labeled. The boundaries of this densely labeled area correspond to the extent of the stimulus and to the electrophysiologically defined organization of area 17 as defined by Gattass et al ('79).

Area 18 in the blindfolded animals is essentially unlabeled except for a faint line in layer IV; in the visually stimulated animal distinct patches of labeling appear, often with a regular configuration which suggests columns. In the portion of area 18 not driven by the moving stimulus, at the 17/18 border there is a decrease in the density of the labeling extending into area 18 as well as into area 17. As one moves into the portion of the area activated by the moving stimulus, the labeling again becomes more dense. The portion of area 18 which is more densely labeled corresponds to the portion of the visual field occupied by the moving stimulus and to the electrophysiological maps of Gattass et al.

The LGNd also shows a difference in the density of the labeling which corresponds to the portion of the visual field stimulated by the moving stimulus and which corresponds to the electrophysiological map of the LGNd by Malpeli et al ('75). We are in the process of analyzing other areas, such as OA, caudal STS, and TE, which appear to be labeled.
(Supported by grants EY05379, RR07083-14, NS5-27301, EY00577)

113.3 A COMPARISON BETWEEN SLEEPING AND WAKING OF SPONTANEOUS AND VISUALLY EVOKED ACTIVITY IN CAT STRIATE CORTEX, EXAMINED BY SINGLE CELL RECORDING AND 2-DEOXYGLUCOSE AUTORADIOGRAPHY. David H. Hubel and Margaret S. Livingstone*. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

We began this study by examining electrophysiologically the influence of waking state on single cells in cat striate cortex. Under halothane anesthesia sleep-deprived cats were implanted with EEG electrodes and a pedestal to hold a microelectrode advancer. All skin incisions were infiltrated with local anesthetic. The trachea was intubated, the animal was paralyzed with intravenous succinylcholine and artificially respirated, and then the anesthetic was discontinued. Single cells were recorded two at a time with tungsten microelectrodes independently positioned by a multiple advancer. Waking state fluctuated spontaneously, or cats could be lulled to sleep or aroused by noise or tactile stimulation. The effects of arousal from slow wave sleep to waking varied from cell to cell but each cell showed a consistent type of change through several sleep/wake cycles. Most often (69/109 cells) an irregular bursty firing during slow wave sleep gave way to a smoother regular firing upon arousal, while the overall rate was unchanged, diminished (sometimes markedly) or occasionally elevated. This confirms previous findings. Visually evoked responses were unchanged (80/109) or enhanced (26/109), only rarely decreased. Usually evoked responses became more obvious against the more regular spontaneous firing. Receptive field properties were generally unchanged. During REM sleep most cells showed very irregular spontaneous activity with high frequency bursts during PGO waves. The spontaneous activity during REM sleep was often so erratic that it obscured any visually evoked response. There were hints that an increase in responsiveness on waking from slow wave sleep was more common in deeper-layer cells. To pursue this further we used the ^{14}C-2-deoxyglucose method in three cats, one awake, one in slow wave sleep, and one in REM sleep, stimulating the left half visual field with vertical stripes. Each brain showed orientation columns on the right. In the awake cat these were densely labeled above and below layer IV, whereas in slow wave sleep the columns were much fainter in the deeper layers than in the superficial layers. The columns in the cat in REM sleep extended through all cortical layers but were less distinct than the columns in the waking animal. (Supported by NIH Grant EY00605).

113.4 DIFFERENCES BETWEEN SLEEPING AND WAKING IN VISUALLY EVOKED CORTICAL ACTIVITY IN THE CAT, MEASURED BY A DOUBLE-LABEL 2-DEOXYGLUCOSE TECHNIQUE. Margaret S. Livingstone* and David H. Hubel (SPON: S. W. Kuffler). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

We have developed a double-label 2-deoxyglucose technique to compare in a single animal the effects of two different stimuli, or a single stimulus under two different conditions. Our immediate objective was to compare visually evoked cortical activity in the cat, awake and in slow wave sleep. Under halothane anesthesia a sleep-deprived cat was implanted with EEG electrodes, intubated, paralyzed with intravenous succinylcholine and artificially respirated. The anesthetic was then discontinued. We waited until the EEG showed normal slow wave sleep, from which the cat could be aroused, then injected a pulse of ^{14}C-deoxyglucose intravenously, and stimulated the left visual field for 45 min. with moving vertical stripes. Then a second pulse, of ^{3}H-deoxyglucose, was followed by 45 min. of right visual-field stimulation during which the cat was kept awake by noise and tactile stimulation as needed. The cat was injected with thiopental, perfused with formalin, and the brain frozen. The heat-dried cryostat sections were exposed first on X-ray film (whose protective coating partially blocks ^{3}H radiation) and then on LKB Ultrafilm ^{3}H, which lacks a protective coating. We determined optimum doses of ^{14}C and ^{3}H to be injected into the cat by exposing the two films to spots of filter paper soaked in graded dilutions of the two labels; such spots were also exposed along with the brain sections to serve as standards for subsequent optical subtraction. On X-ray film the right (^{14}C, asleep) cortex showed much denser orientation columns than the left (^{3}H, awake) while on the unprotected film columns were denser on the left. The two autoradiographs from a given section were subtracted optically by making a negative of one and superimposing its projected image on the original (positive) of the other: the relative intensities of the two images were determined from the autoradiographed filter-paper standards--for example, enough of the LKB negative image was added to the X-ray positive image to make several successive ^{14}C spots in the intermediate density range equal in brightness. At these intensities columns were then seen only in the left hemisphere. Similarly when the process was repeated so as to equalize the ^{3}H spots, columns were seen only on the right. The results confirm our previous findings: columns in the waking state were about equally distinct above and below layer IV, whereas in slow wave sleep they were well labeled in the upper layers but much fainter in V and VI. Slow wave sleep would thus seem to produce a selective decline in activity in layers V and VI of area 17. (Supported by NIH Grant EY00605).

113.5 CYTOCHROME OXIDASE STAIN PREFERENTIALLY LABELS INTERSECTION OF OCULAR DOMINANCE AND VERTICAL ORIENTATION COLUMNS IN MACAQUE STRIATE CORTEX. Jonathan C. Horton* and David H. Hubel (SPON: Edwin J. Furshpan). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Cytochrome oxidase histochemistry has been introduced as a method for mapping oxidative metabolism in the central nervous system (Wong-Riley, 1979). We have examined the pattern of cytochrome oxidase staining in the macaque striate cortex. In coronal section heavier staining was visible in those layers receiving geniculate input: VI, IVC, and an upper tier in IVA. In layers II, III there was a distinct periodic fluctuation in density of reaction product which appeared in tangential section as a network of dark oval patches, roughly 200 × 150 µm. These patches were aligned in rows spaced about 550 µm apart, intersected by rows 350 µm apart. The second, more closely spaced rows resembled ocular dominance columns in spacing, regularity, and by running orthogonal to the 17-18 border. To test this impression we examined cytochrome oxidase staining in a monkey 10 days after damaging one eye. Tangential reconstruction of layer IVC showed dark and light bands arrayed in the characteristic ocular dominance pattern; in layers II, III alternating rows of light and dark patches were in register with the light and dark bands in IVC. Another monkey was stimulated with moving vertical stripes following a ^{14}C-2-deoxyglucose injection. Alternate tangential sections, processed for autoradiography and cytochrome oxidase histochemistry, showed that the rows of patches spaced 550 µm apart matched the pattern formed by the vertical orientation columns. It thus appears that in normal macaque striate cortex, regions of higher oxidative metabolic activity are located at the intersection of the ocular dominance and vertical orientation columns.

Cytochrome oxidase staining of human striate cortex obtained from autopsy material revealed a similar although less regular network of patches in layers II, III. The rows approaching perpendicular to the 17-18 border were spaced about 900 µm apart, corresponding to the width of human ocular dominance columns (Hitchcock & Hickey, 1980). (Supported by NIH Grant EY00605).

113.6 RADIAL ZONES OF HIGH METABOLIC ACTIVITY IN SQUIRREL MONKEY STRIATE CORTEX. A. L. Humphrey & A. E. Hendrickson, Department of Ophthalmology, University of Washington, Seattle, WA 98195.

Hendrickson and Wilson (Brain Res., 170, '79) recently showed that squirrel monkeys had radial zones of increased(^{14}C)-2-deoxyglucose (2DG) uptake in their striate cortices after monocularly viewing a visual scene having many orientations. The radial zones were most distinct in layers II & III; in tangential sections the zones formed separate spots roughly 500µm apart. Layers IVa & IVc were continuously and densely labeled, raising a question as to whether the radial zones above layer IV represented ocular dominance columns or another functional system. We addressed this question in further (14C)-2DG experiments using alert, restrained squirrel monkeys, and we also examined a second metabolic label, cytochrome oxidase (Wong-Riley, Brain Res., 171, '79), in the same animals' cortices.

Radial zones of increased 2DG uptake, roughly 500µm apart, were present in autoradiographs from animals that binocularly viewed black and white stripes (4' to 2^{o} vis. arc) at all orientations. The radial zones still were present, though less distinct, in an animal binocularly stimulated by diffuse light in a ganzfeld, but they were not present in a binocularly occluded animal. In all conditions in which the radial zones were elicited, they were the same in shape, laminar location and spacing.

A laminar and radial pattern of cytochrome oxidase activity virtually identical to the 2DG pattern was present throughout the binocular and monocular regions of normal squirrel monkey striate cortex. Single sections of striate cortex sequentially processed for 2DG and cytochrome oxidase showed that the two labels mark the same spots in cortex. The two labels are dissociable, however, since in the binocularly occluded animal that showed no radial zones of high 2DG uptake radial zones of high cytochrome oxidase activity were present. Preliminary EM indicates that the cytochrome oxidase reaction is labeling mitochondria primarily in postsynaptic, presumably dendritic, processes. These results show that in squirrel monkey striate cortex there are regularly spaced radial zones of high metabolic activity which can be triggered by diffuse light activation of the visual system and which are not dependent on eye dominance, stimulus orientation or spatial frequency for their demonstration.

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113.7 ORGANIZATION OF OCULAR DOMINANCE COLUMNS IN GALAGO DEMONSTRATED BY AUTORADIOGRAPHIC AND DEOXYGLUCOSE METHODS. V. A. Casagrande and L. C. Skeen. Depts. Anat. & Psych., Vanderbilt U., Nashville, TN 37232 and Dept. Psych., Univ. Delaware, Newark, DE 19711

Previous investigators have demonstrated the presence of ocular dominance columns in the striate cortex of the galago using both anterograde degeneration and transneuronal autoradiographic methods (Glendenning et al., '75; Casagrande et al., '77; Hubel and Wiesel, '77). However, while degeneration techniques suggest that input from the two eyes is almost completely segregated in cortex, transneuronal autoradiographic evidence shows a partial segregation of geniculo-cortical terminals which is more pronounced for the ipsilateral cortex. In this experiment, we used two additional methods to determine the degree of segregation of ocular input within the cortex. In the first study, we injected ^{3}H-proline into laminae of the lateral geniculate nucleus (LGN) in galagos under electrophysiological guidance. These animals were sacrificed 24-48 hours later and the brains processed according to standard autoradiographic techniques. In the second study, galagos were given I.V. injections of ^{14}C-2-deoxy-D-glucose (2-DG) and monocularly exposed for 45 minutes to moving stripes at all orientations. Immediately thereafter, the animals were sacrificed, the brains cut, and sections were exposed to X-ray film (Kennedy et al., '76). Laminar injections of ^{3}H-proline produced one or more 300 µ wide labelled patches in layer IV of striate cortex. No differences in patterns of label were observed following injections of ipsilaterally versus contralaterlly innervated LGN layers. Consistent with previous evidence we found that the parvocellular LGN layers (3 and 6) projected to the ventral tier of cortical layer IV, the magnocellular layers (1 and 2) projected to the dorsal tier of layer IV and the small celled layers (4 and 5) projected to layer I (Glendenning et al., '76; Carey et al., '79). Large LGN injections involving all layers produced additional labelled patches in cortical layer III where label was continuous in layer IV indicating that some periodicity of LGN afferents may not reflect ocular segregation. The 2-DG experiments revealed the following results: In layer IV, sharp, alternating 300 µ wide bands of high and low activity were apparent in layer VI. Consistent with the results of the LGN laminar injections, no differences in periodicity were observed between ipsilateral and contralateral retinal pathways. Taken together, our main results suggest that ocular segregation in galago visual cortex may be more complete and more balanced with respect to the two eyes than originally reported.

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113.8 INTERLEAVING PROJECTION BANDS IN CORTICO-CORTICAL CONNECTIONS. Gilbert, C.D. and Wiesel, T.N. Dept. of Neurobiology, Harvard Medical School, Boston, Mass.

Cortico-cortical connections are made between topographically corresponding sites. On a smaller scale, the cells in the superficial layers of a given cortical area that project to a restricted location in another cortical area are distributed in cell-dense patches which are separated by cell-sparse intervening zones (Gilbert and Kelly, 1975). In the present study we have examined the cortical projection patches in greater detail.

Area 17 of the cat's visual cortex projects to area 19, which in turn sends a recurrent projection to area 17. Injecting a small (.05 ul) volume of a 15% solution of horseradish peroxidase (HRP) in area 19 resulted in labeling a large number of cells in area 17 by retrograde transport. These cells were grouped into distinct patches, located approximately 600 micrometers apart. The patches tended to line up, from one coronal section to the next, and when reconstructed from serial sections formed parallel bands running in an anteroposterior direction. The intervening bands also projected to area 19, but to a site adjacent to the site to which the labeled bands projected. This was demonstrated by making large (0.5 ul) injections of HRP in area 19, which resulted in a continuous band of labeled cells in area 17. The recurrent projection from 19 to 17 gave a complementary picture, with patches in 19 resulting from localized HRP injections in 17.

A similar phenomenon was observed in the anterograde direction, between areas 17 and 19. A small patch of one area projected to bands within the receiving area. This was shown by anterograde transport of HRP or of 3H-proline. Thus there was a combination of divergent and convergent connections between bands in areas 17 and 19. Our results suggest that in the projection from 17 to 19 convergence predominates, with a larger number of bands in 17 projecting to a smaller number of bands in 19. Conversely, in the projection from 19 to 17 divergence is predominant. This was not unexpected, since area 17 covers a larger area, has a larger magnification factor, and consequently has many more bands than area 19.

It appeared, furthermore, that the anterograde and retrograde systems of bands were in register, as shown by combining HRP and 3H-proline in a single injection: a given site in one area received input from the bands to which it projected.

This banding pattern of interconnections is a general phenomenon which we observed in several visual cortical areas of the cat and monkey. Thus the columnar system, which is initiated in the cortex by the thalamocortical projection, is repeated in subsequent stages of processing. Supported by NEI grants EY00606 and EY001995 and by a grant from the Medical Foundation.

114.1

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114.3 SACCADIC EYE MOVEMENTS IN PATIENTS WITH DISCRETE UNILATERAL FRONTAL-LOBE REMOVALS. H.A. Buchtel* and D. Guitton (SPON: B. Milner). Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4

In primates, visually-guided saccades can apparently be controlled either by a visual cortex-superior colliculus-brainstem system, or by a frontal cortex-brainstem system (Schiller, P.H. et al., Science, 206: 590, 1979). It seems probable that these two systems usually work together in producing appropriate eye movements. In an attempt to clarify the particular role of eye-movement related mechanisms located in the frontal lobes, we have studied the saccades of patients who have had discrete unilateral removals of frontal-lobe tissue for the relief of intractable epilepsy.

Two tasks were used. In the first task, the patient was asked to look toward or away (anti-saccade) from a tachistoscopically-presented target (small open circle) located at various eccentricities from central fixation. In the second task, the patient was always required to look at the target but on some trials a distractor (a filled circle of the same size) was presented in the opposite visual field. Eye movements were monitored by electro-oculography.

In both tasks, the patients showed disturbances of eye movements that were related to the direction of the required saccade. For example, in the anti-saccade task, they experienced great difficulty in suppressing a reflex-like saccade to the stimulus when it was located in the visual field contralateral to their lesion. When the stimulus was presented to their ipsilateral field, and therefore projected initially to their intact cerebral hemisphere, they were better able to initiate an eye movement in the opposite direction. The data on 6 patients to date suggest that eye-movement related mechanisms of the frontal lobes are useful in inhibiting saccades to inappropriate but visually-attractive stimuli.

114.4 DIFFERENTIAL AFFERENT INPUT TO SUBDIVISONS WITHIN THE FRONTAL EYE FIELDS (AREA 8) OF MACAQUES. H. Barbas and M-M. Mesulam. Harvard Neurol. Unit, Beth Israel Hospital, Boston, MA 02215.

The sources of ipsilateral cortical afferents to subdivisions of the frontal eye fields (Walker's area 8) were studied with horseradish peroxidase (HRP) in macaques. Labeled neurons were noted in: 1- visual association areas of peristriate and inferior temporal cortex; 2- auditory association cortex of the superior temporal gyrus; 3- the ventral bank of the intraparietal sulcus, the inferior parietal lobule, and medial parietal cortex; 4- the depths of the superior temporal sulcus; 5- prefrontal cortex; 6- paralimbic regions of cingulate and retrosplenial cortex.

There were striking differences when the distribution of cells projecting to the caudal part of area 8 was compared with the distribution of cells projecting to its more rostral part. To demonstrate these differences, the number of labeled cells within a specific area was expressed as a percentage of the total number of labeled cortical cells. In a case with HRP injections within the caudal part of area 8, most labeled cells (53%) were in visual association cortices. An additional 23% were in the caudal half of the ventral bank of the intraparietal sulcus, where neurons have predominantly visual and visuo-motor properties. Most other labeled cells were in regions which may be considered high order association areas (the banks of the superior temporal sulcus, and prefrontal cortex). In sharp contrast, when the HRP injections covered the rostral part of area 8, a much lower percentage of labeled neurons (5%) was in visual association areas, with an additional 8% in the caudal ventral bank of the intraparietal sulcus. In addition, there were labeled cells in auditory (21%) and paralimbic (13%) regions. The number of cells in high order association areas (banks of superior temporal sulcus, inferior parietal lobule, medial parietal cortex, and prefrontal cortex) was higher (55%) than in the case with a caudal injection.

The results indicate that most of the cells projecting to caudal area 8 are situated in visual association areas. In contrast, rostral parts of area 8 receive projections from cells that are mostly in high order association areas but also from those in auditory association cortex and paralimbic regions. Differences in neuronal response properties in subdivisions of area 8 may reflect these differences in the source of their cortical afferents. The caudal parts of area 8 may be involved in head and eye movements in response to visual stimuli, while its anterior subdivisions may play a role in directing the head and eyes in response to auditory stimuli. Furthermore, limbic input may also be relevant to the neural processing occurring in the anterior part of the frontal eye fields.

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114.5 AN ANTEROGRADE HRP STUDY OF PREFRONTAL EFFERENTS TO BRAINSTEM OCULOMOTOR CENTERS IN THE MONKEY. G.R. Leichnetz, Department of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.

Using cortical horseradish peroxidase gel implants (Griffin et al 1979), TMB neurohistochemistry (Mesulam 1978) and dark-field microscopy, we have been able to achieve remarkable visualization of anterogradely-labelled corticofugal systems. In particular, the technique has led us to a new appreciation for, and description of, the projections from the monkey prefrontal cortex to several preoculomotor and oculomotor targets in the brainstem which have been directly or indirectly associated with eye movement. These projections not only reach the midbrain and pons through the classically-defined "capsular-peduncular-tegmental" route, but also through the internal medullary lamina (and paralaminar regions of VA and MD) of the thalamus. After principal sulcus and "frontal eye field" (FEF) implants, a prominent bilateral prefrontal-oculomotor bundle was observed in the medial subthalamic region (dorsomedial to the fasciculus retroflexus and ventral to the interstitial nucleus of the MLF) - an area involved in vertical eye movements (Graybiel 1977; Buttner-Ennerver 1978), the bilateral lesion of which produces downward gaze paralysis (Kompf et al 1979). This same bundle was followed caudally into the rostral oculomotor nucleus. Prefronto-tectal projections appeared to be topographically organized. A considerable portion of these, particularly from FEF, reached the midbrain through the "trans-thalamic" pathway, probably giving off fibers (perhaps collaterals) into the intralaminar complex, MDmf, medial pulvinar and pretectum, before terminating in the superior colliculus (SC) and dorsolateral central gray. The capsular-peduncular prefronto-fugal projection traversed the medialmost posterior limb of the internal capsule and medialmost crus cerebri, and thus would not have been interrupted in reported lesions of the internal capsule (Bender and Shanzer 1964) which produced "no defect in gaze." This ipsilateral bundle was followed caudally into the rostral medial pontine reticular formation (PPRF) where some of the fibers decussated. Lesions of PPRF have been reported to produce defects involving both horizontal and vertical gaze. The findings may explain the experiments of Conway and Schiller (1979), demonstrating that combined bilateral FEF and SC lesions in monkeys were required to produce a severe, non-recoverable deficit in visually-triggered saccades, by describing an independent pathway from FEF to oculomotor centers. Thus the efferents of the prefrontal cortex, including FEF, provide several pathways through which a direct cortical influence may be exerted on eye movement mechanisms. Supported by NSF Grant BNS 7822971.

114.7 THALAMIC NEURONS CODING EYE POSITION IN MONKEY. J. Schlag and M. Schlag-Rey. Dept. of Anatomy and BRI, UCLA, Los Angeles, CA 90024.

In the course of microelectrode descents to explore the region of the thalamic internal medullary lamina (IML) in alert monkeys, units firing in relation with eye position were found at the most dorsal aspect of the thalamus. These cells were among the first encountered after leaving the ventricle. By histological control, the majority were located in n. anterior dorsalis; yet some may have been situated in the rostral part of n. lateralis dorsalis or in the capsule formed by the IML around the anterior nuclei. These units discharged with great regularity at rates varying from 0/sec with the eyes near the center in the orbit to 150/sec for gaze deviations of 35 degrees. All had a horizontal preferred direction, 93% contraversive, 7% ipsiversive. Stable rates were maintained sometimes for several seconds at any eye position along the axis of preferred direction. The activity was the same in complete darkness as in light or in presence of discrete photic stimuli. The frequency was modulated as a function of eye position when the animals followed a sinusoidally moving target. Changes of firing levels occurred abruptly in the course of saccades, with no bursts or pauses. The exact timing of the changes seemed to depend primarily on the prior eye position. With respect to the saccades, the latency was shortest when the eyes were already deviated toward the preferred direction before moving further in this direction. In such cases, changes of firing could even precede saccades. In contrast, changes of firing were delayed after saccades starting far away from ipsilateral sites and not reaching much further than the midline. As for neurons of the cortical frontal eye field described by Bizzi, the time relations with eye movements neither support an hypothesis of motor command nor one of proprioceptive feedback.

Further down in the same tracks, other cells were found in the IML itself, which also discharged as a function of eye position. In general, their firing pattern was less regular, and bursts or pauses accompanied saccades, especially in the on- or off-direction of the cells.

These new observations add evidence on the existence of cell populations involved in gaze control in the medial thalamus. Such populations seem to expand further than the boundaries of the IML itself. (Supported by USPHS Grant EY 02305).

114.6 SACCADE-RELATED NEURONS IN PRIMATE THALAMUS. M. Schlag-Rey and J. Schlag. Dept. of Anatomy and BRI, UCLA, Los Angeles, CA 90024.

Previous research has revealed the existence of saccade-related (S-R) neurons in the internal medullary lamina (IML) of the thalamus in cats. The present experiment was undertaken to determine if such neurons exist in primates. Four Macaca Nemestrina were trained to fixate stationary and moving targets presented in dim red light or complete darkness. Between conditioned trials, single unit activity was studied in relation to spontaneous saccades, monitored by DC electro-oculography. Histologically reconstructed microelectrode tracks explored an area extending frontally from planes F 4.5 to F 8.7 (Olszewski), laterally from 2 to 6 mm from the midline and down from the floor of the third ventricle to 4 mm below.

In and above the IML region, three general types of unit firing were found specifically related to saccades in complete darkness as well as in light. 47% of S-R units discharged before and during saccades in light or dark, with or without triggering stimuli. Most of these units showed a sharp tuning in their on-direction. Vertical-oblique preferences were more frequent than purely horizontal ones in our restricted sample of IML neurons. The majority of presaccadic units were of the long lead burst variety, with lead time often exceeding 250 ms. Quantitative analyses of the discharges revealed that in many cases a position component was mixed with the velocity or amplitude component in the preferred direction. These units were found mainly in n. centralis superior lateralis and the upper part of the IML.

41% of the S-R units, typically found in n. lateralis dorsalis stopped firing during saccades. Thalamic omnipausers differed from brain stem and cerebellar omnipausers by the timing of the pause which often led and outlasted saccades and by the presence of rebound activity. Thus, there were pause-rebound and rebound-only types. While pauses were omnidirectional in all cases, rebounds sometimes were not.

The remaining 12% of the S-R units were seen active only during (not before) saccades and were scattered in the region explored. These results support the hypothesis that the medial thalamus, in particular the IML region, is involved in the control of gaze, in primates as well as in cats. (Supported by USPHS Grant EY 02305).

115.1 NALOXONE-SENSITIVE EEG AND BEHAVIORAL EFFECTS OF ECS IN RATS. F.C. Tortella, E.F. Berman*, A. Cowan, M.W. Adler, G.L. Belenky and J.W. Holaday, Dept. of Pharmacology, Temple University School of Medicine, Philadelphia, Pa. 19140 and Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012.

Electroconvulsive shock (ECS) produces opiate-like effects in rats which are attenuated by naloxone (Holaday et al, Soc. Neurosci. Abstr. 4: 409, 1978). In the present study we characterized the EEG and behavioral effects of ECS and provide additional evidence for a functional role of opioid peptides in postictal events.

Male S.D. rats (250-300 g) were prepared with chronic cerebrocortical electrodes. Three to five days after surgery, the rats were placed in individual recording chambers and allowed to acclimate and move freely while control EEG recordings were taken. Rats were subjected to transauricular ECS (0.2 sec, 50 mA, 60 Hz) and the effects of electroshock studied after (a) single shock (acute ECS) and (b) following 10 consecutive once-daily shocks (chronic ECS). ECS produced a generalized convulsion marked by a polyspike EEG seizure. The seizure was followed by a period of postictal depression (PID) characterized by EEG high-voltage synchrony. During the postictal period following acute ECS, quantitation of the EEG voltage output (V.O.) revealed a significant increase in power to 27 ± 7% (S.E.) above control. The duration of the associated PID was 2675 ± 658 sec. Chronic ECS increased the EEG V.O. and duration of PID 55 ± 16% above control and 3310 ± 697 sec., respectively. This represented a statistically significant increase compared to the acute ECS response. Pretreatment with naloxone (0.3-10 mg/kg, s.c.), 10 min before acute ECS, antagonized the postictal increase in EEG V.O. and duration of depression, implicating involvement of endogenous opioid peptides. Naloxone did not markedly alter the EEG or behavioral pattern of the seizure itself, however, nor did it (3 and 10 mg/kg, s.c.) significantly antagonize the chronic ECS responses. Possible explanations for these effects are currently being investigated.

The ECS-induced EEG and behavioral changes in the rat reported here are similar to those seen after the icv administration of morphine and opioid peptides (Tortella et al, JPET 206: 636, 1978). Furthermore, the postictal events appear analogous to the EEG synchrony and depression reported in man following generalized seizures. ECS in the rat thus appears to be a potentially useful model for the study of the interrelationships of endogenous opiate peptides and seizure events.

This work was supported by Grant DA 00376 from NIDA.

115.2 BEHAVIORAL EFFECT OF β-ENDORPHIN (β-EP) AND NOREPINEPHRINE (NE) IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN). S. F. Leibowitz and L. Hor*. The Rockefeller Univ., New York, NY 10021.

Hypothalamic NE injection in the rat is known to elicit feeding through α-adrenergic receptor stimulation. The most sensitive site for observing this phenomenon is the PVN, a brain area which receives a dense innervation of noradrenergic fibers. Evidence from this laboratory suggests that NE release from these fibers mediates the increased feeding observed with local injection of tricyclic antidepressants and also possibly contributes to the increased feeding observed after glucoprivation. In line with this latter suggestion is the finding that noradrenergic feeding is abolished by hypophysectomy and adrenalectomy and is specifically related in magnitude to circulating levels of glucocorticosteroids.

The relationship of this noradrenergic system to brain endorphins was examined in the present study, in which male Sprague Dawley rats (350 g) were stereotaxically implanted with chronic brain cannulas aimed at the PVN. All drugs were injected directly into the PVN through this cannula, and the rats' eating behavior (of lab chow pellets) was measured over the next 2 hr. During this period, satiated rats, after injection of the saline vehicle, generally ate relatively little, approximately 0.6 g. Injection of NE into the PVN produced a significant feeding response of 2.7 g, with a latency of 1 to 2 min and a duration of 15-20 min. Injection of β-EP (1 nmole) also elicited a reliable feeding response (2.0 g), although the latency of this response was approximately 30 min and sometimes longer. (Also see Grandison and Guidotti, 1977.) The magnitude of the NE and β-EP feeding responses in these rats was significantly correlated (r = +.75), suggesting a possible relationship between these two phenomena.

Further support for this relationship was obtained with the α-adrenergic receptor blocker phentolamine (60 nmoles) which inhibited the β-EP feeding response as well as the NE response. This contrasts with the dopamine receptor antagonist fluphenazine (10 nmoles), which had no effect on either response. While this suggests that β-EP may ultimately act through endogenous noradrenergic neurons to alter behavior, evidence obtained with opiate antagonist naloxone (100 and 200 nmoles) indicates that β-EP's immediate action involves local opiate receptors. PVN injection of naxolone abolished the eating response of β-EP but had no effect on NE's action or on spontaneous feeding in hungry rats.

This evidence reveals a possible relationship between opiate and noradrenergic neurons within a discrete region of the hypothalamus. This relationship may broaden our understanding of the neurochemical mechanisms through which this brain area operates physiologically to maintain and achieve energy homeostasis, during normal and perhaps stressful conditions. (Research supported by MH 22879 and grant from Whitehall Foundation)

115.3 EFFECTS OF SYSTEMIC ADMINISTRATION OF α-ENDORPHIN (β-LIPOTROPIN 61-76) ON INTRACRANIAL SELF-STIMULATION IN THE RAT. George T. Bain*, Ralph U. Esposito and Conan Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118

This report concerns an attempt to determine if systemically administered α-endorphin (β-lipotropin 61-76) would affect intracranial self-stimulation behavior in the rat. Subjects were implanted with electrodes aimed at the ventral tegmentum and intracranial reinforcing thresholds were determined by means of a modification of the classical psychophysical method of limits (Esposito and Kornetsky, Science 195:189, 1977). Alterations of reinforcing thresholds were observed after microgram doses (25-75 µg/kg) of the peptide were administered subcutaneously. Both significant increases and decreases of threshold occurred, dependent on dose and time after administration. These threshold effects occurred within 1.5 hours after injection and were not accompanied by any gross behavior changes. The findings demonstrate that systemically administered α-endorphin induces specific alterations in central reward processes.
(Supported by NIDA Grant DA 02326 and NIMH Grant MH 12568 and Research Scientist Awardee MH 1759 - CK).

115.4 INDUCTION OF MATERNAL BEHAVIOR FOLLOWING INTRACEREBROVENTRICULAR ADMINISTRATION OF POSTERIOR PITUITARY HORMONES. C. A. Pedersen*, and A. J. Prange, Jr.* (SPON: G. H. Greeley, Jr.). Biol. Sci. Res. Center and the Neurobiology Program, Univ. North Carolina School of Medicine, Chapel Hill, North Carolina 27514.

Since oxytocin (OXY) is released during birth and nursing, we tested, in virgin female Sprague-Dawley rats, the possibility that this nanopeptide would induce maternal behavior.

Informal experiments revealed that OXY (0.4 µg) given through a lateral ventricular cannula often produced maternal behavior. In all subsequent experiments this route of administration was employed. Behavior was assessed by observers ignorant of treatments.

Intact rats were given 0.4 µg OXY in normal saline (n=36), or normal saline alone (10 µl) (n=12) and immediately placed in individual cages containing nest material and three pups. Quantitative criteria in all of five behavioral categories (grouping, licking, crouching, nest building, retrieval) had to be fulfilled by an animal within two h of injection for its behavior to be considered fully maternal. Fifteen (42%) of OXY injected animals showed full maternal behavior while none of the saline treated animals displayed this effect.

Estrogen dependence of the OXY effect was suggested by the observation that, with one exception, all animals that responded fully maternally to OXY injection (n=15) were in the last day of diestrus, or in proestrus or estrus, when estrogen levels are rising, elevated, or recently elevated respectively. To test the possibility that estrogen is necessary to permit OXY-induced maternal behavior, a number of cannulated virgin females were ovariectomized (OVX). Immediately after operation 27 animals were injected sc with 100 µg/kg of estradiol benzoate (EB) in corn oil (0.1 ml) or corn oil vehicle alone. Forty-eight h later all subjects received 0.4 µg of OXY icv. Eleven of 13 EB primed virgins and none of 14 nonprimed virgins displayed full maternal behavior.

Specificity of the OXY effect was investigated by administering either 0.4 µg OXY or equimolar quantities of other hormones into OVXed, EB primed virgins. OXY produced a 76% rate of full maternal behavior (22/29) while arginine vasopressin induced a 52% response rate (11/21). Lysine vasopressin as well as a large number of peptides, steroids, and prostaglandins were no more effective than saline in inducing maternal behavior.

Dose-response relationships were studied in rats given 100 µg/kg EB at OVX, followed 48 h later by doses of OXY between 0.1 and 0.4 µg. The incidence of full maternal response was linearly related to log dose OXY.

This work was supported by NIMH MH-32316, NH-22536, MH-33127 and NICHHD HD-03110.

115.5 BEHAVIORAL EFFECTS OF PERIPHERAL ADMINISTRATION OF ENDORPHINS: MEMORY OR MOTIVATION? M. Le Moal, O. Gaffori*, J. Rossier, N. Ling*, G. F. Koob, and F. E. Bloom. Laboratoire de Neurobiologie des Comportements, Université de Bordeaux II, Bordeaux, FRANCE, and A. V. Davis Center for Behavioral Neurobiology, Salk Inst., San Diego, CA, 92138.

Subcutaneous injections of 1 to 10 µg per rat of alpha (β-LPH_{61-76}) and gamma(β-LPH_{61-77}) endorphin significantly altered the extinction of aversively and appetitively motivated learning. Alpha endorphin, when injected immediately after the first 10 trials of extinction of an active (pole jump) avoidance task,significantly prolonged extinction on subsequent trials. When injected 1 hour prior to the test trial of a passive avoidance task, alpha endorphin significantly inhibited extinction, but facilitated extinction when injected immediately after the training (shock) trial. In contrast gamma endorphin, when injected immediately after the first 10 trials of extinction of the active avoidance task, significantly facilitated extinction on subequent trials, and when injected 1 hour prior to the test trial of a passive avoidance task, facilitated extinction. Des-tyr^1-gamma (β-LPH_{62-77}) endorphin at doses of 1 to 10 µg per rat failed to alter the extinction of either active or passive avoidance. Both alpha and gamma endorphin, when injected after the first extinction trial of a water- motivated runway task, significantly delayed extinction on subsequent trials. These effects were not reversible by pretreatment with naloxone (5 mg/kg). Measurements of alpha endorphin in the blood and brain with radioimmunoassay following subcutaneous injection revealed measurable levels in the blood at 5 and 10 min. post- injection, but virtual dissappearance after 20 minutes. No detectable levels were found in the brain at any time post- injection. Results confirm that the peripheral administration of minute amounts of endorphins can produce significant behavioral effects, but suggest that these effects are independent of a memory substrate. In addition, the results fail to confirm a behavioral role for des- tyr^1- gamma endorphin. Thus, it appears that the endorphin analogs, alpha and gamma endorphin, act independently of metabolism via removal of the N- terminal tyrosine and act independently of traditional opiate receptors to produce subtle motivational effects.

115.6 DIFFERENTIAL EFFECTS OF MET- AND LEU-ENKEPHALIN ON BEHAVIOR: NALOXONE REVERSIBILITY AND THE DEVELOPMENT OF TOLERANCE. Henk Rigter and Joe L. Martinez, Jr. CNS Pharmacology Department, Organon, Oss, The Netherlands, and Department of Psychobiology, University of California, Irvine, CA 92717 USA.

We have previously shown that Met- and Leu-enkephalin (M-ENK and L-ENK) impair acquisition of an active avoidance response when injected i.p. before training (Rigter et al., Life Sciences, 1980, 26, 337-345). The purpose of the present study was to further characterize the nature of the opiate receptor mediating this response by studying: 1) several opioid agonists, 2) naloxone reversibility of the enkephalin effect and 3) the development of behavioral tolerance by repeated administration of enkephalins.

Male Wistar rats were trained to avoid electric shock by stepping on a platform in the middle of a conditioning box. Shock (0.3 mA) was turned on 10 sec after the rat was placed in the apparatus unless the rat made an avoidance response by stepping on the platform. Rats were trained in a single 10-trial session and drug treatments were given i.p., 5 min before training.

In the first series of studies we found that µ agonists, such as etorphine and morphine, did not affect acquisition of the avoidance response. Also, long-chain endorphins, such as α-, β-, and γ-endorphin, were inactive. Thus, the behavioral effect of enkephalins is probably not due to an interaction with µ opiate receptors. We feel that enkephalin effects on avoidance conditioning are related to δ opiate receptor activity since D-Ala-D-L-ENK was active at a dose level 10-100x lower than the lowest effective dose of L-ENK (Rigter et al., 1980).

In order to further investigate this question we attempted to block the enkephalin effects with naloxone. $ACTH_{4-10}$ was included in this study because pilot work had shown it impaired acquisition at equivalent doses to the enkephalins. It was found that 10 µg/ kg of L-ENK, M-ENK and $ACTH_{4-10}$ given i.p., 5 min before training significantly impaired acquisition. Naloxone (10 mg/kg), given as a cocktail with the peptides, reversed the effect of M-ENK, but failed to antagonize the effect of L-ENK. Surprisingly, naloxone potentiated the behavioral effect of $ACTH_{4-10}$. Thus, it is possible to differentiate between the behavioral effects of different classes of peptides by examining naloxone reversibility.

Tolerance studies also differentiated between M-ENK and L-ENK. While no tolerance developed to the effect of M-ENK during 7 treatments with the peptide, tolerance to L-ENK did develop. The results point to the existence of complex physiological differences between related peptides.

115.7 ENKEPHALIN AND $ACTH_{4-10}$ EFFECTS ON ACTIVE AVOIDANCE CONDITIONING ARE ATTENUATED BY ADRENAL DEMEDULLATION. Joe L. Martinez, Jr. and Henk Rigter. Department of Psychobiology, University of California, Irvine, CA 92717 USA, and CNS Pharmacology Department, Oss, The Netherlands.

Previous research has shown that small amounts of systemically administered Leu-enkephalin (Rigter et al., Life Sciences, 1980, 26, 337-345) and $ACTH_{4-10}$ (Rigter and Martinez, this meeting) impair acquisition of an active avoidance response. Other findings showing both Leu- and Met-enkephalin immunoreactive material in chromaffin granules of adrenal cells suggested that the adrenal medulla may be important for enkephalin effects on avoidance conditioning (Schultzberg et al., Neuroscience, 1978, 3, 1169-1186; Viveros et al., Molecular Pharmacology, 1979, 16, 1101-1108). Thus, we investigated whether Met- or Leu-enkephalin impaired acquisition of an avoidance response in adrenal demedullated (ADXM) rats. Also, we included $ACTH_{4-10}$ in this study because its behavioral actions in this task are similar to the enkephalins.

Male Wistar rats, either ADXM or sham operated (SHAM), were trained to avoid footshock (0.3 mA) in a step-up active avoidance task. Ten µg/kg of either Leu- or Met-enkephalin, or $ACTH_{4-10}$ given i.p. 5 min before training significantly reduced the number of avoidance responses made by the SHAM groups in comparison to saline-injected animals. Peptide-treated ADXM rats did not differ from saline-treated controls. Thus, adrenal demedullation abolished the effects of the peptides.

In a further experiment, we examined whether in ADXM rats increasing the dose of either Leu- or Met-enkephalin would restore their behavioral actions. ADXM or SHAM rats were administered either 100 or 1000 µg/kg Leu- or Met-enkephalin i.p. and trained as described previously. Both the 100 and 1000 µg/kg dose of Leu-enkephalin impaired acquisition of the avoidance response in ADXM rats, whereas neither dose of Met-enkephalin affected the response in ADXM animals.

These results suggest that the behavioral effects of enkephalins and $ACTH_{4-10}$ in this task may be related to peripheral mechanisms. The fact that adrenal medullectomy shifts the dose response function for Leu- but not Met- enkephalin suggests that their behavioral actions are related to distinct physiological processes.

This research was conducted while JLM was a Postdoctoral Fellow at Organon International, B.V.

116.1 NON-ENDORPHIN PATHWAYS SUPPRESS PAIN IN HUMANS. J.B. WALKER* AND R.L. KATZ, DEPARTMENT OF ANESTHESIOLOGY, UCLA SCHOOL OF MEDICINE, LOS ANGELES, CALIFORNIA 90024.

Subcutaneous electrical stimulation (20 HZ) of median, radial, and saphenous nerves produces prolonged analgesia. This effect of subcutaneous nerve stimulation (SCNS) is not mediated by opiate receptors: (1) there is no cross-tolerance with opiates, and SCNS suppresses pain in patients who have received chronic administration and meperidine every two hours for months (2) patients unresponsive to morphine benefit from SCNS (3) there is no tolerance to SCNS, and (4) SCNS, can be administered with oral analgesics (both opiate and non-opiate) to increase patient comfort. These observations suggest that non-endorphin pathways produce powerful analgesia.

A follow-up of patients treated for surgically documented gynecological pain (endometriosis) shows them to be pain free 5 years after cessation of treatment, as measured by verbal report, attendance at work, and analgesic abstinence. To our knowledge, this represents the first such long term follow-up on the clinical pain literature.

Although the mode of action of SCNS is unknown, it decreases the excitability of spinal reflexes. In a double blind study, SCNS suppresses clonus in spastic patients for three hours. The effect is contralateral so that each patient can serve as his own control. Suppression is not mediated by opiate receptors because it is not mimicked by opiates nor is it naloxone-reversible. After multiple treatments, clonus suppression occurs for months. We are currently investigating the neurochemical basis for the long duration of SCNS-induced alterations in neural excitability.

116.2 CORTICAL PROJECTIONS TO THE PERIAQUEDUCTAL GRAY IN MONKEYS: A POTENTIAL ROUTE FOR INFLUENCING NOCICEPTION. S.G.P. Hardy* and G.R. Leichnetz (SPON: J.A. Astruc). Dept. of Anatomy, Med. Col. of Va., Richmond, VA. 23298.

Numerous behavioral manifestations have been associated with the periaqueductal gray (PAG). In recent years the PAG has been shown to participate in analgesic mechanisms. In order to assess the potential influence that the cortex may exert upon this structure a study was made of the cortical efferents to the PAG in the monkey using the horseradish peroxidase (HRP) technique. In a series of Old and New World monkeys, including Macaca fascicularis and Cebus albifrons, stereotaxic HRP fluid injections (0.01-0.05 µl 25% HRP in sterile saline) were made into the PAG. HRP gel implants were subsequently made in various regions of the cerebral cortex. Fluid injection cases were studied with regard to retrograde transport and gel implant cases were used to study orthograde transport. After survival periods of 24-48 hrs. the monkeys were processed according to the tetramethylbenzidine protocol (Mesulam 1978) and sections were studied under bright and dark field microscopy. Results from this study indicate that the prefrontal cortex projects significantly to the PAG, whereas few cortical areas outside the frontal region are involved. The prefrontal-PAG projection arises primarily from small lamina V pyramids (8-15 µ) in the rostral medial prefrontal and dorsal convexity cortices. Other areas of the prefrontal cortex also contributed projections to the PAG, with the exception of the medial orbitofrontal cortex. Prefrontal-PAG fibers were found to terminate primarily in the dorsolateral aspect of the PAG, an area which has been shown to receive nociceptive input via the spinothalamic tract (Mehler 1969). In view of the heavy termination in this precise PAG region we are led to speculate that prefrontal-PAG projections may influence nociception by acting either directly or indirectly upon the spinothalamic system.

116.3 DISRUPTION OF STIMULATION-PRODUCED ANALGESIA BY LESIONS OF THE NUCLEUS RAPHE MAGNUS. J. Timothy Cannon, Guadalupe J. Prieto*, and John C. Liebeskind. Dept. Psychol., UCLA, Los Angeles, CA 90024.

The n. raphe magnus (NRM) has been proposed as a relay carrying pain inhibitory messages from the periaqueductal gray matter (PAG) to the spinal cord. Supporting this view, it was shown, for example, that NRM lesions disrupt analgesia caused by glutamate injections in PAG (Behbehani and Fields, 1979). However, we found that NRM lesions between the inferior olive and the trapezoid body fail to alter analgesia from PAG electrical stimulation (Prieto et al., 1979). In subsequent work, we have extended our efforts in two ways: 1. We find that distinct loci exist within and near PAG, stimulation of which causes analgesia that is differentially sensitive to naloxone blockade. Thus, analgesia from stimulating in or just below the dorsal raphe n. is reliably blocked by naloxone, but that deriving from more dorsal PAG sites is not (Cannon et al., 1980; Prieto et al., 1979). In the present work, we have studied the naloxone-sensitive ventral sites. 2. Recent anatomical studies (Basbaum et al., 1978; Watkins et al., 1980) show a cluster of NRM cells extending over the decussation of the trapezoid body that went undamaged in our previous work. Consequently, lesions were modified in the present study to include these cells.

Rats were implanted with bipolar stimulating electrodes in or just below dorsal raphe n. One week later, current thresholds were established for inhibition of the tail-flick response to radiant heat (7 sec cutoff) by use of a modified ascending method of limits. Brain stimulation consisted of constant current, biphasic pulse pairs (20/sec). Each pulse was 50 µsec in length with a 100 µsec interpulse interval. Stimulation was delivered for 10 sec prior to and during each tail-flick trial. Following threshold determinations, animals received one of the following NRM lesions: 1. On midline between trapezoid body and inferior olive; 2. As (1) but bilateral and displaced .5 mm off midline; 3. As (1) but with a second lesion involving NRM cells above trapezoid body. One week later, analgesic thresholds were determined as before.

All animals sustaining NRM damage from the level of the inferior olive through the region of the trapezoid body exhibited large elevations in analgesia thresholds ($n = 6$, mean = 97%, $p < .05$). No other lesion produced comparable effects in any animal ($n = 25$). Preliminary evidence indicates that these same NRM lesions do not disrupt analgesia from more dorsal PAG placements, those yielding analgesia insensitive to naloxone blockade. (Supported by NIH grant NSO7628 and CONACYT-Mexico)

116.4 ANALGESIA PRODUCED BY MICROSTIMULATION IN THE MEDULLA OF THE RAT. G. Zorman*, I.D. Hentall, J.E. Adams* and H.L. Fields. Depts. of Neurosurgery, Neurology and Physiology, Univ. of California San Francisco, CA 94143.

Extensive evidence implicates the ventromedial medulla in opiate and stimulation produced analgesia (SPA). In the rat, the nucleus raphe magnus (NRM) and adjacent reticular formation ventral to reticularis gigantocellularis (Rgc) are rich in enkephalin fibers and perikarya, and contribute to medullospinal pathways. This ventral region includes the reticularis magnocellularis and pars α, collectively referred to as the nucleus reticularis paragigantocellularis (NRPG). Inhibition of nociceptive reflexes results from opiate microinjection and electrical stimulation of these sites; however, there is disagreement about whether NRM or NRPG is the more effective site. To resolve this issue, we systematically mapped the rat medulla using low-intensity monopolar microstimulation. No marked difference between NRM and NRPG was observed. Male Sprague-Dawley rats (290-340g) were anesthetized initially with 70 mg/kg pentobarbital i.p.. 1µ tip stainless steel stimulating electrodes were stereotaxically placed. Tail-flick latency to noxious heat was used as the measure of analgesia. After stable baseline latencies ($\leq$4sec) were obtained, conditioning medullary stimulation (50Hz, 400µ sec continuous pulse trains) was applied for 5 sec before and during application of noxious heat. Stimulation was considered effective if the tail remained motionless for 10 sec (cut-off). Threshold current for SPA was determined in each animal at 9 sites in a 3x3, transverse grid. In each experiment iron was deposited at three sites, allowing reconstruction of the grid from Nissl-stained sections. The lowest threshold (5-10µA) sites for SPA were found only in NRM and NRPG. Current intensities $\geq$20µA were necessary for tail-flick suppression from other regions including Rgc. Low threshold SPA from NRM and NRPG was naloxone reversible (5 mg/kg i.p.), an effect which usually had a 5 min onset and 20 min duration. Raising stimulus intensity to 20µA at these sites could reestablish SPA. The threshold current values make it unlikely that SPA from microelectrode placements in NRPG is due to current spread to NRM, and the converse is also true. Thus both NRM and NRPG can elicit SPA. Since naloxone reversibility was demonstrated in NRM and NRPG, both nuclei may produce effects mediated by endogenous opioids. Tail-flick suppression from Rgc was obtained only at stimulation intensities $\geq$20µA and was not blocked by naloxone. This suggests that Rgc suppresses tail-flick by a non-opioid mechanism. These results support the concept that NRM and NRPG but not Rgc contribute to an opioid-mediated descending system controlling spinal pain transmission neurons.

116.5 ANALGESIC RESPONSES TO STRESS AND OPIATES FOLLOWING SELECTIVE DESTRUCTION OF EITHER CATHECHOLAMINERGIC, SEROTONERGIC OR SUBSTANCE P PATHWAYS. R.J. Bodnar, M.M. Wallace, J.H. Kordower*, A. Kirschgessner*, D. Simone*, K. Merrigan*, J. Scalisi* and M. Lattner*. Dept. of Psychology, Queens College, C.U.N.Y., Flushing, NY 11367, U.S.A.

Extensive evidence suggest that the analgesic responses to opiates and electrical stimulation of the periaqueductal gray are mediated by an interaction of endorphinergic and serotonergic descending systems which inhibit nociceptive afferents in the dorsal horn. Moreover, acute exposure to a number of stressful events results in a transient analgesia. While certain stressors, such as 2-deoxy-D-glucose (2-DG)-induced glucoprivation share common analgesic characteristics with opiates, other stressors, such as cold-water swims (CWS), appear to act independently of this system, and rather rely in part upon the hypothalamo-hypophyseal axis for their effects. To determine further the properties of each of these analgesic manipulations, separate experiments investigated whether morphine, CWS and 2-DG analgesia were affected by a) systemic administration of parachlorophenylalanine (PCPA - 350 mg/kg), b) bilateral lateral hypothalamic (LH) lesions, c) bilateral locus coeruleus (LC) lesions, and d) intraventricular capsaicin injections. PCPA lowered basal pain thresholds, attenuated morphine analgesia, but did not alter CWS or 2-DG analgesia 48 hours after injection. LH lesions, which produced aphagia for two post-operative weeks, failed to alter any of the analgesic responses following recovery of normal eating patterns. By contrast, LC lesions diminished the analgesic responses to morphine and both stressors. Finally, intraventricular capsaicin, which produces hyperalgesic responses when applied intrathecally, did not affect basal pain thresholds or morphine analgesia, but lowered substantially CWS and 2-DG antinociception. Data will be discussed in terms of heterogeneous mechanisms modulating pain-inhibition. (Supported by NIH Grant NS 14449, NYSHRC Grant 1518 and NIH GRSG 5-S05-PR-07064.)

116.6 ENHANCEMENT OF STRESS-INDUCED ANALGESIA BY ADRENALECTOMY IN THE RAT. M. Glusman, R. J. Bodnar, A. Mansour* and D. D. Kelly. Dept. of Psychiatry, Columbia Univ., and N.Y. State Psychiatric Institute, New York, NY 10032.

We previously reported that stress-induced analgesia (SIA) was greatly attenuated by hypophysectomy (1); and further, that it was dependent on the anterior pituitary (2). The possibility, however, was not excluded that the reduction of SIA was not a primary effect due to loss of anterior pituitary function, but a secondary effect of hypophysectomy in impairing adrenal function.

To test this possibility we investigated the effect of adrenalectomy on SIA in 18 adrenalectomized (ADX) rats and 16 sham operated (SADX) controls. Basal pain thresholds, measured by flinch-jump and tail-flick were not significantly different between the ADX and SADX groups. Cold water swims at 2°C for 3.5 min (CWS) significantly elevated flinch-jump thresholds for both groups; but the elevations for the ADX animals were significantly greater than for the SADX controls. Naloxone 10 mgm/kilo 5 min pre-stress did not significantly alter the responses of either the ADX or SADX animals. CWS similarly increased thresholds for tail-flick; again,the increase was greater for the ADX than for the SADX animals although not statistically significant. The ADX animals also responded with greater SIA than the SADX animals to a second stressor, foot-shock. At 1.0 mA for either 20 sec or 5 sec, foot-shock produced significantly greater analgesia, measured by tail-flick, in the ADX animals than in the SADX controls. Naloxone 10 mgm/kilo did not significantly decrease SIA in the ADX animals, although it reversed the analgesia produced by 3 mg morphine in both the ADX and SADX animals.

Our study demonstrated that SIA following cold water swims or foot-shock is greater in adrenalectomized rats than normals (3). Further SIA in both groups was not mediated by endogenous opioid mechanisms because it was not significantly decreased by Naloxone. Taken with our earlier findings (1,2) this study supports the conclusion that SIA is mediated by endocrine mechanisms involving the anterior pituitary not the adrenals. The enhancement of SIA in the adrenalectomized animals probably reflects compensatory responsivity of the anterior pituitary following adrenalectomy.

(1) Bodnar, R.J., Glusman, M., Brutus, M., Spiaggia, A. and Kelly, D.D. Physiol. Behav. 23:53-62, 1979. (2) Glusman, M., Bodnar, R.J., Kelly, D.D., Sirio, C., Stern, J., and Zimmerman,E.A. Soc. Neurosci. Abstr. 5: #2069, 609, 1979. (3) A. Pert noted similarly that adrenalectomy enhanced cold water swim analgesia (unpublished observations). Supported by NIH Grants MH 15174 and NS 14449.

116.7 CROSS-TOLERANCE BETWEEN MORPHINE AND ONLY THAT FORM OF STRESS ANALGESIA ANTAGONIZED BY NALOXONE. J. W. Lewis, J. E. Sherman* J. C. Liebeskind. Dept. Psychol., UCLA, Los Angeles, CA 90024.

Exposure to a variety of stressful manipulations has been shown to induce analgesia. It has been suggested that opioid peptides mediate this phenomenon, although evidence on this point has been contradictory. We have shown (Lewis et al., Science, in press) that by varying only the temporal parameters of inescapable footshock stress, profound analgesia is elicited that is either opioid or nonopioid in nature: prolonged intermittent footshock causes analgesia that is powerfully antagonized by naloxone, whereas brief continuous footshock provides comparable analgesia that is not. These data suggest the existence of separate analgesia systems selectively activated by different stress parameters. To characterize further these systems, we have examined the effect of prior exposure to morphine on the analgesic response to both types of stress.

Male, Sprague-Dawley rats were assigned to 4 groups (n = 6) each receiving 5 daily injections of either morphine (5 mg/kg) or isotonic saline followed 3 days later by either brief (2.5 ma, 3 min) or prolonged (2.5 ma, 1 sec pulse/5 sec for 20 min) stress. Two measures of pain responsiveness were used, the tail-flick and hot-plate tests. Prior to stress, baseline latencies indicated no differences among groups. Immediately after footshock, each rat received a single hot-plate test followed by 12 tail-flick trials, then another hot-plate test.

In saline treated animals, both types of stress induced potent analgesia in hot-plate and tail-flick tests. Prior exposure to morphine, however, differentially affected tail-flick latency in brief versus prolonged stress groups. Thus, in morphine treated animals, analgesia induced by prolonged stress was severely abbreviated compared to saline controls ($p < .05$) whereas that induced by brief stress was unaffected. This differential effect was not observed on the hot-plate, possibly due to the time of testing. Both groups showed hot-plate analgesia on the first test; neither group did on the second. That morphine treated rats were tolerant to the drug's analgesic effect was indicated by their being significantly less analgesic than saline treated animals to a test dose of morphine (5 mg/kg) given 24 h after stress.

Cross-tolerance between morphine analgesia and only that form of stress analgesia antagonized by naloxone provides additional evidence for the existence of an endorphinergic pain-inhibitory system. Similarly, the lack of effect of morphine tolerance on the naloxone-insensitive stress analgesia emphasizes the importance of nonopioid mechanisms in antinociception.
(Supported by NIH grant NS07628. JWL was supported by MHTP grant MH15345)

116.8 THE ANALGESIA OF DEFEAT IS BLOCKED BY NALOXONE AND BY MORPHINE TOLERANCE. Klaus A. Miczek, Michael L. Thompson* and Louis Shuster. Depts. Psychology, Biochemistry and Pharmacology, Tufts University, Boston, MA 02111.

The role of endogenous opioids in stress-induced behavioral analgesia is still unclear. We have produced analgesia in mice by exposing them to a social stress, that is, defeat of an intruder by a resident attacker. The intruder is introduced into 5 different home cages for successive one minute periods. In each cage he is subjected to repeated attacks by the resident male mouse. Male mice subjected to extensive defeat (a total of 70 attacks in 5 minutes) showed a significant prolongation of latency to tail-flick in response to radiant heat (5.95 ± 0.7 vs. $1.23 \pm .02$ seconds). Analgesia was most pronounced immediately after the first experience of defeat in mice that had previously not been subjected to attack.

When naloxone, between 1 and 10 mg per kg i.p., was administered before fighting, there was no change in the frequency of defensive upright postures, escape leaps, audible vocalizations and non-agonistic locomotor activity. However the analgesia produced by defeat was effectively blocked by naloxone in a dose-dependent manner.

In additional experiments, mice were implanted with pellets containing 75 mg morphine base or placebo pellets. They were subjected to defeat on the 7th day after pellet implantation. The morphine-treated mice exhibited an intact pattern of defensive and flight behavior when attacked, but did not show any analgesia after defeat. We are presently examining whether mice that have been subjected repeatedly to defeat may show decreased analgesia and/or cross tolerance to morphine.

Our results suggest that social stress, in the form of repeated attacks, can produce marked analgesia in mice. In contrast to most other types of analgesia induced by behavioral stress, the analgesia produced by defeat is effectively antagonized by naloxone, and is not observed in morphine-tolerant animals. These results suggest that endogenous opioids may play a role in the analgesia of defeat.

116.9 EFFECTS OF NALOXONE ON THRESHOLDS FOR ESCAPE BEHAVIOR MAINTAINED BY INTRACRANIAL STIMULATION. Stephen Sasson* and Conan Kornetsky (SPON: N.K. Mello). Laboratory of Behavioral Pharmacology, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118.

Natural ligands of the opiate receptor have received attention relative to their role in the modulation of painful stimuli. Recent evidence has indicated a critical role for the reticular formation (RF) in the central processing of nociceptive input. Electrical stimulation to the RF is a reliable stimulus for maintaining escape behavior in rats. We have previously demonstrated that the threshold for escape from RF stimulation increases after morphine (Marcus and Kornetsky, Psychopharm. 38: 1, 1974) and recently reported tolerance to this effect with repeated injections (Sasson, Wheeling and Kornetsky, Soc. Neurosci. Abst. Vol. 5, p. 2254, 1979). Considering the putative involvement of the endogenous opioids in pain perception, we conducted this experiment to determine if drug naive animals would show a decrease in escape thresholds when challenged with naloxone.

Male albino rats were implanted with bipolar electrodes aimed at the RF and subsequently trained to turn a wheel manipulandum to escape RF stimulation. Threshold determination involved varying the current intensity by means of a modification of the psychophysical method of limits. Results indicated that naloxone (2-16 mg/kg, s.c.) produced dose-dependent decreases in escape thresholds. These findings suggest that naloxone may enhance the aversiveness of RF stimulation by blockade of a central opiate receptor.

(Supported by NIDA Grant DA 02326 and Research Scientist Awardee MH 1759 - CK).

116.10 NEUROCHEMICAL SUBSTRATES OF KETAMINE AND MORPHINE ANALGESIA. G.M. Pekoe* & D.J. Smith, WVU Medical Ctr., Morgantown, WV 26506

Two potential mechanisms for ketamine hydrochloride (KH) analgesia are its interaction with opiate receptors (Smith et al, Life Sci. 26:489, 1980) and inhibition of biogenic amine (norepinephrine, NE & serotonin, 5HT) neuronal reuptake (Azzaro & Smith, Neuropharm. 16:349, 1977). Morphine sulfate (MS) analgesia is also mediated by opiate and 5HT systems (Yaksh & Rudy, Pain 4:299, 1978). MS analgesia uses central gray (PAG) opiate receptors which activate the nucleus raphe magnus (NRM) to inhibit pain transmission in the dorsal horn, and spinal opiate receptors (Basbaum & Fields, Ann. Neurol. 4:451, 1978). MS-PAG interaction also causes release of spinal NE which inhibits pain transmission (Yaksh, Brain Res. 160:180, 1979). KH may also use the PAG-NRM-dorsal horn pathway, spinal opiate receptors, and NE to produce its analgesia. KH and MS analgesia were compared in the rat using the tail-flick test after biogenic amine depletion, spinal transection (T4-6) and receptor antagonism. A 70% analgesic dose of KH (160 mg/kg) or MS (4 mg/kg) was administered i.p. Antagonists were given 10 min prior to KH or 5 min after MS.

KH analgesia was antagonized by 4 fold less methysergide (ID50 0.41 mg/kg), 47 times less phentolamine (ID50 0.18) and 20 times more naloxone (ID50 0.46) than that needed to inhibit MS analgesia. Depletion of 5HT by p-chlorophenylalanine (300 mg/kg, 48h prior) attenuated KH and MS analgesia. Depletion of NE by FLA-63 (25 mg/kg, 6h prior) enhanced KH analgesia while not affecting MS. The increase with KH was reduced by naloxone (1 mg/kg) or methysergide (1 mg/kg) suggesting KH interacts with opiate and 5HT processes independent of NE. NE involvement is important but paradoxical in KH analgesia, perhaps due to opposing central and spinal NE action.

Spinal transection increased the sensitivity of rats to KH's analgesic effect 8 fold but reduced 2 fold their response to MS. In these animals KH was antagonized only by methysergide suggesting a local 5HT influence and indicating that KH may not utilize spinal opiate receptors for its analgesia. MS was antagonized by naloxone, with only the higher dose of MS affected by methysergide. These data suggest that the opiate component of KH analgesia is centrally located, as naloxone is effective in intact but not transected rats. Transection revealed a spinal 5HTergic component of KH and MS analgesia which does not appear dominant in intact rats and requires further study. It is likely that the central KH-opiate interaction in intact rats modulates the KH-5HT interaction, probably by activating the spinofugal NRM-dorsal horn pathway. KH may activate this system via PAG opiate stimulation and simultaneously enhance spinally-released 5HT action by neuronal reuptake blockade. A contribution may also be made by a spinofugal NE influence. (USPHS #5 T32 GM07039 & Anes. Res. Fund)

117.1 PHARMACOLOGICAL EVIDENCE FOR A STIMULATORY EFFECT OF SEROTONIN ON RENIN SECRETION IN RATS. L.D. Van de Kar*, C.W. Wilkinson*, and W.F. Ganong. Department of Physiology, University of California, School of Medicine, San Francisco, CA 94143.

There is evidence that central release of serotonin in dogs stimulates renin secretion via a neural pathway (Zimmermann and Ganong, Neuroendocrinology 30:101, 1980). In rats, administration of the serotonin synthesis inhibitor parachlorophenylalanine (pCPA; 300 mg/Kg i.p. 64 and 40 hours before sacrifice) caused a significant decrease in plasma renin activity and hypothalamic serotonin content. Subsequent administration of 5-hydroxytryptophan (L-5HTP; 30 mg/Kg i.p. 2 hours before sacrifice) restored the serotonin content of the hypothalamus, and plasma renin activity was increased above control level. 14 days after injection of the serotonin neurotoxin 5,7-dihydroxytryptamine (5,7-DHT; 150 µg/20 µl) into the lateral cerebral ventricle of rats pretreated with desmethylimipramine (25 mg/Kg i.p.), there was a significant decrease in plasma renin activity and the serotonin content of the hypothalamus and midbrain. The norepinephrine and dopamine content of the hypothalamus remained unchanged. In rats treated with the serotonin agonist quipazine (10 mg/kg i.p.), plasma renin activity was elevated at 30 and 45 min compared to saline controls at 45 min, with a return to control values at 60-120 min. Administration of the serotonin releaser parachloroamphetamine (PCA; 10 mg/Kg i.p.) caused a significant, but slower increase in plasma renin activity, which reached a peak 2 hours after administration. Pretreatment of the rats with pCPA (300 mg/Kg i.p. 72 hours before sacrifice) abolished the effect of PCA on plasma renin activity. These results show that serotonin stimulates renin secretion in rats as well as dogs, and that the effect is probably due to release of this amine from serotonergic neurons in the central nervous system. (Supported by USPHS Grant AM06704 and a fellowship from the Sloan Foundation.)

117.2 THE EFFECT OF HYPOTHALAMIC KNIFE CUTS IN THE REGION OF THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) ON THE PRESSOR RESPONSES TO CENTRAL ANGIOTENSIN II. Steven L. Bealer, Dept. Physiology and Biophysics, University of Tennesee Center for the Health Sciences, Memphis, TN 38163

Electrolyte lesions of the tissue surrounding the anteroventral third ventricle (AV3V) region have been shown to have profound effects on fluid-electrolyte balance, including adipsia, hypernatremia, and attenuated pressor responses to intraventricularly (IVT) administered angiotensin II (AII) (Bealer et al., Am. J. Physiol., 236: E610, 1979; Johnson et al., Brain Res., 157: 61, 1978.). The present experiment tested whether discrete knife cuts surrounding the AV3V region would also effect pressor responses to central AII. An encephalatome was used to produce knife cuts in the coronal plane just posterior to the organum vasculosum of the lamina terminalis (OVLT), extending ventrally from the level of the anterior commissure to the optic chiasm,and bilaterally 2.0 mm from the midline. Cuts in this region did not damage either the OVLT or the supraoptic nucleus. Each animal was subsequently implanted with a cannula in the lateral cerebral ventricle, and allowed to fully recover from surgery. On the day before testing, a catheter was placed in the femoral artery to record blood pressure. During testing, 100 ng and 500 ng AII were administered IVT in awake, unrestrained animals. Control pressures were 118 ± 6 mmHg in animals which had received cuts, and 116 ± 4 mmHg in animals which had undergone control surgical procedures. Following IVT administration of 100 ng AII, pressure in control animals rose to 133 ± 6 mmHg, while rats with cuts showed only a slight pressor response, with peak pressures of 120 ± 5 mmHg. A similar pattern was seen following stimulation with 500 ng AII, with mean blood pressure in control animals reaching 138 ± 8 mmHg, while rats with cuts increased to 125 ± 6 mmHg. Rats which had received coronal knife cuts posterior to the OVLT showed significantly attenuated pressor responses to IVT AII when compared to animals which had undergone control surgical procedures. These data indicate that the neural substrates which mediate the pressor response to IVT AII pass through this portion of the hypothalamus. (This research was supported in part by an award from the UTCHS New Faculty Research Grant Program and a grant from the American Heart Association-Tennessee Affiliate.)

117.3 DRINKING, VASOPRESSIN, AND BLOOD PRESSURE DURING CHRONIC CENTRAL INFUSION OF ANGIOTENSIN II IN THE RAT. Tommy A. Brock* and M. Ian Phillips (SPON: R. K. Wong) Dept. of Physiology, Louisiana State University Medical Center, Shreveport, LA 71130 and Dept. of Physiology, University of Iowa, Iowa City, IA 52242.

Acute injections of the peptide angiotensin II (A-II) into the central nervous system of the rat indicate that A-II can act as a stimulus to increase drinking, vasopressin release, and blood pressure in conscious animals. Since the components of a complete renin-angiotensin system endogenous to the brain have been characterized, theseobservations suggest that A-II formed locally in the brain may be involved in the regulation of these phenomena. In the present study, we have examined the effects of prolonged one to two week infusions of A-II into the lateral ventricle of the rat in order to determine whether or not A-II is effective in producing sustained elevations in drinking, vasopressin and blood pressure.

Male Sprague-Dawley rats, 300-400g, were implanted with chronic ICV cannulas which were attached to an Alzet osmotic minipump, filled with either saline or A-II (6µg/µl). Infusion of A-II (6 µg/hr) lasted for either 6 or 12 days. Daily water intake, food consumption and systolic pressure (BP) were measured. At the end of the test period, rats were decapitated and trunk blood was collected for measurements of arginine vasopressin (AVP) plasma renin activity (PRA) and angiotensin II.

Drinking in the A-II rats was increased following the first 24 hr of infusion and remained significantly elevated throughout the 6 or 12 day test period ($p<.001$). Food consumption and body weight of the A-II rats were decreased during the first few days of infusion, but A-II rats exhibited a slightly increased weight gain as soon as the appetite returned to normal. BP was increased in the A-II group after the third day of infusion and continued to increase throughout both test periods ($p<0.001$). Plasma AVP levels were increased in both 6 day ($p<0.02$) and 12 day ($p<0.05$) A-II infused rats when compared to control animals. Plasma AVP and BP exhibited a high positive degree of correlation. Plasma Na^+ concentration and osmolality were significantly decreased. PRA was less than 10% of control after 6 days of A-II infusion, while plasma A-II levels in the A-II infused group were increased 10 fold above that seen in control animals.

These results indicate that chronic central infusion of A-II can result in sustained increases in drinking, plasma AVP, and blood pressure. The increase in drinking and plasma AVP is most probably due to activation of specific receptors located in the subfornical organ or the OVLT, while the increase in blood pressure was due both to high plasma AVP and A-II levels. The data suggest that the increase in plasma A-II was not due to increased PRA, but may be due to spillover of A-II from the CNS.

117.4 A DISCONTINUOUS PATTERN OF NUCLEAR ESTRADIOL BINDING IN BRAIN IS SUFFICIENT TO ACTIVATE THE LORDOSIS REFLEX AND INCREASE PROGESTIN RECEPTORS IN THE RAT. B. Parsons*, D.W. Pfaff and B.S. McEwen. The Rockefeller University, New York NY 10021.

We have defined a minimum period of estradiol (E_2) treatment which is sufficient to induce sexual receptivity in the female rat and used this paradigm to measure associated changes in nuclear E_2 and cytosol progestin receptors (PRs) in the mediobasal hypothalamus-preoptic (MBH-POA) and pituitary (PIT).

Female rats ovariectomized for 5-7 days were implanted with 5mm Silastic capsules of E_2. For behavior, females were tested with males 24h after initiation of E_2 treatment. Four h prior to testing, each animal received 500µg progesterone. PRs in the MBH-POA and PIT were measured in vitro using ^{3}H-R5020 as a radioligand, 24h after initiation of E_2 treatment. Animals received no exogenous progesterone. Nuclear E_2 receptors (NRs) were measured in the MBH-POA and PIT using an in vitro exchange assay. NRs were measured 1/2h and 1h after implantation of capsules, as well as 3 and 6h after their removal.

Findings: 1) Lordosis quotient (LQ) scores of animals given E_2 for 6h or 24h are equivalent at 24h; 2) PRs in MBH-POA and PIT in animals given E_2 for 6h or 24h are equivalent at 24h; 3) Receptivity comparable to 6 or 24h of continuous E_2 treatment is seen if animals receive only two 1h periods of E_2 treatment, provided that the second treatment period is not fewer than 4 or not greater than 13h after the first period (Sufficient Treatment). If animals receive two 1/2h periods of E_2 during these times, receptivity is not observed (Insufficient Treatment ; 4) Sufficient treatment is associated with ≃30% increase in PRs in MBH-POA and PIT. Insufficient treatment does not significantly increase PRs in these tissues; 5) One h of E_2 treatment produces twice the levels of NRs (≃60% saturation capacity) as does 1/2h of E_2 (≃30%) in MBH-POA and PIT. After capsule removal, levels of NRs sharply decreased, although the ratio of the differences in NRs produced by these two stimuli is maintained at 3 and 6h post E_2. 6) Anisomycin (100mg/kg), which inhibits protein synthesis in the brain for 6h, blocks lordosis when administered 15min. prior to either of the 1h periods of E_2 exposure, and, at any point in the 4-13h interval between the 1h periods of E_2.

We conclude that 2 pulses of E_2 treatment with total exposure time of 2h during 24h is sufficient to activate the lordosis reflex. The discontinuous presence of nuclear E_2 receptor occupation is sufficient to effect more continuous changes in macromolecular synthesis in the MBH-POA, which are necessary for the activation of the lordosis response.

Supported by USPHS grant NS07080 and Institutional Grant RF70095 from the Rockefeller Foundation.

117.5 AN INTERACTION BETWEEN THE PINEAL GLAND, PHOTOPERIOD AND NUTRITIONAL STATUS IN THE NEUROENDOCRINE REGULATION OF MAMMARY TUMORIGENESIS AND PROLACTIN IN THE RAT. David E. Blask* (SPON: D. Stuart). Dept. of Anatomy, Univ. of Arizona, Coll. of Med., Tucson, AZ 85724.

In female rats, mammary tumorigenesis induced by 7,12-dimethylbenz(a)anthracene (DMBA) is dependent upon prolactin (Prl). Environmental factors such as nutritional intake influence tumor growth presumably by altering neuroendocrine mechanisms controlling Prl secretion. The pineal gland, whose activity is markedly potentiated in undernourished rats deprived of photic input, represents a potentially important extrahypothalamic regulator of Prl release. Inasmuch as the pineal has been implicated as having anti-tumor effects, it was of interest to examine a possible interaction between the pineal gland, photoperiod and dietary intake with respect to mammary tumorigenesis. At 50 days of age female rats received an i.v. injection of a lipid emulsion containing 5 mg of DMBA. Three weeks later the animals were either pinealectomized (Px), sham-pinealectomized (S-Px), blinded (B) + S-Px, B + Px or left intact (I). Four days later all operated rats began receiving 50% of the intake of a stock diet consumed daily by the I controls for a 6 week period. From the end of this period until the termination of the experiment rats received 75% of the dietary intake of the controls. All rats were palpated weekly for the presence of mammary tumors (MT) until the end of the experimental period 18 weeks after DMBA treatment. Upon termination of the study half the rats in each group were killed during the day (D) (1300-1400 h) while the other half was killed during the night (N) (2400-0100 h). All animals were maintained on 14L:10D (lights on 0600-2000 h) for the duration of the experiment. Serum Prl levels were measured via a double antibody radioimmunoassay. For the entire experimental period the prevalence of palpable tumors was 44.4% in the I group. Underfeeding reduced the prevalence of tumors to 20.0% in the S-Px group while in the Px rats the prevalence remained at about the same level (22.2%). In the B + S-Px group no tumors developed whereas in the B + Px animals the prevalence of tumors was 41.6%. The B + S-Px rats exhibited a 66% ($p < 0.001$) depression in N levels of Prl versus D levels not seen in the B + Px rats with tumors. Also the combined D + N Prl levels in B + S-Px rats were 43% ($p < 0.02$) lower than in sighted + S-Px rats exhibiting significantly ($p < 0.02$) higher N levels of Prl as compared with D levels. These data show that the reduced development of MT in underfed rats is completely obviated in rats which are also deprived of light. This is a pineal-mediated effect since pinealectomy reversed the effects of blinding and underfeeding. The absence of MT in B + S-Px rats may be related to an overall reduction in serum Prl titers. (Supported by ACS Institutional Grant #IN-110C).

117.6 ENDOGENOUS CEREBROSPINAL FLUID PEPTIDES MONITORED IN THE CONSCIOUS ANIMAL. R. Roger Barnard* and Mariana Morris* (SPON: James G. McCormick). Dept. Physiol. Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

A push-pull technique was used to sample third ventricular cerebrospinal fluid (CSF) in the conscious, unrestrained male rat (250 gms). Cannulae were placed in the anterior (above the optic chiasm, A3V) or middle third ventricle (medial basal hypothalamic region, MBH-3V) and the animals were perfused with artificial CSF at a rate of 10 µl/min. The perfusate was acidified to .1 N HCl, boiled for 10 min, and vasopressin (VP) and oxytocin determined by radioimmunoassay (RIA).

Measureable amounts of VP and oxytocin were consistently found when the cannula tip was located in the mid-hypothalamic region. Levels were 282.7 ± 48.5 pg VP/ml perfusate (n=13) and 285.9 ± 80.5 pg oxytocin/ml perfusate (n=7) (mean ± SEM). Evidence of specificity includes a lack of assay crossreactivity with extracted artificial CSF or a perfusate from the cerebral cortex. Furthermore, dilutions of the MBH perfusate produced a dose-response parallel to the RIA standard curves. Significantly lower amounts of VP were measured when the cannula was located in the anterior 3rd ventricle (39.7 ± 10.4 pg/ml, n=12). Many of the samples collected from this region were below the sensitivity of the RIA. Measurement of timed perfusate samples (15 min periods) illustrates the consistency of the vasopressin levels when using this approach:

Cannula Location	Perfusion Period 1	2	3	4
Anterior 3rd Ventricle	29*	36	43	38
MBH 3rd Ventricle	461	438	390	209

*pg VP/ml

In addition to the presence of VP and oxytocin in the CSF, significant amounts of angiotensin II were also detectable. Extraction of control solutions and perfusion of other brain areas support the conclusion that this was angiotensin itself and not due to artifactual interactions with the RIA.

These results demonstrate that using a push-pull technique vasopressin, oxytocin and angiotensin II can be reliably measured in third ventricular CSF. Secondly, they suggest that this approach may be useful in evaluating dynamic changes in central peptides in response to various physiological manipulations. (Supported by NIH grant HL-22411)

117.7 VASOPRESSIN INVOLVEMENT IN FEBRILE CONVULSIONS. W.L. Veale, N.W. Kasting, K.E. Cooper, K. Lederis*. Div. Medical Physiology and Div. Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta.

There is evidence that the neuropeptide, arginine vasopressin (AVP), is intimately involved in the brain in the negative control or modulation of fever. It has also been observed that AVP can cause convulsions when administered into the lateral cerebral ventricles of rats. This study sought to determine if AVP may be involved in the mediation of febrile convulsions by using a hyperthermia-induced convulsion model.

Long Evans (LE) and homozygous Brattleboro (DI) rats of 250-300 g body weight were used for these experiments. Some LE rats were stereotaxically implanted with guide cannulae directed towards the lateral cerebral ventricles. Body temperature (Tb) was recorded by a rectal thermistor probe. Hyperthermia was induced by placing the rats in an environmental chamber at 50°C. Blood samples were assayed for AVP by a specific and sensitive RIA. Four groups of rats were observed. Group 1 consisted of LE rats whose Tb increased until 42.7°C and they were then removed from the heat and guillotined for blood collection. Group 2 consisted of LE rats whose Tb increased until convulsions began and then they were guillotined for blood samples. Group 3 was DI rats whose Tb increased until either convulsions began or death occurred and then they were guillotined for blood collection. Group 4 was LE rats given anti-AVP antiserum icv and put into the heat. Their Tb increased until convulsions began or death occurred.

Group 2 LE rats all convulsed and these convulsions occurred at a mean Tb of 43.19 ± 0.12°C. Group 3 DI rats did not all convulse and the mean Tb of convulsions or death was 44.40 ± 0.18°C which was statistically higher than Group 2. Group 4 LE rats given anti-AVP antiserum icv did not all convulse and the mean Tb of convulsions or death was 44.00 ± 0.20°C which was also statistically higher than Group 2 LE rats. Mean serum levels of AVP in Group 1 LE rats which were hyperthermic but did not convulse was 56.1 ± 24.1 pg/ml whereas Group 2 LE rats which had convulsed had levels of 325.5 ± 124.6 pg/ml. DI rats which convulsed or died had AVP levels not statistically different from the limit of the assay.

These data suggest that AVP may play a role in the brain to mediate or contribute to the convulsions of hyperthermic animals.

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117.8 THE TRH TEST IN THE DIFFERENTIAL DIAGNOSIS OF DEPRESSION. A.L.C. Pottash, I. Extein, and M.S. Gold. Fair Oaks Hospital and Psychiatric Diagnostic Laboratories of America, Summit, NJ 07901, and Yale Univ. School of Medicine, New Haven, CT 06510

Prange et al. (Lancet 2:999,1972) were the first of many groups to report that depression may be associated with a decreased release of thyroid-stimulating hormone (TSH) by the pituitary after infusion of thyrotropin-releasing hormone (TRH). We studied the TRH-induced TSH response in subtypes of depression because of the data of Prange, studies suggesting antidepressant efficacy for T-3 and TRH. The discovery of stereospecific TRH receptors in the brain, the extrahypothalamic distribution of TRH, the data suggesting a role for TRH as a neurotransmitter, and Hofelt's hypothesis that TRH is an "arousal" peptide. We performed the TRH test on 134 consecutive inpatients who met Research Diagnostic Criteria for a depressive disorder: 99 major depression (81 unipolar, 18 bipolar) and 35 minor or intermittent depressions. Patients with thyroid disease, drug or alcohol addiction, OBS, or recent lithium use were excluded. All patients were free of all medications except flurazepam for at least one week prior to the TRH test. 500 ug of synthetic TRH was administered via an indwelling venous catheter to patients at bed rest after an overnight fast. Blood samples were taken before and 15,30,60, and 90 minutes after TRH infusion for determination of serum TSH in duplicate by radioimmunoassay. Maximum TSH response (ΔTSH) was determined for each patient by subtraction of the baseline TSH from the peak TSH after TRH infusion. Mean ΔTSH (uIU/ml ± SEM) of 7.5 ± 0.6 for unipolars was significantly lower than that of 11.7 ± 1.2 for bipolars ($p < .01$ by t-test) was demonstrated. All patients with a ΔTSH $\leq$ 7 uIU/ml were considered to have a decreased TSH response to TRH. 62 patients had a decreased TSH response to TRH, including 57 patients with unipolar depression, 3 with bipolar depression, and 2 with minor depression. These data suggesting group differences for similarly appearing depressed patients supports the biochemical concept of heterogeneity. In addition, these TRH test data suggest that this test may help confirm the clinical diagnosis of major unipolar depression, and distinguish it from bipolar and minor depressions. If a decreased TSH response to TRH is used to test for a major unipolar depression in patients with depressive disorder, the false-positive rate in our series is 5 in 62 (8%), and the false-negative rate is 24 in 81 (30%). Norepinephrine and dopamine stimulate and serotonin inhibits the release of the hypothalamic tripeptide TRH. Hence, the decreased TSH response to TRH in unipolar depression may reflect changes in brain monoaminergic neurons or release of endogenous TRH in this disorder. The TRH test may identify biologically homogeneous subgroups of depressed patients for research purposes and help in treatment decisions.

118.1 MEMBRANE STRUCTURE IN DEVELOPING, MATURE, AND CULTURED ASTROCYTES
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Certain regions of the membranes of astrocytic processes in mammalian central nervous system are found, when freeze-fractured, to contain "assemblies", aggregates of uniform intramembranous particles packed in orthogonal array with a center-to-center spacing of 4-6nM. The function of these aggregates is unknown, but they are concentrated in astrocytic membranes facing blood vessels and the subarachnoid space, and so are nicely situated to transport substances into or out of the blood and CSF compartments. We have found the structure of assemblies to be the same in tissue prepared for freeze-fracture by standard aldehyde fixation and glycerol cryoprotection as in tissue rapidly frozen against copper cooled by liquid helium. Preliminary studies of rapidly-frozen, deep-etched tissue indicate some representation of the particle array on the true outer surface of the membrane. Assemblies are unusually labile, and begin to clump and disappear from tissue slices excised and rapidly frozen 35 to 45 seconds after decapitation. Loss of assemblies is progressively more complete with increasing interval between decapitation and excision for rapid freezing, but there is no change in the morphology of particles constituting tight junctions, gap junctions, and postsynaptic aggregates. The loss of assemblies can be prevented by incubating the slices *in vitro* in the presence of bubbling oxygen for 30 minutes, but incubation in bubbling nitrogen or with dinitrophenol (100μM) results in loss of virtually all assemblies. This membrane specialization is characteristic of mature, differentiated cells. In developing postnatal cerebellar cortex, processes of Bergmann astrocytes span the molecular layer, penetrate the proliferating external granular layer, and over 14 days acquire increasing numbers of assemblies as they spread out over the surface of the cerebellum. The vertical portion of these processes appears to guide migrating granule cells from external to internal granular layers, but there is no change in the concentration of assemblies or other specialization of membranes at the juxtaposition of astrocytic and neuronal processes. Assemblies appear in the astrocytic processes of cerebellar explant cultures from neonatal mice. Primary astrocyte cultures derived from newborn rats contain scattered assemblies only under restricted conditions, while rodent C-6 glioma and human astrocytoma cell lines appear not to express the membrane specialization at all. We anticipate that the formation of assemblies in primary cultures may permit the studies necessary to define the nature and function of this membrane specialization.

118.2 GAMMA-GLUTAMYL TRANSPEPTIDASE IN GLIA: IMMUNOCYTOCHEMICAL LOCALIZATION. H. D. SHINE, J. D. COULTER, AND B. HABER. Depts. of Physiol. and Biophys., Pysch. and Behavioral Sciences, Biochem. and Human Genetics, Neurology, and Marine Biomedical Inst., Univ. of Texas Medical Branch, Galveston, TX.

Gamma-glutamyl transpeptidase (E.C. 2.3.2.2., γ-GTP) may participate in the transport of amino acids and peptides across cell membranes, in mechanisms of detoxification, or in the metabolism of neuropeptides. Precise determinations of the cellular localization of γ-GTP activity in the brain have been hampered by the lack of sensitivity and specificity of histochemical methods used to identify the enzyme in nervous tissue. We have employed the peroxidase anti-peroxidase (PAP) technique of Sternberger and the fluorescent technique of Coons to identify sites of γ-GTP within the rat CNS. The antibody was made to γ-GTP isolated from the rat kidney. The isolation procedure included deoxycholate extraction, ammonium sulfate precipitation, papian digestion, ion exchange chromatography, gel filtration, and concanavallin A affinity chromatography. The isolated γ-GTP used for immunization was homogeneous as shown by PAG electrophoresis which produced one band that stained for protein and γ-GTP activity. The antibody (IgG fraction only) raised in rabbits to γ-GTP cross-reacted with rat tissues only and upon immunoelectrophoresis produced one arc without spurs that stained both for protein and γ-GTP activity. Enzymatic activity of solubilized rat γ-GTP was inhibited by the addition of the anti-γ-GTP in a dose dependent manner. Immunocytochemical techniques using this antibody demostrated the presence of γ-GTP in microcapillaries (isolated and in situ), choroid plexus, and ependymal cells of the rat brain. In sections of rat CNS, immunoreactive cells were found both in gray and white matter, which were identified as glia. The immunocytochemical localization of γ-GTP in glial cells supports our previous suggestion of the preferential localization of γ-GTP in glial cells

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118.3 PRELIMINARY CHARACTERIZATION OF MÜLLER CELLS ISOLATED FROM RABBIT RETINA. M.C. Trachtenberg and D.J. Packey. Div. of Neurosurgery and Dept. of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX., 77550.

Studies of the regulation of the extracellular ionic milieu in the mammalian CNS require an examination of the cellular contributions to ion and water homeostasis. The complex morphology of the cellular interactions in the brain make in vivo determination of relative glial and neuronal contributions of other than single cells so difficult as to be beyond currently available technology. Therefore, in order to investigate the glial contribution to ion regulation in nervous tissue, we have developed a procedure for the bulk isolation of Müller cells (glia) from the retina of the rabbit.

We have modified the retinal dissociation procedure of Sarthy and Lam (Brain Res. 176:208, 1979) to give a rapid yield of about 4 million Müller cells per rabbit. Purification of the crude dissociate by centrifugation on continuous density gradients of either BSA or percoll provides approximately 3 million cells. These yields correspond to a final wet weight of 34 mg of Müller cells per 100 mg of retina with about 2 mg of protein present in each preparation. At the completion of the purification the cells appear to be intact as judged by exclusion of vital dye. Light microscopy reveals the cell morphology to be strikingly similar to that demonstrated in fixed tissue by Ramon y Cajal. Fine lamellar and filamentous processes are clearly visible. Electron micrographs show the cell membranes to be intact and numerous microprojections are evident. The cells contain both 8-9 nm filaments and glycogen, both characteristic of glia. Two enzymes taken to be glial specific markers (carbonic anhydrase and glutamine synthetase) have been assayed in these cells and show significant enrichment over the values for intact retina. Carbonic anhydrase activity is measured to be 1068 U/μg protein in isolated Müller cells (49% of which is membrane bound) as compared to 546 U/μg protein in the whole retina (39% membrane bound). Glutamine synthetase activity also is relatively enriched in the isolated Müller cells as compared with the whole retina - 1000 U/μg protein are present in Müller cells while only 783 U/μg protein are found in retina.

Glial cells respond to elevations of extracellular $[K^+]$ with an increase in metabolic activity, as evidenced by K-stimulated 0_2 consumption (Hertz, J. Neurochem. 13:1373, 1966). We find that isolated Müller cells show an increase in glucose uptake in 53 mM $[K^+]$ to a rate double that seen in 3 mM $[K^+]$.

This work was supported by DHEW Program Project 5P50 NS 07377-10.

118.4 MYELIN BASIC PROTEIN (MBP) IN DEVELOPING ALBINO RAT OPTIC NERVE: AN IMMUNOPEROXIDASE STUDY OF PARAFFIN EMBEDDED MATERIAL.
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The distribution of MBP in the optic nerve of the developing albino rat was studied by the peroxidase-anti-peroxidase method of Sternberger using paraffin embedded material from animals ranging in age from 0 to 14 days of age. The eyes with attached optic nerves were removed immediately after decapitation and fixed with Perfix for 30 min. at room temperature and were then embedded in paraffin using a 30 min. processing schedule. With this method MBP activity could be demonstrated in perikarya of immature oligodendrocytes without the use of $HgCl_2$/formaldehyde fixation. It was found that cytoplasmic MBP antigenicity was somewhat less sensitive to the deleterious effects of formaldehyde fixation than is the case for glial fibrillary acidic protein, and that the antigenicity of MBP within the myelin sheath is quite stable with respect to formaldehyde fixation.

As in other regions of the central nervous system, MBP activity could be detected in immature oligodencrocytes prior to the formation of myelin as judged by the appearance of periaxonal sheaths in electronmicrographs (Sternberger et al, J. Cell. Neurocytol., 7:251, 1978; Proc. Natl. Acad. Sci., 75:2521, 1978). A correlation was found between the distribution of MBP containing cells and the early deposition of myelin reported by previous investigators (Skoff et al, Neurosci. Letters, 7:191, 1978). They first appeared as small islands of MBP containing cells which then expanded until assuming the distribution found in the mature optic nerve.

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118.5 GLIAL MEMBRANE POTENTIALS AND THEIR RELATIONSHIP TO $(K^+)_o$ IN GUINEA PIG AND MAN: A COMPARATIVE STUDY OF INTRACELLULARLY MARKED NORMAL, REACTIVE AND NEOPLASTIC GLIA. S. Picker*, C.F. Pieper* and S. Goldring. Dept. of Neurol.& Neurol. Surg., Wash. Univ. Sch. Med., St. Louis, Mo. 63110

Cells were studied in-virto using physiologically viable brain slices (Gibson, I.M. and McIlwain, H., J. Physiol., 176:261-283, 1965). After recording the resting membrane potential (RMP) or the relationship of RMP to changes in $(K^+)_o$ in the cells with very stable RMPs, the cell was injected iontophoretically with HRP for later visualization and correlation with the physiologic data.

Normal glial cells were studied in cortical tissue obtained from the occipital pole of guinea pig (GP), and in man from cortex overlying tumor tissue or from an epileptogenic focus where the tissue was microscopically normal but electrically abnormal. Reactive glial cells were studied in cortical slices from human epileptogenic foci which showed neuronal drop-out and astrocytosis. Neoplastic cells were studied in tissue obtained from one human glioblastoma multiforme (53 cells) and one mixed glioma (7 astroglia and 5 oligodendroglia).

The average RMP of 335 GP glia was -70 ± 7mV and -69 ± 8mV in 68 cells from histologically normal human cortex. The cells in both GP and man were protoplasmic astrocytes, appearing either as densely labeled cells with short processes, or cells with small somas and delicate veil-like processes. The average RMP of 59 reactive glia was -63 ± 9mV. The cell population consisted of fibrous astrocytes with long tortuous processes exhibiting varying degrees of swelling, glia with short thickened processes containing rounded nodules, and some normal protoplasmic astrocytes.

In the glioblastoma multiforme the average RMP from 53 cells was 32 ± 7mV. These cells were large, varied in size, resembled more the fibrous astrocyte and had numerous radiating processes covered with irregular nodules. The labeled cells from the mixed glial tumor showed oligodendroglia, and astroglia resembling some of the glioblastoma cells. The average RMPs of the astrocytic and oligodendroglial cells were -50 ± 11mV and -53 ± 3mV respectively.

In 13 GP protoplasmic astrocytes RMP was recorded while the perfusate (4mM K^+) was exchanged for solutions having (K^+) of 7, 12 and 20 mM. The slope of the RMP vs. log $(K^+)_o$ was 58 ± 2mV/dec approximately the value predicted by the Nernst equation if glia are permeable only to K^+. In 8 human protoplasmic astrocytes from histologically normal cortex a slope of 56 ± 5mV/dec was obtained when a (K^+) was changed from 4-12 mM. In 11 human reactive glia a similar change in (K^+) yielded a slope of 32 ± 8mV/dec.

118.6 ANALYSIS OF GLYCOPROTEINS SYNTHESIZED AND RELEASED BY C6 GLIOMA CELLS. N. Shitara*, P. E. McKeever*, B. H. Smith, R. E. Pleasants*, M. A. Banks*, and P. L. Kornblith*. Surgical Neurology Branch, NINCDS, NIH, Bethesda, MD 20205.

As part of a study of glycoproteins characterizing malignant glial cells, the synthesis and extracellular release of ^3H-fucose labeled glycoproteins by the C6 glioma cell line was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography.

Cultured C6 glioma cells (10^6 cells at passage 64) in Ham's F-10 medium plus 1% fetal calf serum in a 95% air:5% CO_2 atmosphere at 37°C show significant glycoprotein synthesis within three hours after addition of 10μC/ml ^3H-fucose. There is a linear increase in synthesized protein from the initiation of labeling to 120 hours. Five major glycoprotein groups can be detected in NP-40 detergent lysates of (washed) cells. Molecular weights were calculated from plots of logs of the known m.w. of protein standards versus the linear migration distances of these proteins. Estimated molecular weights are 82,000; 110,000; 120,000; 140,000 and 160,000 daltons.

Six hours after initiation of fucose labeling, the C6 cells begin to release six major glycoproteins into the extracellular medium. These released glycoproteins accumulate linearly from 12 to 120 hours. The most prominent band (GP85) comprises 80% of the total released glycoprotein and occurs at 85,000 daltons. The other five bands occur at 55,000; 115,000; 130,000; 150,000; and 170,000 daltons in 7.5% SDS-PAGE. The ratio of TCA-precipitable released glycoprotein to total synthesized glycoprotein at 24-120 hours is 52.5% which is greater than the 39.1% ratio of 3-24 hours. Cycloheximide (100μg/ml) resulted in 98% reduction in both intracellular and released glycoprotein.

Little or no fucosylated GP85 was synthesized or released by a syngeneic normal fibroblast line or by a cell culture derived from normal rat cortex, suggesting that GP85 may be specific to malignant C6 glioma cells. Further characterization of GP85 with respect to its possible functions and/or antigenicity is now underway.

118.7 INDUCTION AND PREVENTION OF ASTROGLIAL SWELLING IN CEREBRAL CORTEX. K.D.Barron, R.S. Bourke*, J.B. Waldman* and S. Easton*. Department of Neurology and Division of Neurosurgery, Albany Medical College, Albany, NY 12208

After placement of plastic perfusion domes (Bourke et al,1970), edema was induced in feline temporoparietal gray matter by superfusion for 60 minutes with artificial CSF (Bourke and Nelson, 1972) containing K+ (54 mM) or adenosine (0.125 to 2.5 mM) or norepinephrine (0.2 mM). Two control cats were superfused with unmodified artificial CSF solution. Vital signs, arterial pH and blood gases, microhematocrits, serum and urine osmolalities and Na+, K+ and Cl- concentrations were monitored at the inception of superfusion and 30 and 60 mins. afterward. At conclusion of superfusion animals were killed by intracardiac formaldehyde-glutaraldehyde perfusion. Samples of cortical ribbon at 3 graded depths beneath the pia (named pial, subpial and deep) were post-fixed and prepared for electron microscopy. Anatomical findings were correlated with estimates of total solids and Na+, K+ and Cl- contents of gray matter of cats treated identically but used for biochemical assays.

Cortex of control cats showed no changes. Both K+ (2 cats) and adenosine (6 cats) induced massive astroglial swelling at pial and subpial levels. Adenosine was about equally effective at 0.125 and 2.5 mM concentrations. Astrocytic enlargement involved perikarya and processes throughout the parenchyma but was greatest perivascularly. In areas of astroglial swelling degeneration of neuronal somas and dendrites was manifested by increased electron-density of hyaloplasm, disintegration of ER etc. Expanded electron-lucent dendrites were rare. Axoplasm and boutons were seemingly little affected but adaxonal, oligoglial invaginations were common. Norepinephrine-induced swelling (3 cats) was practically limited to tissue within 300 um of the pia and was mild.

Acylaryloxyacetic acid derivatives included in the adenosine superfusates greatly reduced astrocytic swelling.

We conclude the following: (1) astroglial edema is induced in superfused cortex by K+ concentrations equivalent to those encountered in traumatic, hypoxic and ischemic insults; (2) increases in extracellular K+ may act indirectly to produce glial swelling by release of putative neurotransmitters; (3) acylaryloxyacetic acid derivatives effectively reduce astrocytic edema; (4)expansion of astroglial cytoplasm, by increasing intercapillary distances (Auen et al, 1979), may critically prolong diffusion pathlengths for O_2 and other vital metabolites and thereby cause neuronal necrosis.

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118.8 PRESERVATION OF PULSATORY AND MIGRATORY ACTIVITIES OF RAT SCHWANN CELLS BEFORE AND AFTER MITOTIC STIMULATION. B. Rentier*, M. Dubois-Dalcq*, A. Baron* (SPON: T.S. Reese). NINCDS, National Institutes of Health, Bethesda, Maryland 20205.

Rat primary (I) and secondary (II) Schwann cells (SC) have been cultured following Brockes et al.(Brain Res. 165:105, 1979). These SC have no basement membrane but contain numerous bundles of intermediate and actin filaments. All motility is reversibly suppressed by cytochalasin B. The motility pattern of I SC was compared to that of II SC after intense mitotic stimulation by choleratoxin and a pituitary extract (Raff et al., Cell 15:813, 1978). Continuous recording by time-lapse video intensification microscopy (VIM) allowed analysis of various SC movements. In I SC, slow rhythmic undulation episodes were observed as described (Pomerat, Science 130:1759, 1959; Forman et al., Soc. Neurosci. Abstr. 5, 1955, 1979) and lasted 2.3 ± 0.2 min. In spite of the extreme variability of the interval between these episodes, the total number of episodes per day remained rigorously identical for different cells (166.3 ± 0.2). Short and long intervals occurred at random. Cycles, consisting of an undulation episode followed by a resting interval, had mean durations of 8.6 ± 4.1 min. and a sharp peak of occurrence at 6 min., with exponential distribution of the longer periods, indicating a non-random event. Migratory events often alternate with undulatory episodes. Migration speed was 135 ± 50 μ per hour and the path of migration was often oriented along a constant axis for every cell, in contrast with the random migratory path of occasional fibroblasts observed in I cultures.

During mitotic stimulation, subcultured SC acquired a "fried egg" shape and both their undulatory and migratory activities were dramatically reduced. Undulation was replaced by a pulsation of approximately the same periodicity, but occurring only in 2% of the cells. Migration was reduced to 24 ± 2 μ per hour. Six to 12 hours after removal of mitogenic factors, 80% of the SC started pulsating twice as fast for 2 to 3 days. When mitosis ceased, SC quickly recovered their spindle shape and rhythmic undulation while their migration speed increased to 92 ± 20 μ per hour. When II SC were seeded over dissociated neuron cultures, they rapidly attached to neurites and showed migratory and undulating movements along these neurites.

In conclusion, VIM revealed that 1) frequency of undulation episodes show a remarkable uniformity, perhaps demonstrating a genotypic basis; 2) in spite of dramatic modification of shape and behavior during mitotic stimulation, SC subsequently recover their unique motility pattern which might be essential for their myelinating function.

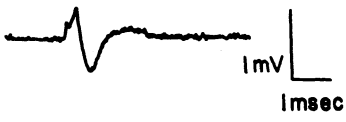
119.1 DEVELOPMENT OF CEREBRAL CORTEX IN VITRO: FUNCTIONAL INFLUENCE OF SUBCORTICAL INPUTS. Arnold L. Leiman & Frederick J. Seil (SPON: Irving Zucker) Department of Psychology, University of California, Berkeley and Department of Neurology, University of Oregon Health Sciences Center and Veterans Administration Hospital Portland, Oregon.

Our previous studies described the functional and structural development of organotypic tissue cultures of fetal mouse cerebral neocortex. Such completely isolated cerebral neocortex shows cellular differentiation and the establishment of cortical networks. Extracellular microelectrode observations reveal similarities and differences with in vivo functional development. This present study examined the possible role of subcortical tissue in the guidance and control of cerebral neocortical functional development in vitro.

Parasagittally oriented explants were obtained from newborn Swiss-Webster mice. They were placed on collagen coated coverslips with nutrient medium and sealed in a Maximow assembly. Some explants were prepared so that they consisted of cortex and attached subcortical tissue which probably included caudate-putamen. Extracellular electrophysiological comparisons were made between completely isolated cerebral neocortical explants and those grown with connected subcortical tissue. At different post explant intervals, responses were recorded to dorsal surface and deep cortical electrical stimulation. Explants that included subcortical inputs/outputs showed the development of larger amplitude extracellular slow wave responses. These responses also displayed a better columnar organization than was evident in explants that were completely isolated cultures where horizontal spread of activity is quite evident. At all stages of post explant development a greater responsiveness to repetitive stimulation was also evident. The basic progression of electrophysiological development was not different in the two explant states nor were there major distinctions in the timetable of functional development. The data suggest that subcortical inputs/outputs may influence the progressive functional development of cerebral neocortical circuitry.

119.2 ELECTROPHYSIOLOGICAL CHARACTERISTICS OF SUBSTANTIA NIGRA BRAIN GRAFTS IN THE LATERAL VENTRICLE. S.M. Wuerthele, L. Olson*, W.J. Freed*, J. Morihisa*, L. Spoor*, R.J. Wyatt, B.J. Hoffer*. Dept. of Pharmacology, University of Colorado, Denver, CO 80262.

Pieces of fetal rat mesencephalon containing the substantia nigra were homologously transplanted into the lateral ventricle of rats pretreated with 6-OHDA injections into the ipsilateral substantia nigra. Dopamine-containing cells from such grafts innervate the adjacent caudate. Apomorphine-induced rotation is significantly reduced in the host animals (Perlow et al., Sci. 204: 643-647, 1979), suggesting that the grafts provide functional input to the denervated striatum as well. Extracellular recordings from nigral grafts, using NaCl-filled micropipettes, revealed spontaneously active neurons with action potential waveforms (1.5-2.5 msec duration; distinct break between initial segment and somatodendritic component) and firing rates (0.5-8.0 Hz) similar to that previously reported for dopaminergic nigral neurons in situ:



Furthermore, local application of dopamine agonists inhibited the activity of such neurons in a dose-dependent manner. These data support the hypothesis that neurons in substantia nigra grafts are functionally active. The data also suggest that some of the mechanisms regulating nigrostriatal neuronal activity present in situ are also present in this preparation. Supported by USPHS Grants DA-07043, NS-09199, and Swedish MRC Grant 04X03185.

119.3 LONG-TERM POTENTIATION OF THE HIPPOCAMPAL SLICE RESULTS IN A STIMULATED SECRETION OF NEWLY SYNTHESIZED PROTEINS. C. Duffy*, T. J. Teyler and V. E. Shashoua (SPON: Timothy J.Teyler). Mailman Research Center, McLean Hospital, Harvard Medical School, Belmont, MA 2178.

Long-term potentiation (LTP) of synaptic responses in the rat hippocampus has been extensively used as a model system for the neurophysiological study of plasticity. Recent studies have demonstrated that LTP can change the pattern of phosphorylation of specific proteins in the hippocampal slice (Browning, M., et al., Science, 203:60, 1979). There have, however, been no reported findings which show a relationship between LTP and the pattern of protein synthesis. We have, therefore, explored this question using the techniques developed in our laboratory in studies of the pattern of protein synthesis in goldfish brain after training. In the goldfish, we found that two specific proteins (Ependymins β and γ) are rapidly labeled and secreted into the extracellular fluid (ECF) after the animals acquire a new pattern of behavior. If LTP in the hippocampal slice is also an indicator of neuronal plasticity, then by analogy to the goldfish data (Shashoua, V. E., Brain Res., 166:349, 1979) some changes in the pattern of synthesis and secretion of proteins might be expected in the rat hippocampal slice after LTP.

Using the double-labeling procedure, in which the pattern of protein synthesis in a single potentiated slice (^{3}H-valine) of rat brain hippocampus is compared with that of an unpotentiated control slice (^{14}C-valine), we found that a 150% increase in the secretion of labeled proteins into the ECF occurs after LTP. An examination of the remaining, unsecreted cytoplasmic proteins showed that the biochemical changes were limited to the area potentiated. Thus potentiation of the CA1 region, by stimulation of Schaffer collaterals, resulted in local protein secretion within the hippocampal region, as compared with the unstimulated dentate area of the same slice. Conversely, when the dentate area was potentiated via the perforant path, enhanced secretion occurred, as compared to the unstimulated CA1 region of the same slice. Control experiments in which nonpotentiated slices received the same number of stimuli as did tetanized slices, but spaced 20 seconds apart, did not show changes in protein synthesis. Also, some slices which received the tetanus but did not develop LTP showed no protein changes. These results suggest that there is a link between the LTP and the metabolic processes that lead to the class of protein synthesis destined for secretion into ECF.

(Supported by a grant from NINCDS #09704.)

119.4 DEVELOPMENTAL DECREASE IN NEURITE EXTENSION IN CULTURED CHICK EMBRYO RETINA AND SPINAL CORD NEURONS. Jeffrey M. Thompson. Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Baltimore, Maryland 21224

Both retina neurons and spinal cord neurons from chick embryos attach to collagen-coated tissue culture dishes and extend neurites. The ability to form neurites in culture as a function of developmental age of the donor was studied. The neurons were dissected from chick embryos of different ages, trypsinized and 1 X 10^{6} cells cultured on collagen-coated 35 mm tissue culture dishes in MEM plus 10% fetal calf serum. After 24 hr, photomicrographs were taken of each culture with phase-contrast optics. The photographic negatives were enlarged and the neurites from each cell were traced. The total number of cells and number of cells with neurites were counted. The length of each neurite was measured with a Graf/Pen-6 Sonic Digitizer. Length measurements made at 24 hr of culture reflect rates of extension rather than the maximum extent which may be achieved. It is not possible, however, to show in this mixed culture which neuronal cell type extends processes and undergoes changes in the number and length of neurites. The percent of chick embryo retina neurons which formed neurites was 12.3% in cultures from 8-day embryos and decreased to 2.4% with 16-day embryos. The average length of retina neurites was 36.2 μm in cultures from 6-day embryos, whereas, 16-day embryo retina neurons only extended neurites an average of 11.8 μm. Thirty-eight percent of spinal cord neurons from 6-day embryos formed neurites, whereas, 31% formed neurites with 16-day embryos. The average length of spinal cord neurites declined from 50 μm with 4-day neurons to 27.8 μm with 14-day neurons. There is a statistically significant age-related decrease in both the percent and average length of neurites formed in cultures of chick embryo retina and spinal cord neurons. The decrease in neurite extension during development may be important in synaptogenesis as it would restrict synapse formation to a discrete period of development. This is consistent with developmental decreases in synapse formation in culture between rat muscle cells and either chick embryo retina neurons (Ruffolo et al., Proc. Nat'l. Acad. Sci., U.S.A. 75:2281-2285, 1978) or chick embryo spinal cord neurons (Thompson et al., manuscript in preparation).

119.5 SYNAPTIC RESPONSE IN A SIMPLE CORTICAL SYSTEM TO THALAMIC REMOVAL: TYPE OF SYNAPSE AND POSTSYNAPTIC TARGETS OF TWO DIFFERENT SPROUTING FIBER SYSTEMS. L.M. Smith and F.F. Ebner. Neurosci. Sect., Div. of Biol. and Med., Brown Univ., Prov., R.I. 02912

Two types of axons increase in numbers in cerebral cortex following thalamic removal in Pseudemys turtles. Quantitative EM analysis at known depths through the molecular layer shows that those axons containing small flattened or pleomorphic vesicles (FVCPs) increase in number in the outer 100um of cortex before the electron dense phase of degeneration and are 3X normal by 14 days postoperative (DPO). Their numbers stay elevated by 180% above normal levels for longer than 90 DPO (from 6.6±1.7/200um^2 to 19.3±1.6/200um^2 at 90 DPO). Substantial increases in FVCPs also occur in the contralateral cortex, and although the increase takes a longer time course, it is ultimately as great. The number of FVCPs that make a symmetrical synapse (FS contacts) in the plane of section increases significantly by 90 DPO, and throughout the molecular layer, that is, on the entire extent of the dendritic tree. These new contacts are mostly on dendrites. Round VCPs contain spherical 50nm lucent vesicles, usually make asymmetrical (RA) contacts, and are depleted by 50% in the subpial zone after thalamectomy. RVCPs return to almost normal levels by 40 DPO (55.0/200um^2 to 25.2 at 14 DPO to 53.5 at 40 DPO), and RVCPs remain at normal levels below the zone of thalamic fiber terminations. RVCPs and RVCP synapses of spines decrease slightly throughout cortex by 90 days. At 90 DPO there is a concomitant increase of RA contacts on dendrites.

Shrinkage of the whole cortex or of the deafferented area does not occur in this system at any of the survival times reported (14, 40, and 90 DPO). Rather cortical thickness increases slightly, but significantly over time. Areas and diameters of RVCPs and FVCPs do not change from unoperated values.

These results support the hypothesis that there are two types of collateral sprouting in turtle cortex following partial deafferentation; one is the RVCPs that replace synapses lost by degeneration and removal of RVCP thalamic fibers and the other is the FVCP response that is triggered by, but partially independent of, the loss of thalamic fiber inputs.

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119.6 DEVELOPMENT OF APPARENT PRESYNAPTIC ELEMENTS. Richard W. Burry, Department of Anatomy, The Ohio State University, College of Medicine, Columbus, Ohio, 43210.

In cell cultures of the rat cerebellum axons have been shown to grow out onto polylysine coated beads and form apparent presynaptic elements with the beads positioned like postsynaptic elements (Burry, Brain Res., 184: 85-98, 1980). These observations were made on cultures at 7 days in vitro (DIV) when neurons have begun to form synaptic contacts between themselves. Sepharose beads coated via cyanogen bromide with poly-L-lysine were then added and the cultures were fixed 24 hrs. later for transmission electron microscopy (TEM). Neuronal elements in contact with the beads displayed both numerous synaptic vesicles and a slight electron density at the site of contact with a bead.

In this report, results show the morphology of the early development of these elements using both the TEM and the scanning electron microscope (SEM).

With cultures, 7 DIV, beads were added for time periods from 1 to 24 hrs. For TEM, sapharose coated beads were used; with SEM, polyacrylamide coated beads were used. Axons appeared to grow up on to beads following the growth of non-neuronal cells. The non-neuronal cells were identified both by their broad, flat processes and by their very fine filopodia (0.1-0.5μm). The cytoplasm of the non-neuronal cells contained small clusters of polysomes. The axons were both large (1-2μm) and round, and, in addition, contained both microtubules and dense core vesicles.

As the axons grew over the non-neuronal cells, they made contact with the beads forming their first apparent presynaptic elements by 3 hrs. In addition to the apparent presynaptic elements seen at 3 hrs., several 1-2μm neurites with only amorphous cytoplasm were seen in contact with the bead. These processes, resembling growing neurites, were seen only rarely after 3 hrs. The apparent presynaptic elements at 3 hrs. had few synaptic vesicles and were smaller in diameter than those seen later. By 9 to 12 hrs. the appearance of the apparent presynaptic elements resembled that of cultures incubated for 24 hrs. with beads. With SEM at 24 hrs. axons were seen to form dense nests of processes and swellings on areas of the bead not covered by non-neuronal cells.

These results show that apparent presynaptic elements have begun to form by 3 hrs. after contact. By 9-12 hrs. apparent presynaptic elements had a stable appearance. In addition, the results suggest that neurons may be able to form a morphologically identifiable contact within 3 hrs. (Supported by a NINCDS Grant No. NS-15894.)

120.1 DUAL EXPRESSION OF TRANSMITTER SYNTHESIS IN CULTURED AUTONOMIC NEURONS. L.I. Iacovitti, T.H. Joh, D.H. Park and R.P. Bunge. Dept. of Anatomy & Neurobiology, Washington University School of Med., St. Louis, MO; Laboratory of Neurobiology, Cornell University, Medical College, New York, NY.

Previous studies have demonstrated that when the predominantly adrenergic neurons of the neonatal rat superior cervical ganglion (SCG) are grown under certain culture conditions, they will express a number of cholinergic functions, including: the development of hexamethonium-sensitive synaptic contents, the accrual of choline acetyltransferase (CAT) activity and the accumulation of clear vesicles in their synaptic endings. In the present study we sought to determine whether, as cultured SCG neurons accumulate these cholinergic characteristics, they simultaneously relinquish or retain certain of their adrenergic properties. To address this issue, we cultured SCG neurons under conditions which foster the accrual of cholinergic properties and measured: a) the activities and amounts of the specific norepinephrine-synthesizing enzymes tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) and the acetylcholine-synthesizing enzyme, CAT and b) the number and distribution of neurons which stain with the antibody to TH.

Dissociated neurons from SCG of perinatal rats were established in culture in the virtual absence of supporting cells for up to 7 weeks. Cultures were maintained in 5% CO_2 using medium containing serum, embryo extract, and NGF. After 1,3,5 or 7 weeks in vitro, the activities of TH, DBH and CAT were measured in cultures of predetermined neuronal number. The results of these studies demonstrate that the activities of TH, DBH and CAT increase in a synchronous and linear fashion: enzyme activities were barely detectable at 1 week in vitro but increased rapidly thereafter with a 3-4 fold increment between weeks 3 and 7. Immunotitration studies using specific antibodies to TH and CAT indicate that the increase in the activities of these enzymes was entirely due to increased amounts of enzyme protein. Furthermore, immunocytochemical staining of sister cultures with the antibody to TH demonstrated that 95-99% of all neurons contained this adrenergic enzyme througout their in vitro development.

We conclude, that under these culture conditions, virtually all SCG neurons retain the enzymatic machinery required for the synthesis of norepinephrine. The simultaneous occurrence in these neurons of high CAT activity strongly suggests that SCG neurons are capable of dual transmitter production. This capability is not restricted to a transient phase, but increases throughout in vitro development.

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120.2 MEMBRANE COMPONENTS OF SATELLITE CELLS WHICH MEDIATE AXON GLIA INTERACTION. H. M. Liu and D. Sackett*. Division of Biomedical Sciences. Brown University, Providence, Rhode Island 02906.

There has been a recent serge of interest toward the production of Nerve Growth Factor (NGF) by many lines of non-neural cells, both normal and neoplastic. In particular, the macromolecular NGF elaborated by neurolemma cells and by glial cells is considered to be of physiological significance as this substance appears to provide the chemical basis for the neurotropism postulated by Ramón y Cajal and confirmed by many recent experimental studies (Windle, W.F., Exp. Neurol., 67:251, 1980). The question of how the NGF molecule fits in with the membrane components of the satellite cells in providing a chemical environment which recognized and attracts the neuraxon has been our main interest in recent studies (Liu, H.M., Exp. Neurol., 66: 123, 1979).

The present report is a preliminary study of the chemical properties of the membrane components of the satellite cells which may have intimate interaction with the NGF molecules produced by the same cells. Two types of cells were used in this study: one a rat glioma cell, C_6 (Am. type culture collection), and the other neurolemma cells from sciatic nerve of 16 day-old chick embryos. The cells were grown on cover-slips placed in Falcon dish; after various period of growth the cells were fixed in formaline and used in a two-step immunocytological study. The cells were first incubated in rabbit anti-NGF serum (Collaborative Research Laboratory) followed by incubation in fluoresein-conjugated horse anti-rabbit immunoglobulin G serum (Sigma). The degree of fluorecence varied considerably among cells of various cultural ages; it was more intense in young cells. A comparable degree of NGF molecules appeared on C_6 and chick embryonic neurolemma cells.

Initial chemical study was performed on conditioned media and membrane extract from both cell lines. The cells were grown to near confluency in culture medium enriched with 10% fetal bovine serum. The cultures were then rinsed thoroughly in saline and maintained in serum-free medium for 3 days, after which the medium was collected, dialyzed against 0.01 M ammonium acetate and lyophilized. Membrane extract of these cells were obtained by incubating the culture in 0.2 M urea for 2 hours; the solution was likewise dialyzed and lyophilized. S.D.S acrylamide gel electrophoresis of samoles from both cell lines showed identical major protein bands, one with molecular weight of approximately 68,000 daltons and a minor band in the range of 55,000 daltons. These protein fractions appear to be membrane in origin and shed into the microenvironment as they appear in both conditioned media and in membrane extract.

120.3 TISSUE INTERACTIONS BETWEEN CO-CULTURED TRIGEMINAL GANGLIA AND CORNEA IN THE CHICK EMBRYO. Betty F. Sisken and Roger W. Beuerman. Wenner-Gren Research Laboratory, University of Kentucky, Lexington, KY 40506 and Div. of Opthalmology, Stanford Medical Center, Stanford, CA 94305.

This study was undertaken to determine if a system could be developed to investigate neuro-epithelial cell relations in the cornea in vitro. Recent work has shown that the trigeminal nerve can exert a trophic effect on the maintenance of the corneal epithelium in vivo (Beuerman and Schimmelpfennig, Exp. Ner., in press). We decided therefore to test the developmental significance of this nerve by co-culturing the trigeminal ganglia with the cornea. These tissues were removed from chick embryos from 6-12 days of incubation and placed 0.5 - 3.0 mm apart in plasma clots. They were grown for periods of 2-10 days and fed with Dulbecco MEM-10% dialyzed fetal bovine serum plus added glucose, glutamine and antibiotics. Neurite outgrowth was promoted by the addition of nerve growth factor (10^{-8}M) or direct current stimulation (10nAmp/dish). Similar cultures were also placed in Rose chambers for time-lapse cinematographic observation. At the conclusion of these experiments, the cultures were fixed and processed for scanning and transmission electron microscopy.

Analyses of movie film demonstrated that within 24 hours the corneal epithelium migrated as a sheet differentially toward the ganglia; the rate approached .02 mm/hour finally overgrowing the ganglia by 72 hours. In contrast, neurite outgrowth did not appear to have a preferred orientation and actually seemed to contain fewer neurites directed toward the cornea. Microcinematography revealed that the limited neurite extension disappeared as the advancing epithelial cells reached them. Electron microscopy verified the absence of neuronal profiles between the explants which contained a bilayered epithelium. At the ganglion, a multilayered, unstratified epithelium was found. The desmosomal junctions of these epithelial cells were developmentally more mature than those found in the bilayered epithelium and in addition contained mats of tonofilaments. Although the underlying ganglion had many degenerating structures, numerous axons containing neurotubules and neurofilaments were found in various stages of Schwann cell developments; this was particularly the situation in NGF-treated cultures. These tissue interactions and the absence of neurites at the cornea are under further investigation.

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120.4 GLIAL GROWTH FACTOR: A NEW COMPONENT OF THE BRAIN AND PITUITARY. G.E.Lemke, D.R.Balzer,Jr*, K.A. Stygall*,and J.P.Brockes*. Div. of Biology 216-76, Calif. Inst. of Technology, Pasadena, CA 91125

In a normal tissue culture medium containing 10% fetal calf serum, purified rat Schwann cells divide very slowly. We have previously reported that the cells are stimulated to divide by an activity present in extracts of the brain and pituitary (Brockes, J.P., Fields,K.L., and Raff,M.C. (1979) Brain Res. 165,105-118) and that this activity appears to be both novel and restricted in its distribution (Raff,M.C., Abney,E.R., Brockes,J.P.,and Hornby-Smith, A., (1978) Cell 15, 813-822). The pituitary activity, assayed by the incorporation of ^{125}I-UdR into the DNA of Schwann cells growing in microwells, has been purified over 4000-fold from a pool of 10kg of frozen glands and 4000 lyophilized anterior lobes. It has an apparent molecular weight of 6 x 10^4 on gel filtration and adheres strongly to the cation-exchange resins CM-cellulose and phosphocellulose. The most purified (phosphocellulose) fraction was analyzed by native gel electrophoresis at pH 4.5, followed by a second dimension of SDS gel electrophoresis and the activity was consistently associated with a component of molecular weight 3 x 10^4, suggesting that the native species is a dimer. The effect of phosphocellulose fraction on proliferation of central glial cells in dissociated cultures of the rat corpus callosum was also investigated. The oligodendrocytes and 'macrophage-like' microglia were not significantly stimulated, but the astrocytes, as well as fibroblasts prepared from neonatal rat muscle, were stimulated over the same range of concentration as the Schwann cells. The activity against astrocytes, fibroblasts, and Schwann cells co-migrated on native gel electrophoresis at pH 4.5, providing strong evidence that the same molecule acts on all three cell types. The activity isolated from bovine brain, as analyzed by phosphocellulose chromatography and native gel electrophoresis at pH 4.5, is closely related, if not identical, to the activity isolated from the pituitary. The brain exhibits a distinct regional variation in growth factor activity, with the caudate nucleus (the most active region assayed) yielding crude extracts of an even higher specific activity than those of pituitary. The question as to whether or not this activity is present in serum is currently under investigation. In view of its derivation and effect on Schwann cells and astrocytes, the activity has been named glial growth factor (GGF).

120.5 MODULATION OF EPIDERMAL GROWTH FACTOR RECEPTORS BY NERVE GROWTH FACTOR IN RAT PHEOCHROMOCYTOMA (PC12) CELLS IN CULTURE. D. End*, K Huff*, and G. Guroff. Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20205.

Previous reports from this laboratory have described the attenuation of biological responses to, and the reduction in binding of, the mitogenic peptide, epidermal growth factor (EGF), following treatment of PC12 cells with nerve growth factor (NGF) (Biochem. Biophys. Res. Comm. 89, 175, 1979). While NGF and EGF share several common effects in PC12 cells including an induction of ornithine decarboxylase and rapid increases in 2-deoxyglucose transport and cellular adhesion, the enhanced cellular proliferation observed with EGF contrasts with the decreased cell division and the morphological differentiation induced by NGF. This report presents a further characterization of both the EGF receptor and its modulation in PC12 cells. Kinetic analysis of the binding of iodinated (^{125}I) EGF to PC12 cell monolayers revealed a single class of binding sites with an apparent dissociation constant (K_d) of 1.75 x 10^{-9} M. A single cell possessed approximately 7200 binding sites. The binding of (^{125}I)-EGF was independent of cell density. (^{125}I)-EGF was specifically displaced by native EGF, but not by NGF, insulin, cytochrome c, or growth hormone. Treatment of PC12 cells with NGF produced a morphological differentiation into sympathetic neuron-like cells with a concomitant decrease in EGF binding. The reduction in EGF binding reached a maximal 90% decrease after 72 hours treatment with NGF with a lag of nine hours preceding the disappearance of binding. Scatchard analysis of (^{125}I)-EGF binding to NGF-treated cells demonstrated that this decreased binding represented a loss of binding sites rather than an alteration of receptor affinity. The loss of EGF binding sites was dependent upon the concentration of NGF with 20 ng/ml producing a maximal effect. When PC12 cells were cultured under conditions which prevented attachment and neurite outgrowth, NGF treatment still induced a loss of EGF binding suggesting that this receptor modulation represented a direct response to NGF rather than an indirect consequence of surface changes accompanying morphological differentiation.

120.6 COMPONENTS IN CHICK EYE EXTRACT THAT STIMULATE GROWTH AND DEVELOPMENT OF CHICK CILIARY GANGLION NEURONS IN DISSOCIATED CELL CULTURE R. Nishi and D. K. Berg, Dept. of Biol., UCSD, La Jolla, CA. 92093

We have previously shown that chick ciliary ganglion (CG) neurons (including those normally destined to die in vivo) can survive and develop for long periods of time in dissociated cell culture when supplied with culture medium supplemented with embryo extract or conditioned by heart or skeletal muscle cells. We have now found conditions permitting neuronal survival in the absence of tissue extracts or conditioning, but growth under such conditions is slow. We have used these observations to construct a bioassay for factors that stimulate development of CG neurons. Extracts prepared from chick eye tissue, the normal target of CG neurons, contain at least two such activities. One activity appears to selectively stimulate cholinergic development, while the other activity stimulates overall growth of the neurons.

Complete survival of the CG neuronal population was achieved for periods of at least 3 weeks in dissociated cell culture using only basic culture medium (MEM + 10% horse serum) supplemented with 20 mM KCl, if the substratum was first prepared with fibroblasts. The fibroblasts were grown to confluency, lysed and removed with distilled water; the neurons were then plated. It is not clear whether the fibroblast treatment contributed a specific component necessary for neuronal survival, or whether it simply promoted neuronal adhesion and subsequent survival in a relatively non-specific way. Cholinergic development as assayed by choline acetyltransferase (CAT) activity and overall growth of the neurons as assayed by lactic dehydrogenase (LDH) activity, a general cytoplasmic marker enzyme, lagged considerably behind that obtained for neurons grown with muscle or conditioned medium.

Inclusion of eye extract in the basic medium resulted in a substantial increase in CAT and LDH levels associated with the neurons (e.g. 3-fold at 9 days). Fractionation of the eye extract by gel filtration resolved two separate activities. One activity, with an apparent mol. wt. of ca. 5x10^4, stimulated CAT levels while having no effect on LDH levels. The other activity, with an apparent mol. wt. of ca. 2x10^4, stimulated overall growth as indicated by LDH levels and relative rates of protein synthesis, but had no effect on CAT levels. The increased growth did not reflect contaminating nonneuronal cells since (1) few nonneuronal cells were present at 9 days, and (2) the increase in LDH levels correlated well with an increase in the mean neuronal soma.

The activities described here may represent factors normally produced by the target tissue to help direct the growth and development of neurons in vivo. It will be of interest to determine the range of cell types responsive to the activities, and to characterize their modes of action on CG neurons. (Supported by USPHS Grant #12601 & The Muscular Dystrophy Assoc.)

120.7 NEUROTROPHIC FACTOR: CHARACTERIZATION AND PARTIAL PURIFICATION. Heinz Popiela and Stanley Ellis*. Biomedical Research Division, NASA-Ames Research Center, Moffett Field, CA 94035.

Recently published in vivo (Popiela, Exp. Neurol. 55:160, 1977) and in vitro evidence (Bonner, Dev. Biol. 66:207, 1978; Popiela, Exp. Neurol. 62:405, 1978) suggests that neurotrophic activity is required for normal proliferation and development of muscle cells. In the present work two independent and quantitative in vitro assay methods are used to monitor partial purification of neurotrophic activity from adult chicken ischiatic-peroneal nerves. The greatest amount of neurotrophic activity is extracted at pH 8. With either increasing or decreasing pH progressively less activity is extracted. Aqueous suspensions of neurotrophic activity are labile to changes in pH but not to longterm storage at room temperature or to storage for more than a week at 37°C. Specific activity is doubled upon precipitation of extract with ammonium sulfate at 50-75% saturation or after gel filtration on Sephadex G-100 columns. Salt gradient elution from DEAE-cellulose columns yields a single active peak with specific activity increased 4-5 fold. The active fraction obtained after gel filtration and rechromatography on DEAE-cellulose yields a single active peak with a 7-10 fold increase in specific activity. Purification of neurotrophic activity, as indicated by an increase in specific activity, is revealed in the lower concentration range of 20-60 µg/ml. At greater concentrations, the more highly purified material shows a plateau in activity which is much lower than in the presence of unfractionated extract. It seems that maximum activity is progressively reduced with increasing purity of active fractions. These observations lead us to postulate the existence of a co-factor necessary for the expression of maximum activity. Purification of inhibitory activity seems unlikely because the partially purified material does not seem to show inhibitory properties.

Heinz Popiela is a National Research Council Resident Research Associate.

120.8 BASAL LAMINA FRACTION FROM THE ELECTRIC ORGAN OF TORPEDO ORGANIZES ACETYLCHOLINE RECEPTORS ON CULTURED MYOTUBES. Lee L. Rubin, Adrienne S. Gordon and U.J. McMahan, Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305 and Dept. of Neurology, UCSF Sch. of Med., San Francisco, CA 94143.

Each skeletal muscle fiber is ensheathed by a basal lamina (BL) which extends through the synaptic cleft at the neuromuscular junction. Associated with the synaptic portion of the BL are factors that direct the formation of active zones in regenerating motor nerve terminals and of dense subsynaptic accumulations of acetylcholine receptors (AChRs) in regenerating myofibers. To examine these factors in detail it would be helpful to have an in vitro preparation where BL, nerve terminals, and muscle cells can be easily manipulated. A particularly rich source of cholinergic synapses and one that may provide large amounts of synaptic BL for in vitro studies is the Torpedo electric organ. The aim of the experiment described here was to determine whether BL fractions from the electric organ induce dense accumulations of AChRs on cultured myotubes.

To obtain electric organ BL we used procedures similar to those of others (Carlson et al, J. Ultrastruct. Res. 62:26-53, 1978) who isolated BL from kidney and blood vessels. The BL fraction consisted of insoluble particles that ranged from less than one micron to 30 microns in their greatest dimension. They contained acetylcholinesterase, an enzyme thought to be associated with the BL. When BL particles were added to cultures of chick myotubes whose surface AChRs had been labelled with ^{125}-α-bungarotoxin, autoradiograms showed that there was an increase in the number of AChR clusters per myotube within 2 hr. By 8 hr the BL particles had induced a 3-5 fold increase in clusters. Thus, the BL fraction had a marked effect on the organization of AChRs. Since the AChRs were labelled prior to addition of particles to the culture, clusters induced by BL particles must have been formed by lateral migration of the receptor molecules. Boiled BL fractions produced only a small increase (<2 fold at 8 hr) in AChR clusters; thus most clusters were induced by a temperature-sensitive component of the BL particles. BL fractions from rat muscle, which contains little synaptic BL, produced a small effect similar to that of the boiled fraction from the Torpedo electric organ. We conclude that the BL fraction from the Torpedo electric organ is rich in factors that organize AChRs on myotubes in vitro, mimicking the influence of the synaptic BL on AChRs of regenerating muscle cells in vivo.

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120.9 PARTIAL PURIFICATION OF A NERVE TROPHIC FACTOR REGULATING MUSCLE ACETYLCHOLINESTERASE ACTIVITY. Thomas L. Lentz and Janice Chester*. Section of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

A fraction influencing acetylcholinesterase (AChE) activity of newt triceps muscle in organ culture has been isolated from rat brain homogenates. Fractions were assayed for effects on AChE by comparing the AChE activity as measured with a photometric method of muscles cultured for one week in the presence of a fraction with the activity of paired muscles cultured without treatment. Rat brains were homogenized in Tris (0.05 M, pH 7.2) buffered saline (0.9%). Ultrafiltration of crude brain extract revealed that a fraction passing through a filter retaining species >100,000 in MW and retained by a filter retaining species >10,000 in MW had an effect on AChE. After gel filtration chromatography on Ultrogel AcA 44, a fraction containing proteins 21,000 to 60,000 in MW maintained AChE activity of cultured muscle. Ion exchange chromatography of the fraction obtained by gel filtration showed that the active material did not bind to DEAE Sephadex A-50 in buffer at 0.1 M ionic strength, pH 8, and bound to CM Sephadex C-50 at 0.1 M and 0.75 M ionic strength and was eluted by 1.5 M buffer, pH 5. The gel filtration fraction was bound to CM-Sephadex C-50 in 0.1 M buffer at pH 6.5 and eluted stepwise at increasing pH. The fraction affecting AChE bound to CM-Sephadex at pH 8 and was eluted at pH 9, indicating the active factor is basic with a pI between 8 and 9. SDS polyacrylamide gel electrophoresis of the pH 9 eluate revealed one major and a few minor bands. The major protein has a MW of 28,000. Muscles treated with the pH 9 fraction had up to 17% higher total AChE activity than paired untreated muscles after one week in organ culture. The fraction was active at a concentration of 5-15 µg protein/ml culture medium. Analysis of the molecular forms of AChE by sucrose gradient centrifugation revealed four major molecular forms of newt triceps muscle AChE with sedimentation coefficients of 16.5 S, 12.6 S, 9.7 S, and 5.2 S and a small amount of a 19.4 S form. The isolated fraction had the greatest effect on the 16.5 S AChE, increasing it more than 100% over untreated controls, and a smaller effect on the 12.6 S form. The fraction prevented the decrease in the 16.5 S form of AChE occurring as a result of denervation and in addition increased the amount of this form relative to normal muscle. This study shows that a protein, termed neurotropin, which mediates neuronal trophic effects on skeletal muscle AChE has been identified and partially purified from nerve tissue. (Supported by NSF Grant BNS 79-12898).

120.10 NEUROTROPHIC CONTROL OF 16S ACETYLCHOLINESTERASE FROM MAMMALIAN SKELETAL MUSCLE IN ORGAN CULTURE. Hugo L. Fernandez and Myron J. Duell. Neuroscience Research Lab., V.A. Medical Center, Kansas City, MO 64128 and Dept. Physiology, Univ. of Kansas Med. Ctr.

The effects of rat obturator nerve extracts on total and 16S acetylcholinesterase (AChE) activity were studied in endplate regions of denervated anterior gracilis muscles maintained in organ culture for 48 hr. The decrease of total AChE activity in cultured muscles was similar to that observed in denervated muscles *in vivo*. This decrease in activity was partly prevented by addition of either 100 µl or 200 µl nerve extract (2.7 mg/ml protein) to the nutrient medium. Nerve extract-treatment also decreased the release of AChE activity from the muscle into the bathing medium. Conversely, rat serum (20 µl; 90 mg/ml protein) had no effect on total AChE activity in muscle endplates, nor on release of the enzyme by the muscle. The 16S form of AChE was confined to motor endplate muscle regions and its activity was drastically decreased by denervation in both organ culture and *in vivo* preparations in a comparable manner. Nerve-extract supplemented cultures contained a significantly ($p << 0.001$) larger amount of endplate 16S AChE activity (140-145%) than the corresponding controls (100%). Our results suggest that some nerve soluble substance, other than serum contaminants or 16S AChE itself, affects the maintenance of 16S AChE at the neuromuscular junction. Whether the nerve extract affected the formation and/or prevented the degradation of 16S AChE is being currently studied.

121.1 AFFERENT AND EFFERENT CONNECTIONS OF THE TORUS SEMICIRCULARIS IN THE ELECTRIC FISH, Eigenmannia virescens. S. O. E. Ebbesson and H. Scheich, Institut für Zoologie, Technische Hochschule, Darmstadt, West Germany.

Teleost fish with an electroceptive sense have highly differentiated cell aggregates related to this system. In order to determine the interconnections of these cell groups, horseradish peroxidase (Miles, 40-50% solution) was injected into the torus semicircularis in 40 animals. Survival times ranged from 1-15 days, and after removal the entire brains were cut on a cryostat and processed according to a modification of the De Olmos procedure (De Olmos, J., Exp. Brain Res. 29:541-551,1977).

A preliminary analysis of our data reveals that the torus receives a topographic input from 1) the stratum griseum centrale of the ipsilateral optic tectum, 2) some of the layers of the contralateral torus, and 3) the ipsi and contralateral posterior lateral line lobe. The torus projects in a topographic manner to 1) three ipsilateral, hitherto unnamed, thalamic cell groups, 2) the stratum centrale and stratum griseum et fibrosum superficiale of the ipsilateral optic tectum, 3) the contralateral cerebellar cortex, 4) the ipsi and contra lateral posterior lateral line lobe, 5) the pacemaker, 6) the anterior nuclei bilaterally, 7) the ipsilateral nucleus praeeminentalis,and 8) several hitherto unidentified cell groups in the rhombencephalon.

These results suggest that the torus serves an important role in the intergration of electrical information from both sides of the body and in the correlation with visual input in relation to orientation. A direct influence on the pacemaker by the torus is also indicated by the findings.

121.2 ORIGINS OF OLIVOCOCHLEAR NEURONS IN THE RAT. J.S. White and W.B. Warr. Human Communications Laboratories, The Boys Town Institute for Communication Disorders in Children, Omaha, NE 68131.

Recent studies of the origins and terminations of olivocochlear neurons in the cat provide evidence that dual efferent systems differentially innervate inner and outer hair cells in the organ of Corti. In order (to begin) to test the generality of the dual nature of the olivocochlear system in other mammals, the cells of origin of the (vestibular and) olivocochlear bundle were labeled in the rat by the method of retrograde axonal transport of horseradish peroxidase (HRP). HRP (20% in saline + 2% DMSO) was injected into the labyrinths of adult rats and after allowing 18-30 hours for transport of the tracer, its presence in neurons of the brain stem was demonstrated histochemically using tetramethyl benzidine as the chromogen. The locations and counts of labeled cells were made on the basis of 2 cases with the greatest number of labeled cells so far obtained. Two groups of labeled cells were found in nuclei of the superior olivary complex, but their numbers and locations differed from that reported in the cat. Approximately 150 small ($15\mu \times 20\mu$) cells were located within the lateral superior olivary nucleus, entirely on the ipsilateral side. Approximately 240 large ($18\mu \times 25\mu$) cells were found bilaterally in the medial periolivary nucleus, the majority of which (approximately two-thirds) were situated on the contralateral side. Another group of labeled neurons (presumably vestibular efferents) was found in the region between the medial vestibular nucleus and the abducens nucleus. These findings show that, as in the cat, two morphologically distinct groups of neurons project to the cochlea in the rat. In contrast to the cat, however, 1) there are more large than small olivocochlear neurons, 2) the large cells are localized in a single nucleus, and 3) the small cells are incorporated directly within the lateral superior olivary nucleus and only on the side ipsilateral to the injection site. These findings suggest that there may be significant species differences in the extent of efferent innervation of inner as compared to outer hair cells.
(Supported by PHS Grant No. NS14832).

121.3 KAINIC ACID: ITS USE AS A LESIONING TECHNIQUE FOR DETERMINING THE ORIGINS OF AUDITORY BRAINSTEM RESPONSES. J.N. Gardi and S.C. Bledsoe, Jr. Coleman Laboratory, University of California, San Francisco, Ca. and Kresge Hearing Research Laboratory, New Orleans, La.

Previous attempts to clarify the origins of the auditory brainstem response (ABR) have produced only general conclusions, with one group's results often contradicting those published by others. In feline and rodent populations, the response waveform consists of four to five waves. Each wave is thought to arise from a progressively higher order relay station in the auditory pathway; such that wave I reflects VIII nerve activity, while wave II cochlear nuclear complex activity, wave III activity from the superior olivary complex, wave IV activity from the nuclei and tracts of the lateral lemnisci, and wave V activity from the inferior colliculi. However, traditional sectioning, aspiration, cooling, or thermal coagulation techniques have not been suited to precise definition of ABR generators because such techniques indescriminately destroy both soma and axons of passage. A more precise technique, which affects cell bodies without significantly disrupting axons of passage, is the micro-infusion or iontophoretic deposition of kainic acid. In the present study, the feasibility of this new procedure was explored in hopes of developing a more punctaform and precise lesioning technique by micro-infusing kainic acid into one of the nuclei of the superior olivary complex - the medial nucleus of the trapezoid body (MNTB).

The scalp, posterior skull, and overlying cerebellum were removed in seventeen guinea pigs. Kainic acid (0.25 - 10.0 nmoles dosage, and 0.1 - 10.0 microliters volume) was infused into the MNTB contralateral to the stimulated ear. Click-evoked, scalp-recorded ABRs were collected before, during and up to two hours after infusion. All brains were fixed in formol-saline, sectioned at 40 microns, and stained with cresyl-violet.

Among the results were the following: 1. Infusion of small doses (0.1-0.2 microliters) of kainic acid in the MNTB produced relatively restricted lesions, approximately 300 microns in diameter. 2. Such restricted lesions produced decrements in the amplitude of waves III (60-80%) and IV (20-30%) without affecting the amplitude of waves I and II. 3. Infusion of larger dosages in larger volumes (1.0 - 10.0 microliters) produced more extensive lesions extending into adjacent portions of the medial superior olive and in some cases portions of the lateral superior olive without producing significantly different ABR amplitude changes other than those observed in the more restricted lesion cases.

These results suggest that: 1. The MNTB is the primary if not exclusive locus of activity that contributes to the generation of the third wave of the guinea pig ABR. 2. Discrete kainic lesions placed all along the auditory pathway should yield valuable information about the contributions that such structures make to the various waves comprising the ABR. Supported by NIH-NS-06094 and NS-07058. Research facilities provided by the Kresge Foundation.

121.4 ELECTROPHYSIOLOGICAL PROPERTIES OF INFERIOR COLLICULAR NEURONES IN THE BIG BROWN BAT, EPTESICUS FUSCUS. P. H.-S. Jen (Div. of Biological Sciences, Univ. of Missouri, Columbia, MO 65211) and P. A. Schlegel* (Fachbereich Biologie, Goethe Univ., Frankfurt/Main, Federal Republic of Germany).

With 3M KCl micropipette electrodes, 230 single unit activity in the inferior colliculus of Eptesicus fuscus were isolated between the depth of 44 and 2092 µm of the brain surface. During acoustic stimulus, 59 (26%) units discharged tonically, 155 (67%) fired phasically, and the remaining 16 (7%) units either showed phasic off response (10 units) or phasic on-off response (3 units) or discharged impulses upon the cessation of the stimulus (3 units). The latency of those tonic and phasic responders ranged from 5 to 35.5 msec, but the majority were below 12 msec. Individual neurons had either broad or sharp or upper threshold tuning curves with Q_{10} dB values ranging from 1.3 to 39.5. Their best frequencies (BF) were between 11.1 and 92.5 kHz with most of them below 70 kHz. A tonotopical organization of units according to their BFs was demonstrated when an electrode was orthogonally penetrated into the brain surface. As the electrode advanced, BF of the encountered units systematically changed from low to high. The lowest minimum threshold (MT) obtained from those units was 4 dB SPL. Those units with higher BFs do not necessarily have higher MT. Properties of these single units are apparently suitable for encoding signals used by the bat. (Work supported by NSF of USA and DFG of FRG.)

121.5 DIFFFERENTIAL ENCODING OF COMMUNICATION AND ECHOLOCATION CRIES IN THE INFERIOR COLLICULUS OF THE BAT. George J. Carman*, George Pollak, Robert Bodenhamer*, and David Marsh*. Department of Zoology, University of Texas, Austin, Texas.

Recent investigations in our laboratory have revealed intriguing differences in the encoding of constant frequency (CF) and frequency modulated (FM) pulses by neurons of the inferior colliculus (IC) of the Mexican free-tailed bat, Tadarida brasiliensis mexicana. Based on their observed characteristic frequencies and response properties, neurons of the medial IC appear to subserve echolocation, whereas those of the lateral IC seem poorly suited for such a function (Marsh, D., Pollak, G., and Bodenhamer, R., in preparation). This finding raised the interesting possibility that lateral neurons may encode communication calls emitted by members of this species in social settings.

In order to investigate this hypothesis, we decided to examine the response of neurons in the IC to recorded communication and echolocation cries. Various calls of this species, recorded from roosts and in the field, as well as in the lab, were selected for use as stimuli. After preparation under anaesthesia, single unit recordings were made from the IC of awake bats using conventional techniques. The responses of neurons to free-field stimuli were determined, using synthesized CF and FM pulses as well as communication and echolocation cries presented iteratively via a Lockheed Store 4D tape recorder under microprocessor control. Data were collected with a Declab 11/03 laboratory computer in the form of post-stimulus histograms having a minimum resolution of one millisecond per bin. At the end of each experiment, animals were sacrificed and prepared for histology.

The results of these experiments clearly indicate that neurons of the lateral IC respond vigorously to one or another of our recorded communication calls. Furthermore, those neurons which responded to synthesized FM pulses were generally found to respond to recorded echolocation calls as well. As yet, no neurons have been found in the lateral IC which respond to our synthesized or recorded echolocation cries to the exclusion of recorded communication signals; however, a more systematic sampling of the IC may yet reveal such units.

These and other results tentatively support the hypothesis of differential encoding of communication and echolocation cries by neurons of the IC. Our findings do not, however, suggest that the lateral IC is so highly specialized as to preclude a role in echolocation. We are pursuing further systematic neurophysiological and neuroanatomical studies which we hope will reveal the mechanisms of this differential encoding.

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121.6 THE RESPONSE PROPERTIES OF CELLS IN THE MEDIAL GENICULATE BODY (MGB) OF THE AWAKE SQUIRREL MONKEY TO SPECIES SPECIFIC VOCALIZATION. N.Allon* and Z. Wollberg. Dept. of Zoology, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv (Israel). (Spon: E.E. Decima).

The possible mechanism underlying the response properties of cells in the MGB of the squirrel monkey to species specific vocalization was studied. The approach used in this work was to record the responses of single cells in the MGB of awaked squirrel monkey to simple acoustic stimuli as pure tones and to compare them to the responses of the same cells to complex acoustic stimuli. The spectral and temporal composition of the complex stimuli, including the species specific vocalizations, were analyzed for correlation with the response patterns of the cells.

The response band widths of cells in the MGB to pure tones presented at 80dB SPL ranged between 1-6 octaves. Forty percent of the cells responded to all the six octaves which were presented (0.5 - 32 KHz).

The best frequencies (BFS) of 28% of cells in the MGB were in the range of the first two octaves (0.5 - 2KHz). Eighteen percent of the cells had their BFS in the range of the two middle octaves (2 - 8KHz), and 54% of the cells in the range of the last two octaves (8 - 32KHz).

Most of the cells responded to most of the vocalizations presented, however, with different response patterns. The response patterns' characteristics were qualitatively and quantitatively dependent upon the stimulus intensity. When the vocalizations were presented at low intensity (near threshold), the cells' response patterns were dominated by the intensity modulations in the vocalizations around the best frequency of the cell.

At high dB levels (80dB) one could find a number of cells which responded with the same response pattern to a given vocalization ("typical pattern"). These cells had similar BFS and response patterns to pure tones. The BFS of these cells did not fall in the range of frequencies of the vocalization which contained the most energy.

Of the 7 vocalizations that were tested the "typical" responses were found most frequently to presentation of "long peep."

It was suggested that the "typical" responses may represent a coding of those features of a vocalization which serves to distinguish the individual monkey's vocalization in a group. It was further suggested that the "long peep" can be a very efficient carrier for this information..

121.7 FUNCTIONAL CLASSES OF AUDITORY CORTICAL NEURONS DISTINGUISHED BY SENSITIVITY TO SOUND LOCATION. J.C. Middlebrooks and J.D. Pettigrew. Beckman Labs, Caltech, Pasadena, CA 91125.

We have explored the possible role of the primary auditory cortex (AI) of the cat in the representation of sound location. We determined the direction (location) sensitivity of cortical neurons and compared these properties with the passive acoustics of the cat's head and pinna.

Tonal stimuli were generated by a loudspeaker that could be positioned in any direction a constant distance from the animal; the animal and loudspeaker were enclosed in an anechoic chamber (see Knudsen et al., Science 198:1278). The activity of single units was recorded from AI of cats lightly anesthetized with Ketamine. The spatial receptive fields (rf's) of neurons were plotted by noting the area of the sound field in which a tone of characteristic frequency elicited a reliable response. Units were grouped into three classes on the basis of spatial location sensitivity. Omnidirectional units (O units) responded to sounds presented anywhere in the sound field. Hemifield units (H) responded only to sounds presented in the contralateral sound hemifield. Axial units (A) had circular or eliptical rf's (as small as 30°) located on the acoustical axis of the contralateral pinna (approx. 30° lateral to the midline and 10-20° above horizontal). In radial electrode penetrations, all units tended to show the same class of rf. Along tangential penetrations, sequences of O, H, and A units were segregated from each other in a manner consistant with the pattern of binaural interaction bands demonstrated with diotic stimulation (Middlebrooks et al., Br.Res. 181:31).

In correlative acoustical experiments, a probe microphone was introduced from behind the pinna into the acoustic meatus. Sound levels were recorded while varying the location of the loudspeaker. Iso-intensity contour plots were derived and compared with the rf's determined physiologically. Many of the characteristics of the measured rf's could be inferred from such contour plots, but neither the H nor the A rf's can be explained entirely by the passive acoustics of the head and pinna.

There was no indication of an orderly map of sound space in AI of the cat. All well-circumscribed rf's lay on the acoustical axis of the pinna. The results suggest the presence of at least three independent processing systems in AI. One system, the O units, does not appear to play a role in sound localization. The H units may be involved in the approximate localization of sounds, while the A units are suited for active scrutiny of relevant sound stimuli.

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121.8 NEURONAL MECHANISMS OF ECHO DELAY PROCESSING IN BAT ECHOLOCATION. W.E. Sullivan*. (SPON: N. Suga). Dept. of Biology, Washington Univ., St. Louis, MO. 63130.

Many neurons in the auditory cortex of the FM bat Myotis lucifugus respond poorly or not at all to synthesized orientation sounds or echoes, but respond vigorously to echoes which follow loud sounds after some preferred time delay. Such neurons are presumably involved in target range analysis since behavioral studies have shown that echo delay is the primary cue used by bats for the determination of target distance. However, it is conceivable that delay sensitive neurons may also participate in other forms of information analysis, because other parameters such as echo intensity will be affected by target distance. In fact, two kinds of delay dependent facilitation have been found, one in which the best delay is insensitive to changes in echo amplitude, and another in which echo amplitude has a marked effect on the best delay.

In studying the response latency of cortical units to single FM sounds, it was found that some units responded at shorter latencies for weaker sounds than for loud sounds. This latency difference appeared as an abrupt change rather than a gradual decline as amplitude was decreased. Often, there was an intermediate amplitude at which no response could be observed. The response latencies to loud sounds were also found to be related to anatomical position, such that there was a progressive increase in latency as the electrode was moved to more posterior locations. The latency for weak sounds remained relatively constant.

Several lines of evidence indicate that these two phenomena are functionally linked. First, for units which demonstrated both a paradoxical latency shift and delay dependent facilitation, the best delay was correlated with the size of the latency shift. Secondly, both the best delay and the response latency for loud sounds increased as the electrode was moved from anterior to posterior. This data supports a model in which facilitation is achieved by the synchronization of synaptic inputs from the loud outgoing sound and the returning echo. Because the orientation cry occurs at an earlier time, it's input must be delayed relative to the input from the weak echo. The length of this input delay, which is mapped on the cortical surface, will then be related to the echo delay which produces maximal facilitation. This suggests that the processing of delay information is related to the existence of a spatial-temporal excitation pattern in the bat's auditory cortex. The latency and best delay maps represent a unique situation in which the cortical representation is produced by neuronal processing rather than by orderly projections from the periphery. (Supported by NSF grant BNS78-12987, PHS BRSG grant 50324C and PHS training grant 1-T32-NS07057-01).

121.9 UNILATERAL REMOVAL OF SUPERIOR TEMPORAL CORTEX IN MACAQUE MONKEYS AFFECTS DELAYED MATCHING PERFORMANCE. J.A. COSTALUPES AND J.H. DEWSON, III. Auditory Neurobiology Laboratories, Hearing and Speech Sciences, Stanford University School of Medicine, Stanford, CA 94305.

In our previous report (Costalupes, et. al., Soc. Neurosci. Abstr., 5, 1979, 360) we suggested that unilateral removal of superior temporal cortex -- the so-called auditory association area -- in macaque monkeys effects a performance decrement on a visual delayed conditional matching task while leaving visual delayed match-to-sample performance unaffected. Earlier work had indicated a comparable deficit on an auditory-to-visual delayed matching task. The present report will compare pre- and post-operative performance on these visual and cross-modal delayed matching tasks following serial removal of right-sided and subsequently of left-sided superior temporal cortex in a single monkey. Previous delayed visual and delayed auditory-to-visual matching performance and confirmation of the locus and extent of the lesions will be considered in view of recent anatomical and physiological evidence of polysensory function in superior temporal cortex.

121.10 AUDITORY FATIGUE: BEHAVIORAL AND NEURAL OBSERVATIONS IN MONKEYS. B.L. Lonsbury-Martin and G.K. Martin*. Dept. Otolaryngology, Univ. Washington Sch. Med., Seattle, WA 98195.

Using a simple auditory reaction-time task, the fatiguing effects of pure tones (100 dB SPL, 3 mins) were quantified in terms of magnitude of threshold shift and duration of recovery time. These psychophysical experiments formed a framework for later physiological observations in the awake monkey investigating changes in level-dependent properties of single neurons in cochlear nucleus (CN) and inferior colliculus (IC) following stimulation with similar pure tones. The behavioral observations indicated that: a) both the magnitude and time course of recovery of the hearing loss were a function of the frequency of the fatiguing stimulus with higher-frequency stimuli producing greater threshold shifts and longer recovery times; b) although the peak hearing loss was located either at or above the frequency of the fatiguing stimulus depending upon the portion of the hearing range stimulated, threshold shifts were always asymmetrically distributed towards frequencies higher than that of the fatiguer; c) alterations in functions relating response latency to stimulus intensity were demonstratable; and, d) recovery-time courses were monotonic and approximately exponential. Similarly, neuronal measures revealed that: a) the magnitude of threshold elevation for CN units did not significantly differ from those measured for IC neurons; b) a decrement in driven discharge rate at all tested intensities was noted for most units, but approximately one-third of our sample demonstrated a decrement in poststimulatory activity for threshold-related test stimuli, while for high-level tone bursts, discharge rate increased above control levels; c) spontaneous activity levels for units with moderate to high discharge rates either increased or decreased, while those for low-spontaneous rate neurons (<2 spikes/s) were generally unchanged; d) short-lasting alterations were also observed in stimulus-level dependent properties as reflected in rate-intensity curves and in functions relating latency of the first evoked spike to signal intensity; and, finally, e) recovery from fatigue was generally more rapid for spontaneous than for driven activity and for IC than for CN units. Although these results indicate that stimulation with fatiguing stimuli reduced the sensitivity and altered the driven and spontaneous neural activity of neurons in brainstem auditory structures in a manner that was consistent with the behavioral symptoms of auditory fatigue, the magnitude and duration of these changes were frequently much greater and longer lasting than those indicated by the pure-tone behavioral threshold measures.

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122.1 STUDY OF MECHANISM(S) OF PEPTIDE MODULATION OF CATECHOLAMINE RELEASE IN BOVINE ADRENAL PARANEURONS. Deanne M. Dean* and Bruce G. Livett. (SPON: M. Rasminsky). Division of Neurology, The Montreal General Hospital and McGill University, Montreal, Canada.

Adrenal chromaffin cells in culture provide a model system for examining the development and function of cells of neural crest origin. Bovine adrenal medullary cells were isolated and purified as previously described, and maintained in a nutrient medium based on Eagle's minimum essential medium, human placental serum and chick embryo extract. Studies were carried out on cells 3-20 days old, during which they attach to a collagen-coated surface and extend processes. For pharmacological studies, the cells were loaded with ^{3}H-norepinephrine (^{3}H-NE) as previously described[1]. These studies have demonstrated that ^{3}H-NE release is mediated by stimulation of nicotinic cholinergic receptors, and that various peptides including substance P (SP), somatostatin, and enkephalins, as well as other opiates (e.g. morphine) inhibit the release of ^{3}H-NE induced by nicotine, but not that induced by K^+[1,2].

We have now examined whether depolarization of the voltage sensitive Na^+ channel is involved in the modulation of catecholamine release by peptides. Tetrodotoxin (10^{-9}-10^{-5}M) inhibited the release of ^{3}H-NE induced by veratridine ($5x10^{-5}$M), but not that induced by nicotine ($5x10^{-6}$M). At a concentration of SP ($1x10^{-5}$M) that abolished the nicotine-induced release of ^{3}H-NE, the veratridine-induced release of ^{3}H-NE was not depressed. Thus, SP modulation of catecholamine release does not involve depolarization of voltage-sensitive Na^+ channels.

Blockade of neuromuscular transmission by agents such as quinacrine, amantadine and procaine is mediated by binding to the ionophore of the receptor-ionophore complex. We have found that these agents also depress the nicotine-induced release of ^{3}H-NE from adrenal chromaffin cells in culture. The depression of ^{3}H-NE release produced by quinacrine and SP is similar. In addition, SP does not add to the depression produced by quinacrine. These studies suggest that SP acts to modulate catecholamine release by regulating the ionophore of the receptor-ionophore complex.

1. Mizobe, F., Kozousek, V., Dean, D.M. and Livett, B.G. (1979) Brain Res. 178: 555-566.
2. Mizobe, F., Dean, D.M. and Livett, B.G. (1979) Society for Neuroscience Abstracts 5: 1801.

(Supported by Canadian MRC)

122.2 REGULATION OF TYROSINE HYDROXYLASE IN ISOLATED CHROMAFFIN CELLS: EFFECTS OF 8Br-cAMP AND ACh ON DEAE-SEPHACEL ELUTION PATTERNS. *John Haycock, Robert George and Jack Waymire*. Dept. Neurobiology & Anatomy, Univ. Texas Medical School, Houston, TX 77025.

Treatment of intact, isolated chromaffin cells with either ACh or cAMP (or analogues) increases catecholamine synthesis rates.[1] 8Br-cAMP, e.g., not only increases catecholamine synthesis but activates tyrosine hydroxylase activity *in vitro* as well.[2] And, such increases in tyrosine hydroxylase (TH) activity have been attributed to increases in ^{32}P incorporation.[3] Thus, the acceleration of catecholamine biosynthesis by cAMP and its analogues seems closely related to phosphorylation processes. On the other hand, the nature of ACh-induced increases in catecholamine biosynthesis in chromaffin cells remains speculative.

A recent report by Sze et al.[4] suggested that DEAE-cellulose chromatography might separate activated and nonactivated TH. Sze and coworkers reported that TH activity from rat striatal supernatants, passed over a DEAE-cellulose column, was eluted as two peaks (corresponding to 0.1M and 0.2M NaCl). Prior phosphorylation shifted TH activity to the 0.2M peak whereas phosphatase or prolonged incubation (0°C) shifted TH activity into the 0.1M peak. Sze and coworkers conclude that the 0.1 and 0.2M peaks correspond to unphosphorylated and phosphorylated TH, respectively.

In the present study, we investigated the effects of ACh and 8Br-cAMP on intact, isolated chromaffin cells on the basis of the TH elution pattern from DEAE-Sephacel columns. Isolated chromaffin cells were incubated in MES-buffered saline (pH 6.2,15',37°C) with or without ACh (100µM) or 8Br-cAMP (1mM). *In vitro* TH activity (100,000g supernatant) was increased by 8Br-cAMP, and the ratio of activity shifted toward the 0.2M peak. On the other hand, although both 8Br-cAMP and ACh increased catecholamine synthesis rates, ACh had no discernible effect upon *in vitro* TH activity.

The present data are consistent, then, with the notion that exogenous 8Br-cAMP alters catecholamine synthesis by increasing the phosphorylation (hence catalytic activity) of tyrosine hydroxylase. To the extent that the elution patterns on DEAE cellulose may reflect the relative phosphorylative state of TH, these data do not support a role for phosphorylation in the increase in catecholamine synthesis produced by exogenous ACh.

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[1]Neurosci Abst 3:319, 1977.
[2]Neurosci Abst 4:316, 1978.
[3]4th Int Catechol Symp, pp. 40-42, 1979, Pergamon, Usdin et al.
[4]Trans Amer Soc Neurochem 11:141, 1980.

122.3 SUGGESTIONS FOR A NEW CLASS OF OPIATE RECEPTORS IN BOVINE CHROMAFFIN CELLS. L. Saiani* and A. Guidotti (SPON: D. L. Cheney). Lab. Preclin. Pharmacol., NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

P-2 membranes prepared from primary culture of bovine adrenal chromaffin cells and from adrenal medulla homogenates contain opiate receptors. This binding was characterized with ^{3}H-dihydromorphine, ^{3}H-D-Ala^2-leu-enkephalin, ^{3}H-etylketazocine and ^{3}H-SKF10047 to label the µ, δ, κ and σ receptors, respectively. ^{3}H-etorphine, which labels both µ and δ receptors, was also studied, as well as the two antagonists, ^{3}H-naloxone and ^{3}H-diprenorphine. The K_D was similar for all the compounds, approximately 2 nM.

A similar K_D was obtained studying the same ^{3}H-ligands in P-2 membranes prepared from bovine frontal cortex. In brain the Bmax for all the compounds ranged from 50 to 80 fmol/mg protein, whereas in adrenal medulla the Bmax of the various compounds ranged from 20 to 200 fmol/mg protein. ^{3}H-etorphine, ^{3}H-naloxone, ^{3}H-diprenorphine and SKF10047 bind with a Bmax of 200, 150, 100 and 90 fmol/mg protein respectively; ^{3}H-dihydromorphine, ^{3}H-D-Ala^2-leu-enkephalin and ^{3}H-etylketazocine bind with a Bmax smaller than 20 fmol/mg protein.

In membrane of adrenal medulla, morphine, D-Ala^2-leu-enkephalin, β-endorphin, naloxone, etylketazocine and pentazocine displaced ^{3}H-etorphine (K_I = 100-400 nM), while diprenorphine, phenazocine and SKF10047 displaced ^{3}H-etorphine with a K_I of 4, 12 and 45 nM, respectively. The potency of morphine and D-Ala^2-leu-enkephalin for displacing ^{3}H-etorphine was greater in membranes prepared from adrenal medulla than in those prepared from frontal cortex.

Kinetic studies revealed that the frontal cortex possesses two populations of receptors, while the adrenal medulla has only one labelled by ^{3}H-etorphine. The ^{3}H-etorphine binding was not displaced by the non-opioid peptides, substance P, bradykinin, oxytocin, neurotensin and cosyntropin; it was decreased by NaCl (100 mM) and GMP-P(NH)P (50 µM). These results suggest that etorphine receptors in the bovine chromaffin cells are different from the ones in the frontal cortex, and that they are not µ, δ or κ type.

The correlation between the potency of different opiate agonists in displacing ^{3}H-etorphine from receptors and in inhibiting nicotine-induced catecholamine release, shown to exist in primary cultured chromaffin cells, indicates a functional role for these receptors.

122.4 UPTAKE OF NUCLEOTIDES INTO CHROMAFFIN GRANULES OF VARIOUS SPECIES. Stephen W. Carmichael* Andreas Weber* and Hans Winkler*. (SPON: C. R. Craig)., Department of Anatomy, West Virginia University, Morgantown, WV 26506 and Department of Pharmacology, University of Innsbruck, Innsbruck, Austria.

ATP is known to be the most abundant nucleotide within the chromaffin granule of the adrenomedullary cell. It is thought to play an important role in the uptake, storage, and secretion of catecholamines. Uptake of ^{3}H-ATP has been demonstrated in isolated bovine chromaffin granules (Kostron et al., Neuroscience 2:159-166,1977). The present study also identifies a similar uptake of ATP in other species and an uptake of other nucleotides into bovine chromaffin granules. Uptake of ^{3}H-ATP was measured in chromaffin granules isolated from the adrenal medulla of pigs and horses. Medullas were separated from fresh adrenals and homogenized. A large granule fraction was isolated and washed by centrifugation. The granules were incubated at 37°C for 5 minutes with tritiated ATP in concentrations of 6, 4, 2, 1, and 0.5mM. Granules were washed and purified by centrifugation over 1.5M sucrose. After measuring the radioactivity taken up into the granules, the apparent Km was 1.34mM for pig and 0.89mM for horse. This is similar to the Km (1.4mM) reported for bovine chromaffin granules (Arberer et al., Biochem. J. 172:353-360,1978). By thin layer chromatography, 66% and 54% of the radioactivity taken up into the granules was identified as ATP for pig and horse, respectively. The uptake of nucleotide was inhibited by atractyloside and uncouplers of oxidative phosphorylation. Reserpine, a specific inhibitor of the carrier for catecholamine uptake, did inhibit ^{14}C-norepinephrine uptake but not ^{3}H-ATP uptake. Bovine chromaffin granule were incubated with labeled UTP and GTP and the uptake was found to be similar to that for ATP. The same nucleotide carrier of the chromaffin granule transports all three nucleotides which contrasts with the mitochondrial ATP/ADP carrier which is specific for adenine nucleotides. $Mg^{++}$and Ca^{++}reduced the affinity of ATP for the carrier by apparently inducing a less favorable configuration of the molecule.

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122.5 ACTIVATION OF TYROSINE 3-MONOOXYGENASE IN PHEOCHROMOCYTOMA CELLS BY ADENOSINE. Raymond Erny*, Michael Berezo*, and Robert L. Perlman* (SPON: Richard E. Zigmond). Committee on Cell and Developmental Biology and Department of Physiology, Harvard Medical School, Boston, MA 02115.

We have been studying the regulation of tyrosine 3-monooxygenase (TH) activity in cell suspensions prepared from a transplantable rat pheochromocytoma. When pheochromocytoma cells are incubated with tyrosine and brocresine (an inhibitor of DOPA decarboxylase), they produce DOPA and release it into the incubation medium. We have used high pressure liquid chromatography with electrochemical detection to measure the rate of DOPA production, and thereby the activity of TH in these cells. The activity of TH in pheochromocytoma cells can be increased acutely by two distinct mechanisms. Incubation of the cells in medium containing depolarizing concentrations of K^+ results in an activation of TH that is dependent upon extracellular Ca^{2+}, that is accompanied by an increase in catecholamine secretion, and that is not associated with a change in cellular adenylate cyclase activity. In contrast, incubation of the cells with cholera toxin causes an activation of TH that is accompanied by a large increase in the cyclic AMP content of the cells, but that is independent of extracellular Ca^{2+}. We now report that adenosine (Ado) also increases the activity of TH in pheochromocytoma cells. Incubation of the cells with 100μM Ado rapidly results in a 40-70% increase in TH activity. The variability of the response to exogenous Ado is due in part to the presence of variable amounts of endogenously produced Ado, since addition of adenosine deaminase to cell suspensions decreases basal TH activity by 10-30%. The effect of added Ado is virtually abolished under these conditions. In contrast, the Ado analog 2-chloroadenosine (2-Cl-Ado) causes a greater than two-fold increase in TH activity in the presence of adenosine deaminase. Half-maximal activation of TH is produced by 100nM 2-Cl-Ado, and maximal activation at approximately 10μM. Activation of TH by Ado and by 2-Cl-Ado is not dependent upon extracellular Ca^{2+}, and is not accompanied by a significant increase in the secretion of catecholamine from the cells. Moreover, the maximal effects of Ado and of 2-Cl-Ado on TH activity are additive with the activation produced by K^+-induced depolarization. Finally, 2-Cl-Ado causes a 6-fold increase in the accumulation of [^{3}H]-cyclic AMP in cells preincubated with [^{3}H]-adenine. We propose that pheochromocytoma cells contain Ado receptors coupled to adenylate cyclase, and that the stimulation of these receptors by Ado or by 2-Cl-Ado causes an increase in intracellular cyclic AMP and thereby activation of TH. Adenosine may be a physiological regulator of adenylate cyclase activity and of TH activity in normal chromaffin cells.

122.6 ACETYLCHOLINE INHIBITS CATECHOLAMINE UPTAKE INTO CHROMAFFIN CELLS Lorna W. Role* and Robert L. Perlman* (SPON:K. Rockland), Department of Physiology, Harvard Medical School, Boston, MA 02115.

We have been studying catecholamine (CA) uptake into isolated chromaffin cells. Chromaffin cells are prepared by collagenase digestion of guinea pig adrenal glands, and purified by isopycnic centrifugation. The cells are pre-incubated with pargyline, to inhibit monoamine oxidase, and are then incubated with l-[^{3}H] norepinephrine (NE) or l-[^{3}H]epinephrine (EPI). We now report that freshly dissociated chromaffin cells have a high affinity uptake system for CA, similar to that described for sympathetic neurons, and that acetylcholine (ACh), the physiological agonist for CA secretion from chromaffin cells, inhibits this CA uptake system.

CA uptake is linear for at least 20 min., is concentration-dependent, and is saturable. CA uptake is energy, temperature, and Na^+-dependent, and is inhibited by imipramine. In the presence of 130 mM Na^+, the apparent K_ms for the uptake of NE and EPI are ∿1 μM and ∿4 μM respectively. The apparent V_{max} for both NE and EPI uptake is in the range of 100-200 pmol/min/mg protein. Half-maximal uptake of 6 μM NE is observed at 34 mM Na^+.

ACh, veratridine and pilocarpine, which cause CA secretion from chromaffin cells, inhibit CA uptake into these cells. ACh (100 μM) and veratridine (25 μM) cause the release of 10-20% of the CA content of the cells, but block uptake by more than 90%. Atropine (50 μM) inhibits the effects of ACh on CA secretion and on CA uptake; tetrodotoxin (1 μM) inhibits the effects of veratridine on these processes. Somatostatin and substance P are present in guinea pig adrenal glands. At a concentration of 10 μM, these peptides partially reverse both ACh-induced CA secretion and the inhibition of CA uptake produced by ACh. Replacement of extracellular Ca^{2+} by Co^{2+} prevents ACh and veratridine-induced CA secretion, but does not block the inhibition of CA uptake caused by these agents. Therefore, the inhibition of CA uptake can not be accounted for by the stimulation of CA secretion. ACh and veratridine may depolarize chromaffin cells, and thereby decrease the electrochemical gradient for the entry of Na^+ into the cells; this decrease in the electrochemical gradient for Na^+ may then cause a decrease in CA uptake.

The Na^+-dependent CA transport system may be important in maintaining a low basal release of CA from the adrenal medulla in vivo. The inhibition of CA uptake by ACh may prevent the reuptake of CA during physiological stimulation of CA secretion.

122.7 SIMULTANEOUS USE OF THE RETROGRADE TRACER 'TRUE BLUE' AND IMMUNOHISTOCHEMISTRY: INNERVATION OF THE RAT ADRENAL MEDULLA. V.R. Holets, T.J. Mullett*, and R.P. Elde. Department of Anatomy, University of Minnesota Medical School, Minneapolis, MN. 55455

The adrenal medulla is innervated by preganglionic sympathetic neurons in the intermediolateral cell column (IML) of the thoracic and upper lumbar spinal cord. This innervation is interesting in that: i) these presumed cholinergic neurons innervate adrenal medullary cells which contain and release enkephalin-like material concomitant with catecholamines; and ii) we have an incomplete understanding of neurotransmitter-specific circuitry impinging upon IML which in turn project to the adrenal medulla. Retrograde axonal transport of the fluorescent dye 'True Blue' was utilized to identify the neurons in IML which project to the adrenal medulla, and by combining this with indirect immunofluorescence, it was possible to visualize met-enkephalin (M-ENK), serotonin (SER), somatostatin (SOM), and substance P (SP) immunoreactive fibers in IML in the same section.

Five days following an injection of 'True Blue' (5 ul 1% 'True Blue') into the left adrenal medulla of rats, the animals were perfused with buffered 4% paraformaldehyde. Serial horizontal or transverse 10 and 30um cryostat sections were taken through IML in the thoracic and upper lumbar spinal cord. Adjacent sections at each level of the spinal cord were processed for immunofluorescence with primary antisera directed against M-ENK, 5-HT, SOM, and SP, with additional sections serving as absorption controls.

The largest number of retrogradely-labeled neurons were observed at T7 and T8, but neurons were observed in a long, continuous column between T1 and L2. Cell clusters could be seen at each spinal cord level. Fusiform (8 x 22 um) to round (12 um) neurons as described by Schramm et al. (Exptl. Neurol. 49:548, 1975) were observed in IML, as well as a small population of large round neurons in the lateral horn-lateral funiculus border. Occasionally, neurons medial to IML were retrogradely-labeled. Axons and dendrites could be seen arising from most perikarya, the latter branched close to the soma and extended either laterally into the white matter, or medially for short distances.

5-HT and SP immunoreactive fibers were prominent in IML, whereas M-ENK and SOM immunoreactive fibers were sparse. These fibers could be seen surrounding the retrogradely-labeled neurons and traveling parallel to the long axis of IML.

The occurrence of several putative neurotransmitters in nerve fibers and terminals suggests diverse control of IML neurons projecting to the adrenal medulla.

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122.8 SPONTANEOUS AND NERVE GROWTH FACTOR INDUCED AXON OUTGROWTH FROM RAT AND BOVINE ADRENAL CHROMAFFIN CELLS IN CULTURE. K. Unsicker and W. Ziegler*. Dept. of Anatomy and Cell Biology, Philipps University, D-3550 Marburg, West Germany.

Adrenal chromaffin cells from young rats have been shown to extend axon-like processes, when grown in low density primary cultures with 2.5 S nerve growth factor (NGF). We have recently observed that processes may also develop spontaneously without exogenous NGF in the presence of large amounts of adrenal non-neuronal (Schwann, fibroblast-like) cells. Spontaneous axon outgrowth could not be blocked by administration of an antiserum to NGF, but was abolished, when non-neuronal cell growth was suppressed by γ-irradiation. Irradiation did not affect the vitality and capacity of chromaffin cells to respond to NGF. The addition of both separately grown intact adrenal non-neuronal cells and diluted high speed supernatants of homogenized cells, but not of conditioned medium, re-established fibre outgrowth from irradiated chromaffin cells.

Chromaffin cells isolated from adult bovine adrenal glands and kept in long-term cultures also exhibited spontaneous axon outgrowth, which was neither blocked by an antiserum to NGF nor enhanced by administration of NGF. The role of non-neuronal adrenal cells in promoting fibre outgrowth from bovine chromaffin cells is under investigation.

We conclude that adrenal non-neuronal cells may be involved in fibre outgrowth from chromaffin cells. Moreover, our results suggest that the response of chromaffin cells to NGF in terms of fibre outgrowth is age- and/or species-dependent.

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122.9 PROLONGED SURVIVAL OF BOVINE ADRENAL CHROMAFFIN CELLS IN RAT CEREBRAL VENTRICLES. M. J. Perlow, K. Kumakura* and A. Guidotti. Lab. of Clinical Psychopharmacology and Lab. of Preclinical Pharmacology, NIMH, St. Eliz. Hosp., Wash. D.C. 20032

Dispersed, cultured bovine adrenal chromaffin cells transplanted into the cerebral ventricles of neonatal and adult rats survive at least two months without evidence of immunological rejection. The cells can be identified for their strong yellow fluorescent reaction using glyoxylic acid, thus suggesting that they maintain intact the capability of synthesizing and storing catecholamines. The cells did not show sprouting or process formation and appear free in the ventricle or aggregated in clusters. This is the first report showing that cells from different animal species and from different tissue origin can be transplanted and survive in the cerebral ventricles.

123.1 STERNBERGER'S UNLABELED ANTIBODY METHOD USED TO STAIN NEURON SPECIFIC ENOLASE AND LYMPHOCYTIC CHORIOMENINGITIS VIRUS ANTIGENS IN TISSUES CONVENTIONALLY PREPARED FOR ELECTRON MICROSCOPY. Manuel del Cerro[1,2], Paul J. Marangos*[3], Robert L. Stoughton[1], Andrew A. Monjan*[4], and Coca del Cerro*[1]. 1)Center for Brain Research, Univ. of Rochester Med. Ctr., Rochester, NY 14642. 2)Center for Visual Science, Univ. of Rochester, Rochester, NY 14627. 3)Clinical Psychobiology Branch, NIMH, Bethesda, MD 20205. 4)Dept. of Epidemiology, School of Hygiene and Public Health, The Johns Hopkins Univ., Baltimore, MD 21205.

The extreme sensitivity of Sternberger's unlabeled antibody method permits detection of antigens in paraffin sections from tissues originally processed by routine histological techniques (retrospective cytochemistry). We thought it of interest to test this technique on one µm thick plastic sections of tissues conventionally prepared for electron microscopy, i.e., fixed with glutaraldehyde-containing mixtures, post-fixed with OsO_4, and stained with uranyl acetate. Antibodies (Ab) against neuron specific enolase (NSE) and lymphocytic choriomeningitis virus (LCMV) were obtained from rabbits by standard immunological procedures. These Ab were used on one µm sections of rat retina and cerebellum from both normal animals and animals infected with LCMV. The tissues had been prepared as described above and embedded in epoxy resin. The plastic was removed from the sections; the tissues were incubated with the Ab in dilutions ranging from 1:100 to 1:2000, and then with anti-immunoglobulin and peroxidase-antiperoxidase, and reacted with diaminobenzidene-hydrogen peroxide. NSE was detected in the somas of bipolar and ganglion cell neurons, and in the neuropil of the inner plexiform layer. LCMV antigens stain most prominently over the internal granular layer and in the white matter. The results suggest that other antigens may be demonstrable retrospectively in tissues conventionally prepared for electron microscopy. The strength of the reaction depends on the fixative used. For example, the LCMV antigens stain more strongly after fixation with 3%paraformaldehyde - 1% glutaraldehyde than with 1% acrolein - 2% paraformaldehyde - 1% glutaraldehyde; nonetheless, positive results are obtained with either fixative. The possibility may exist of performing retrospective ultrastructural studies, even on glutaraldehyde fixed material, using this approach.

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123.2 STUDIES ON THE SUBCELLULAR DISTRIBUTION OF THE MAJOR POSTSYNAPTIC DENSITY PROTEIN: A SYNAPSE SPECIFIC PROTEIN? Paul T. Kelly. Division of Biol., Kansas State Univ., Manhattan, KS 66506.

The subcellular distribution of the major PSD protein (M_r=52K) in both bovine and rat brain was examined in subcellular fractions enriched in myelin, mitochondria, microsomes, synaptic membranes, synaptic junctions (SJ)/postsynaptic membrane specializations (PSMS) and soluble brain proteins (cytosol). The unambiguous identification of the major PSD protein among all other subcellular fraction proteins in the 49,000-55,000 M_r range was accomplished by the following biochemical and morphological analyses: (1) estimates of M_r by gel electrophoresis, (2) 2-dimensional peptide fingerprints of individual proteins, (3) interprotein cross-linking resulting from disulfide bond formation, (4) insolubility in SDS of protein-protein complexes produced in (3) above, and (5) examination of subcellular fractions by EM. In addition to the fractions listed above, purified mitochondria, microsomes, cytosolic proteins and a light plasma membrane fraction (0.8-1.0M sucrose interface) were individually treated with sulphydryl cross-linking reagents, extracted with Triton X-100 (0.4%, v/v) and their respective detergent insoluble counterparts were examined for major PSD protein content. Additional experiments, employing similar strategies, were carried out on a plasma membrane/gap junction fraction isolated from chick lens as well as plasma membranes purified from rat liver.

With the exception of synaptic membranes and synaptic junctions, the only fraction that contained significant amounts of the major PSD protein were microsomes (P_3B fraction) prepared by the method of de Blas and Mahler (J. Neurochem., 30:563, 1978); and then, only after these microsomes were treated with INT (p-iodonitrotetrazolium violet) and extracted with Triton X-100. In addition to containing large amounts of the major PSD protein, the resulting Triton-insoluble P_3B material displayed endless numbers of structures that resembled isolated PSDs when examined by EM. Interestingly, the P_3B fraction has been shown to be enriched in transmitter receptors of presumptive post-synaptic origin (de Blas & Mahler, J. Neurochem., 30:563, 1978).

These results suggest that the major PSD protein in brain is confined to structures of postsynaptic origin in either synaptic membrane (SPM) or P_3B (microsomal) fractions. Previous studies of synaptic junctional polypeptides during brain maturation have shown that the appearance and accumulation of the major PSD protein parallels the later periods of synaptogenesis (postnatal days 17 through 30) (Kelly, P. & C. Cotman, Neurosci. Abstr., 4:117, 1978). Together, these results support the notion that the major PSD protein is a molecular marker specific to asymmetric synapses (Gray Type II) in the CNS. This work was supported by NIH grant NS 15554.

123.3 INDIVIDUAL NERVE CELLS ARE IMMUNOLOGICALLY DISTINGUISHABLE IN THE LEECH. Birgit Zipser, Ron McKay*, John Farrar*. Cold Spring Harbor Laboratory, N.Y. 11724, Uniformed Services University of Health Sciences, Bethesda, Maryland 20014.

Immunological techniques showed that leech neurons are characterized by macromolecular specificity labels. Both conventional antisera raised against purified antigens and monoclonal antibodies raised against the entire leech nerve cord distinguish individual neurons in the stereotypic 400 neuron leech ganglion. The possibility exists that such antigens constitute cell markers for specific cell to cell interaction. Extensive data will be presented showing immunocytochemical staining of identified and identifiable neurons by monoclonal antibodies and conventional antisera against peptides. Our preliminary results strongly suggest that every leech neuron has one or more immunologically recognizable unique macromolecules.

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123.4 THE MORPHOLOGY OF NOCICEPTIVE NEURONS IN THE SKIN OF THE LEECH. Susanna Blackshaw* (SPON: J. G. Nicholls). Dept. Neurobiol., Stanford Med. Sch., Stanford, CA 94305.

The cell bodies of nociceptive or "N" cells in the leech lie within the central nervous system. They respond to noxious or painful stimulation of the skin. A great deal is known about the electrical properties of N cells, the morphology and physiology of their synapses, and the way in which they regenerate connections after injury. The receptive fields of individual N cells have been mapped physiologically and each of the 4 neurons within a ganglion innervates a specific quadrant of the body wall. There is however little information on the way in which individual axons branch and terminate within their receptive fields. N cells have now been visualised directly in the skin of the leech by injecting horseradish peroxidase into their cell bodies within the ganglion. Branches of the axon run within the network of peripheral nerves at deep levels of the body wall. The N cell terminals consist of fine axon branches that form continuous loops and spirals in close association with the hillock region of large neuron cell bodies that lie within the sheath of a peripheral nerve. Previous experiments have shown that N cells respond to noxious stimulation of the skin, and are not activated by temperature, pH, osmotic pressure or stretching the body wall (Nicholls, J. G. and Baylor, D. A., J. Neurophysiol., 31: 740-756, 1968). The finding that the N cell terminates on peripheral neurons raises the question of the role of the peripheral cells, and also the possibility that the N cells repond to some other form of stimulation in addition to noxious stimulation of the skin.

A striking feature of the N cell morphology was that the axon terminals were found at specific locations within the receptive field in animal after animal. This emphasizes the high degree of specificity of the connections of these neurons with their peripheral targets.

123.5 LECTINS AS MARKERS FOR STUDIES OF NEURONAL CONNECTIVITY. J.D. Coulter, M.C. Sullivan* and M.A. Ruda. Marine Biomedical Inst., and Depts. of Physiol. and Psychiat., Univ. of Texas Med. Branch, Galveston, TX. 77550 and Nat'l Inst. Dental Res., NIH, Bethesda, MD. 20014.

Lectins, a class of proteins with selective affinities for carbohydrates, were investigated as axoplasmic transport markers for light and electron microscopy. Two lectins, wheat germ agglutinin and concanavalin A, were found to be transported in both anterograde and retrograde directions, as well as, transneuronally. In rats, 0.1-2.0% solutions of lectin were injected (0.1-50 µl) into various well-defined neuronal pathways. These included subcutaneous injections in the vibrissal region for retrograde labeling of facial motoneurons and for anterograde labeling in trigeminal pathways, and intraocular injections for anterograde labeling in retino-geniculo-tectal pathways. Immunohistochemical (Sternberger, PAP method) localization of transported lectin was found to be superior to localization by histochemical methods for lectin-peroxidase conjugate. In facial motoneurons the subcellular localization of retrogradely transported lectin was examined in the electron microscope. Punctate reaction product occurred in proximal dendrites and the cell body cytoplasm, exclusive of the nucleus, and could be identified in lysosomes, multivesicular bodies, and discrete, circular foci over saccule-like profiles. In the spinal trigeminal nucleus anterogradely transported lectin was localized to the superficial laminae. Cell somata in this same region also contained reaction product suggesting transneuronal labeling. Intraocular injections of lectin anterogradely labeled retinal projections to the lateral geniculate and tectum. In the superior colliculus, reaction product was most dense in the stratum griseum superficiale, but also occurred, presumably by transneuronal transport, in cell bodies of the superficial layers and the stratum griseum intermediale. Suppression of anterograde/transneuronal labeling, while preserving substantial retrograde labeling, can be achieved by decreasing the sensitivity of the histochemical reactions. Neurons projecting to the spinal cord were retrogradely labeled with lectin in this way using $CoCl_2$ to yield a black, punctate reaction product in labeled cells. Sections were then stained with an antiserum to serotonin to give a brown reaction product (Bowker et al., this volume). In the raphe nuclei, spinally projecting neurons containing serotonin were identified by the presence of both the brown and black labels in the same cell. These results indicate that lectins are sensitive markers and show considerable promise for studies of neuronal connectivity in the light and electron microscope.

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123.6 WHY ARE NERVE CELL DENDRITES ARRANGED IN TREE SHAPES? J. D. Daniels. Division of Engineering and Center for Neuroscience, Brown University, Providence, RI 02912.

Notions that dendrites support surface area increase or enable algebraic summation of inputs are dismissed as inadequate to provide a necessary condition for the bifurcating structure of dendritic trees. We offer instead the idea that bifurcations are involved in a nonlinear, possibly binary logic, action upon the two distal dendritic branch signals which enter the bifurcation. Considering activity within the dendrites to be represented by positive logic only, we classify bifurcations as OR or AND. We then suggest that the bifurcation may be an important site for external influence of dendritic signals, and we offer a truth table definition for competitive inhibition, assuming inhibitory synapses make contact at the bifurcation.

We show an example which demonstrates the power of dendritic logic to solve problems of pattern recognition. Such solutions require the hierarchical tree structure which dendrites provide.

Dendo-dendritic synapses have a ready explanation from our point of view. Information partially processed by one section of a dendritic tree can be made available to a neighboring dendrite before the information is combined with other signals in the main part of the tree.

We review physiological data which demonstrate nonlinear dendritic "spikes". Such behavior could be a consequence of regenerative logic processing at bifurcations. We searched electron microscopic data and found that dendritic bifurcations are structurally different from other regions of dendrites. Bifurcations contain less microtubule material and often harbor Nissl bodies and endoplasmic reticulum.

A model is set forth which shows the neuron to be a biological digital-to-analog-to-frequency converter, with digital processes in the dendrites, analog conversion at the cell body, and voltage-controlled oscillation at the axon hillock.

We surveyed Golgi material from Cajal's studies of cortical neurons to compute an average of 4.4 separate dendrite trunks per cell body. The relative influences of these trunks on the cell body determine the digital-to-analog transfer function. We conjecture that changes in these relative influences can be an important basis for modification of a cell's response. For example, a binocular neuron may have separate trunks for left and right eye inputs; monocular deprivation may affect the relative strengths of the two trunk-to-cell body contacts without any necessary modification of the distal synaptic contacts.

123.7 ANALYSIS OF THE GEOMETRIC PARAMETERS WHICH GOVERN THE SHAPE OF DENTATE GRANULE CELLS IN THE MOUSE. R. S. Williams*& S. B. Matthysse, Harvard Medical School, Boston.

The mammalian central nervous system is composed of literally billions of neurons which are so complex in their branching structure that essentially no two are alike. Nevertheless, most share similarities in the size and shape of their somas and the tree-like structure of their neurites which permit a parcellation into morphologic subclasses. The geometric parameters of neuronal trees are governed to some extent by the genome, but in large part their characteristic shape is governed by the environmental mileu in which growth and differentiation occurs. Most quantitative studies of dendritic branching have concentrated on analysis of geometric parameters which govern size but are independent of shape. To date, no information is available as to which parameters govern the characteristic shapes of neurons regardless of their size.

In this pilot study granule cells of the mouse dentate gyrus were examined in Golgi impregnations using a computer-assisted microscope. One or more primary dendrites exit from the superior pole of each cell to enter the molecular layer. Multipolar neurons are confined to the outer 1/3 of the cellular layer. Over 50% of all branch points are found in the inner 20% of the molecular layer, the zone of convergence of association and commissural afferents. In general, the dendritic arbor inscribes a cone with its apex at the superior pole of the cell, and its base at the pial surface. The base of the cone is compressed into an ellipse the long axis of which bears no systematic relationship to the septo-temporal axis. Dendritic segments in the walls of the conical ellipse are highly axial. This characteristic shape derives from the narrow range of the angle of inclination made by the planes of the daughter branches with the ideal axis, and of the angle formed by the bisector of that triangular plane and a projection of the axis. Values for the angle between the daughter branches vary widely but cluster about a mean of 90^{o}. The range of values for these three angles coupled with the more widely varing values for numbers of segments and segment lengths can be used to synthesize tree-like structures of similar characteristic shape by computer simulation. Future studies will analyze how these shape parameters vary in response to genetic and environmental manipulation.

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123.8 DENDRITIC VARICOSITIES AND MICROTUBULE DISARRAY IN HUMAN CORTICAL NEURONS IN DEVELOPMENTAL FAILURE. D.P. Purpura, K. Suzuki, I. Rapin* and S. Wurzelmann*. Dept. of Neuroscience, Rose F. Kennedy Center, Albert Einstein College of Medicine, Bronx, NY 10461.

Although more than 200 causes of neurobehavioral deterioration in infancy have been catalogued there are few clues to common mechanisms whereby these diverse factors influence brain maturation. That dendrites of cortical neurons are particularly vulnerable to developmental perturbations has long been appreciated, but why this is so remains unknown. Studies of cerebral biopsy tissue from 5 infants with developmental failure suggest that mechanisms regulating microtubule orientation may be especially sensitive links in the chain of developmental events underlying dendritic growth and maintenance. Golgi studies of cortical tissue obtained at the time of diagnostic biopsy revealed variable dendritic abnormalities including reduced branching, decreased and abnormal spines, and short segment lengths in all subjects (ages 5-21 months). The most striking changes in dendrites occurred in neurons with otherwise robust dendritic arbors such as layers II and III pyramidal cells. In these neurons distal dendritic branches exhibited 'string-of-beads' varicosities, each 'bead' ranging from 1-3µm with intervening 'string' segments less than 1µm. Both the varicosities and intervening thin dendritic segments bristled with hairlike spines. Electron microscopy confirmed the presence of long thin spines, many with ribosomes present in the spine necks. Bundles of microtubules exhibited S- and U-shaped orientation patterns beginning at somadendritic junctions. In distal dendrites microtubule bundles criss-crossed in jumbled arrays in normal-appearing dendritic shafts and particularly in varicosities. Intervening 'string' segments were devoid of microtubules. Organelles usually confined to the perikaryon or proximal dendrites were frequently encountered in distal dendrites. While it is unlikely that the neurobehavioral deterioration in these infants is due to similar causes, nonspecific precipitating factors appear to induce a specific cytological lesion, microtubule disarray. Hence, disorganization of cytoskeletal elements may be a primary pathogenetic event underlying dendritic morphological abnormalities in developmental failure and profound mental retardation.

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123.9 THE NUCLEUS RAPHE DORSALIS: A MORPHOMETRIC GOLGI STUDY IN RATS OF THREE AGE GROUPS. S. Díaz-Cintra*, L. Cintra*, T. Kemper*, O. Resnick* and P.J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545.

Using Rapid Golgi and Nissl techniques, three major cell types: fusiform, multipolar and ovoid were identified in the nucleus raphe dorsalis (NRD) of male rats at 30, 90 and 220 days of age. One hundred neurons per age group encompassing all three cell types were selected for quantitative study. The major and minor axes of the cell body were measured at their maximal extent and the number of spines on the somal surface was counted. Dendritic number, extent, thickness and the number of spines along a 50 μ segment near the midpoint of the dendritic length in primary and secondary dendrites were quantified in each age group. The origin of the axon was characteristic for each cell type. All cell types showed an increase in the linear extent of their primary dendrites and spine density on primary and secondary dendrites between 30 and 90 days. The most striking age related changes were in the multipolar and ovoid cells involving the dendritic number, length, thickness and spine number and in the number of perisomatic spines. Between 90 and 220 days we found a significant decrease in the linear extent of primary dendrites in ovoid cells. Between 90 and 220 days there is an increase in the number of primary dendrites on the ovoid cells and in the number of secondary dendrites on multipolar cells. Between 30 and 90 days multipolar and ovoid cells show an increase in perisomatic spines. Between 90 and 220 days both multipolar and ovoid cells show a dendrite and somal spine loss, particularly in the multipolar cells. In the present study we found special relationships between the raphe cell dendrites and blood vessels throughout the NRD. In the ovoid and multipolar cells this relationship was established by dendrites at 30 days. At 90 and 220 days of age some cell bodies of ovoid cells were found to be closely applied to raphe blood vessels. During the period 30-220 days there is progressive increase in rostro-caudal length of NRD which is proportional to the increase in growth of the brain stem. The cells of the NRD mostly have characteristic reticular features, i.e., few, long, straight and poorly ramified dendrites. The fusiform cells have a special tangential orientation with respect to the medial longitudinal fasciculus (MLF) and were the most frequently encountered cell in the entire NRD. Some of the dendrites of these cells extend into and through the MLF. The dendrites of the multipolar cells of the NRD overlap with dendrites of the nucleus raphe medianus and with dendrites of both the dorsal and ventral nuclei of Gudden. (Supported by grants BNS 77-16512 (NSF), HD-06364 (NICHHD) and PHS International Fellowship 5 F05 TWO 2693-02).

123.10 STIMULATION OF NEURONAL GLUCOSE UTILIZATION BY ANTIDROMIC ELECTRICAL STIMULATION IN THE SUPERIOR CERVICAL GANGLION OF THE RAT. P. Yarowsky, A.M. Crane*, and L. Sokoloff. Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

Previous studies with the 2-deoxyglucose method demonstrated a linear relationship between frequency of electrical stimulation of the cervical sympathetic trunk and rate of glucose utilization (LCGU) in the superior cervical ganglion (SCG) of the rat (Yarowsky et al., Soc. Neurosci. Abstr. 5:421, 1979). The linear relationship was confined to the physiological range of impulse frequency of the SCG. The orthodromic stimulation resulted in functional activation of presynaptic and postsynaptic elements, as manifested by physiological responses in the distribution of the efferent nerves of the SCG; it was, therefore, uncertain whether the metabolic activation occurred in presynaptic or postsynaptic elements or both. Earlier studies have provided evidence that functional activation stimulates LCGU in presynaptic terminals (Schwartz et al., Science 205:723-725, 1979), but it has been uncertain whether cell bodies are similarly metabolically activated. The SCG appeared to offer an appropriate model system to resolve this question. The horseradish peroxidase technique has shown that the cell bodies of origin of the axons of the efferent internal and external carotid nerves are located in different regions of the SCG. (Bowers and Zigmond, J. Comp. Neurol. 288: 227, 1979). The 2-[^{14}C]deoxyglucose method was, therefore, applied to measure LCGU in the SCG of the rat during antidromic stimulation of the external carotid nerve (ECN). The experiments were carried out under combined urethane (700mg/kg) and α-chloralose (40mg/kg) anesthesia. The SCG was acutely deafferented bilaterally by section of the cervical sympathetic trunks. An intact branch of the ECN of the experimental side was placed on platinum electrodes and electrically stimulated for 45 minutes via a stimulus isolation unit. The control SCG was left unstimulated. Stimulation was initiated 5 minutes prior to the 45 minute period of measurement of LCGU. Effectiveness of stimulation was ascertained by recording of the compound action potential on the surface of the ganglion and by impedance plethysmographic monitoring of blood volume in the extracerebral tissues. Electrical stimulation resulted in increased LCGU above the control level only in the region of the SCG containing the cell bodies of origin of the ECN. The activation of LCGU was frequency-dependent; LCGU was linearly correlated with frequency of stimulation in the range of 0-15 hz ($r = 0.80$, $p < 0.001$). These results indicate that the energy metabolism of the perikaryon and/or dendrites is coupled to functional activity and that the 2-deoxyglucose method, when combined with antidromic stimulation, can be used to identify sites of origin of neurons of antidromically stimulated fiber tracts.

124.1 ULTRASTRUCTURE OF ENKEPHALIN NEURONS IN THE MONKEY NEOSTRIATUM AND GLOBUS PALLIDUS. M. DiFiglia, N. Aronin*, A. Liotta* and J.B. Martin. Dept. of Neurology, Mass. General Hosp. Boston, MA and the Endocrine Div., Mount Sinai Sch. of Med., N.Y.C., N.Y.

The immunoperoxidase method was used to localize leu-enkephalin (leu-enk) in the monkey basal ganglia. The leu-enk antiserum cross-reacts less than 0.1% with met enkephalin in radioimmunoassay. We studied affinity of the antiserum to extracts of monkey neostriatum by immunoprecipitation with excess leu-enk antiserum, followed by various separation methods. Preliminary results show that at the high concentrations (1:500) used in the immunoperoxidase method, leu-enk antiserum can bind with relatively high affinity to intact leu-enk and several larger, related species (4620 and 31,000 Dal) and with lower affinity to a peptide (1900 Dal) with separation characteristics of leu-enk-arg6.

Following colchicine pretreatment the neostriatum exhibits an intense immunostaining within numerous medium-size neurons (10-20 μm) and fine beaded axonal processes. The globus pallidus shows a dense plexus of fibers primarily in the outer segment. At the electron microscope level, caudate leu-enk somata have relatively large nuclei with little surrounding cytoplasm. They receive many synaptic contacts which are also present on proximal dendrites. Distal dendrites have many spines and both are contacted by various types of axon terminals. Such features are characteristic of neurons identified as spiny type I using the Golgi-EM method (DiFiglia et al, J. Neurocytol., in press). Immunoreactive axon terminals (1 μm) are abundant and contain many ovoid (50 nm) and some dense core (100 nm) vesicles. They form synapses with unlabeled cell bodies and primary dendrites. Less frequently they contact the distal shafts of unstained spiny dendrites and dendritic spines. Finally some leu-enk boutons synapse with axon initial segments of medium size cells. Most of the above contacts appear to be symmetric and at the synaptic site some of the postsynaptic elements contain subsurface cisterns or form slight swellings which invaginate the enkephalin profile. Peroxidase reaction product is also present in myelinated axons (0.5 μm) which are present throughout the neostriatal neuropil and collected into bundles. In the globus pallidus similarly labeled fibers are present, and in addition fine, unmyelinated immunostained axons (0.15 μm) are found. Leu-enk axon terminals ensheath and synapse with the shafts of long pallidal dendrites and occasionally with their emerging spines. The present results suggest that in the monkey neostriatum enkephalin-like peptides are contained within long axon spiny type I neurons. Moreover, it is likely that intrinsic leu-enk terminals belong to axon collaterals known to arise from these output cells which may also contribute enkephalin fibers to the globus pallidus. Supported by grants # 1-RO1-AM-26252-01 and # 1-F32-AM06143-01.

124.2 APOMORPHINE AND AMPHETAMINE INDUCE ASYMMETRIC CHANGES IN CEREBRAL GLUCOSE UTILIZATION IN RATS WITH UNILATERAL SUBSTANTIA NIGRA LESIONS. G.F. Wooten and R.C. Collins; Depts. of Neurology and Pharmacology; Wash. Univ. Sch. of Med. St. Louis, Mo. 63110

We have studied cerebral glucose utilization with the ^{14}C-2 deoxyglucose autoradiographic method in rats 21 days after unilateral 6-hydroxydopamine-induced lesions of the substantia nigra (SN) following treatment with apomorphine (Apo) or amphetamine (Amph). In "control" rats with only a unilateral SN lesion there was a 30-40% increase in glucose utilization in the ipsilateral globus pallidus (GP) and a 15-25% increase in the ipsilateral lateral habenular nucleus (LH).

Glucose Utilization after Treatment with Apo or Amph Compared to Unilateral 6-hydroxydopamine Lesioned Controls

	Relative to Lesion	Apo (0.5mg/kgSC)	Amph (2.5mg/kgSC)
Substantia Nigra	Ipsi	↑ 313%	↑ 53%
Pars Reticulata	Contra	↑ 50%	↑ 109%
Corpus Striatum	Ipsi	↑ 20%	NC
	Contra	NC	↑ 43%
Globus Pallidus	Ipsi	NC	↑ 18%
	Contra	↑ 13%	↑ 45%
Entopeduncularis	Ipsi	↑ 168%	↑ 27%
	Contra	↑ 33%	66%
Subthalamic Nucleus	Ipsi	↑ 47%	↑ 42%
	Contra	↑ 69%	↑ 89%
Lateral Habenular Nucleus	Ipsi	↓ 43%	↑ 33%
	Contra	↓ 24%	↓ 10%
Turning		Contraversive	Ipsiversive

No asymmetrical changes were noted elsewhere including neocortex, thalamus, or hippocampus.

Apo, a direct dopamine agonist, is thought to produce contraversive turning in rats with unilateral nigral lesions by stimulating supersensitive dopamine receptors in the ipsilateral CS. Amph, in contrast, presumably produces ipsiversive turning by releasing endogenous dopamine from nigrostriatal neuron terminals in the contralateral CS. It follows that the most striking changes produced by Apo were ipsilateral, and by Amph, contralateral to the nigral lesion. Elucidation of the exact significance of these alterations in glucose utilization in the local neuronal circuits of the basal ganglia awaits a precise determination of the neural elements in which these dramatic changes are occurring.

124.3 INTRACAUDATE DOPAMINE INJECTION BLOCKS APOMORPHINE-INDUCED GNAWING. R. E. Davis, J. N. Joyce, C. J. Rogers*, L. Kuhn*, and C. Van Hartesveldt. Psychology Dept., University of Florida, Gainesville, FLA. 32611.

High doses of apomorphine (APO) are known to induce stereotyped, repetitive behavior, characterized in the rat by continuous gnawing, licking, or sniffing over restricted regions of the environment. At least some components of these stereotypes may be influenced by dopaminergic mechanisms in the neostriatum. Large intrastriatal injections of dopamine (DA) produce intense gnawing behavior in rats pretreated with MAO inhibitors. Since the pretreatment may alter other neurotransmitters in various brain regions, we decided to investigate the effects of discrete intracaudate DA injections on both spontaneous and APO-induced behavior in the normal rat.

Male Long-Evans hooded rats were implanted bilaterally with 27 ga stainless steel cannulae stereotaxically directed at the dorsal or ventral anterior caudate nucleus (CN). Through these cannulae rats were administered either DA (100 μg/ .5 μl) or isotonic saline (.5 μl) followed 20 min later by a subcutaneous (SC) injection of either APO or saline. All rats received all drug combinations in a counterbalanced order. Immediately after intracaudate injection rats were observed for 20 min in a rectangular environment; they were then removed, given the SC injection, and returned to the same test chamber where they were observed continuously for 60 min. During these intervals the durations and frequencies of 10 different behaviors were recorded.

Intracaudate DA injections elicited frequent shifts from immobile, "trance-like" states to bursts of sniffing directed toward the floor and walls during rapid movements about the cage. Gnawing and licking behavior were never seen.

Intracaudate DA injections also influenced APO-induced stereotyped behavior. The appearance of stereotyped gnawing was blocked and the duration of stereotyped sniffing was increased after DA injection into the dorsal but not the ventral anterior CN. This shift in APO-induced stereotypes by intracaudate DA is similar to that seen after large 6-OHDA lesions of the CN. This evidence suggests that small, discrete injections of DA into the dorsal anterior CN may increase local recurrent inhibition in areas outside the sphere of our injections.

124.4 SYSTEMIC APOMORPHINE REVERSES INTRACAUDATE DOPAMINE-INDUCED CONTRALATERAL DEVIATION. J. N. Joyce, R. E. Davis, C. J. Rogers*, P. Craig*, C. Rodrigues*, and C. Van Hartesveldt. Psychology Dept., University of Florida, Gainesville, FLA 32611.

In previous work we have shown that dopamine (DA) injected unilaterally into the dorsal anterior caudate nucleus (CN) induces contralateral asymmetry in spontaneous ongoing behaviors. In order to further explore this phenomenon we administered apomorphine (APO) systemically to rats with unilateral intracaudate DA injections to determine whether the stereotyped behaviors elicited would be expressed contralaterally.

Male Long-Evans hooded rats were implanted bilaterally with 27 ga cannulae directed either at the dorsal or ventral anterior CN. Through these cannulae either DA (100 ug/ .5 μl) or isotonic saline (.5 μl) was injected unilaterally, and time spent in ipsilateral or contralateral postural deviation was recorded. Twenty min later 1 mg/kg APO was administered subcutaneously, and postural asymmetry recorded for 60 min.

Consistent with previous research, unilateral DA injection into the dorsal anterior CN induced significant durations of contralateral deviation; unilateral DA injection into the ventral anterior CN was less effective. Neither DA injection outside the CN nor vehicle injection inside or outside the CN induced contralateral asymmetry of behavior.

Subcutaneous injection of APO unexpectedly reversed the contralateral deviation induced by unilateral intracaudate DA injection. Stereotyped sniffing ipsilateral to the side of intracaudate DA injection was recorded. The ipsilateral asymmetry was of greater duration when DA was injected into the dorsal rather than ventral anterior CN. The reversal of postural deviation induced by systemic APO is consistent with the hypothesis that small injections of DA into the dorsal anterior CN may increase local recurrent inhibition of regions outside the sphere of our injections.

124.5 NON-DOPAMINERGIC NIGRO-STRIATAL NEURONS IN THE RAT. P.G. Guyenet, J.K. Crane* (SPON = P.E. Gold), University of Virginia, Department of Pharmacology, Charlottesville, VA 22908.

A double fluorescence technique was used to simultaneously examine in the same substantia nigra (SN) sections the cellular localization of a retrogradely transported fluorescent label (Evans Blue, EB) introduced in the striatum and the topography of the dopamine-containing cell bodies (DA). The animals were perfused according to the Faglu method of Blessing et al. (Neurosci. lett (1978) 9, 311) for DA histofluorescence and 25 µ frontal sections were cut on a vibratome, mounted and coverslipped in mineral oil.

Each fluorochrome could be visualized independently of the other by an appropriate selection of excitation wavelengths and filters. The location of single (EB+) and double (EB+DA+) labelled neurons was recorded throughout the SN on charts drawn from Nissl and Woelcke stained sections.

The unilateral injection of 0.2 µl of EB in various striatal locations (10 rats; the globus pallidus and overlying cortex but not the thalamus nor limbic areas were occasionally involved) resulted in 30 to 50% of the SN DA+ cells being EB+ ipsilaterally (60-100 cells/section). An average of 3 to 9 cells per section were EB+ but DA-; these were predominantly located in the upper half of the pars reticulata from its medial to its lateral edge depending on the injection site and were occasionally found in the pars compacta (PC).

In two animals 6-hydroxy-dopamine (6OH-DA, 1.2 µl, 4.8 µg in saline) was pressure injected 0.5 mm above the PC 10 days before introducing the 0.2 µl of EB in the striatum. The 6-OHDA resulted in the total (98%) disappearance of DA+ cells in all but the medial border of the SN and the adjoining A10 area. In the area of the SN essentially devoid of DA+ cells, 3 to 10 cells per section were EB+ and over 90% of these were DA-. Their general location was identical to that of the EB+ DA- cells found in control rats.

In 2 additional rats, 6 OHDA (1.2 µl, 4.8 µg) was injected 0.5 mm above the median forebrain bundle (level of subthal. nuc.) 6 days before injecting EB (0.2 µl, 10%) in the striatum. This procedure did not decrease significantly the number of DA+ cells ipsilaterally but decreased the total number of EB+ cells to 20-40 per section. The number and location of EB+ DA- neurons was the same as in non-6-OHDA treated animals (4-9 cells per section) but they accounted for 10 to 20% of all EB positive cells instead of 3 to 5% in non 6-OHDA treated rats.

In conclusion, the present study establishes the existence of a non-DA nigrostriatal pathway. The actual number of SN cells belonging to this pathway is still uncertain. Supported by a BRS award #5 S07 RR 05431-18.

124.6 OPIOID PEPTIDES, SUBSTANCE P AND SOMATOSTATIN IN THE STRIATUM: AN IMMUNOHISTOCHEMICAL STUDY IN THE CAT AND KITTEN. A.M. Graybiel, C.W. Ragsdale*, E.S. Yoneoka* & R.P. Elde. Dept. Psych.,MIT, Cambridge, MA 02139 & Dept. Anat., U. Minn., Minneapolis, MN 55455.

Immunohistochemical localization of met- and leu-enkephalin (ENK), substance P(SP), and somatostatin(SS) in the striatum was attempted in 5 cats and 2 kittens. Frozen 30-50µm sections were processed by the peroxidase-anti-peroxidase(PAP) technique and,in selected sections,by immunohistofluorescence with FITC-IgG. Control procedures included serial dilutions, substrate blocks, and omission of the primary antisera.

In the caudate nucleus of both cat and kitten distinct macroscopically visible inhomogeneities appeared in the distribution of enkephalin-like and SP-like immunoreactivity. In PAP cross sections ENK-immunoreactivity was organized into variably shaped patches and bands, 100-400µm wide and up to 1mm long, in which reaction product was more dense than in the surrounding matrix. The patches were darkest ventrally and many, especially dorsally, were bounded by thin (ca. 50µm) very pale septa. These were prominent in the kittens. In the adults, stain in the caudate nucleus was present both in cell bodies (8 to 26µm) and in fine granular material; the stained neuropil appeared to account for the darkness of the patches. Reaction product in the n. accumbens was darker than in the caudate nucleus and interrupted by 200-300µm bands of light staining. ENK-positive patches rarely appeared in the putamen. In two cats, sets of serial sections were stained alternately for acetylcholinesterase and enkephalin. There was a striking correspondence between the AchE-poor zones in the caudate nucleus ("striosomes") and the patches of dense opioid immunoreactivity in every pair of sections studied; matches also occurred in the n. accumbens.

Patches of SP-reaction product (50-200µm, up to 30 per section, some surrounded by pale septa) marked the caudate nucleus in the kittens. In the adults circumscript SP-positive 200-400µm wide zones were prominent dorsally in the caudate nucleus, while ventrally (and in n. accumbens) pale zones of low SP-like immunoreactivity were present. The SP-positive patches contained dark granular material in one case while SP-positive perikarya accounted for the darkness of the patches in a second case. SP-inhomogeneities could in many instances be lined up with AchE-poor zones visible in serially adjoining sections.

SS-like immunoreactivity was mainly confined to perikarya (cat: 8-20µm; kitten: 7-16µm) scattered throughout the striatum. Although some clustering of SS-positive cells was present, striosomal organization was not obvious in the material so far prepared.

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124.7 AXON COLLATERALS OF SUBSTANTIA NIGRA PARS RETICULATA NEURONS. A.B. Karabelas* and D.P. Purpura. (SPON. S.U. Walkley). Dept. of Neuroscience, Rose F. Kennedy Center for Research in Mental Retardation, Albert Einstein College of Medicine, Bronx, NY 10461.

Intracellular recording and intracellular HRP staining was employed to study the axonal branching of substantia nigra pars reticulata (SNR) neurons in barbiturate anesthetized cats. SNR neurons were antidromically activated following stimulation of the VM-VL thalamic nuclei and the intermediate layer of the superior colliculus with latencies of 0.6-2.8msec and 0.5-2.5msec, respectively. For up to 60% of the physiologically tested SNR neurons, axonal branching was demonstrated with antidromic activation from both sites. Caudate stimulation elicited IPSPs with latencies of 4-14msec in SNR neurons.

Axons of HRP-stained SNR neurons emerge from the soma or a primary dendrite. They run caudally and eventually turn dorsally. During their initial trajectory within the substantia nigra all the SNR axons emit 1-8 collaterals originating at distances of 90µm to 1850µm from the axon hillock. The majority of SNR axons has 4-8 collaterals of varying diameter. After branching, most SNR axons pursue a dorsal course towards the collicular intermediate layer, while some axons follow indirect routes. The collaterals of a single SNR neuron can be classified as local, ascending and descending. Local collaterals exhibit extensive branching and emit numerous terminals within the vicinity of the parent cell. Light microscopy, strongly suggests that en passage and terminal boutons of a local collateral contact dendrites of the parent cell. The ascending collaterals may bifurcate once or twice within the SNR and/or at long distances from the SNR. One such collateral has been observed to arborize and give off terminals within the dorsal pole of the subthalamic nucleus. At the thalamic level most ascending collaterals turn dorsally towards the ventral thalamic complex, while some continue more rostrally. Most of the SNR axons emit one long descending collateral which travels through the substantia nigra pars compacta where it may branch and give off terminals. These data indicate that a single SNR neuron 1) can send axonal branches to at least three separate locations and 2) can provide an extensive local plexus of terminals for intrinsic synaptic operations. (Supported by NIH NS-07512 and NIH HD-01799)

124.8 RECOVERY FOLLOWING DAMAGE TO THE ASCENDING DA SYSTEM: REVERSAL OF LESION-INDUCED CHANGES IN CEREBRAL ^{14}C-2DG UPTAKE. Michael R. Kozlowski*, Kim A. Neve*, John F. Marshall. Dept of Psychobiology, Univ. of California, Irvine, CA 92717.

Damage to the ascending dopamine (DA) system of the rat brain produces impaired orientation to sensory stimulation. Remarkably, almost complete behavioral recovery can occur postoperatively. Such damage also alters the ^{14}C-2-deoxy-D-glucose (2DG) uptake into the basal ganglia and associated brain regions (Kozlowski and Marshall, Brain Res., in press). We now report that many of these metabolic changes are reversed in animals which show behavioral recovery.

Male rats were given unilateral tegmental injections of 6-hydroxydopamine (6-OH-DA, 4.5 µg) into the ascending DA system. One group showed a profound deficit in orienting to contralateral somatosensory stimulation that persisted for the next six weeks (Unrecovered group). Other animals gradually recovered orientation over the next two weeks (Recovered group). The time course of the recovery is similar to the development of behavioral supersensitivity to i.p. apomorphine, measured using rotational behavior. ^{14}C-2DG (15 µCi/100 g, i.v.) was given either 3 d or 6 weeks after the 6-OH-DA injection. The animals were sacrificed 45 m later and their brains processed for autoradiography.

Three days after the 6-OH-DA injection, all animals which exhibited a loss of somatosensory orientation showed a characteristic pattern of altered cerebral ^{14}C-2DG incorporation. An ipsilateral decrease of ^{14}C-2DG uptake was found in several forebrain structures normally innervated by the ascending DA system (neostriatum, olfactory tubercle (OT), nucleus accumbens septi (NAS), and central amygdaloid nucleus). An increased labeling was observed in structures which receive striatal efferents (globus pallidus (GP), entopedunclear nucleus (EN), and substantia nigra, pars reticulata (SNr)). In addition, there was a decrease of ^{14}C-2DG uptake in the ventral tip of the internal capsule, an area through which striatal efferent fibers course. These changes in 2DG uptake persisted for at least six weeks in animals of the Unrecovered group.

In contrast, many of these asymmetries in ^{14}C-2DG uptake were reversed by six weeks in animals of the Recovered group. The asymmetries of labeling in the striatum, internal capsule, and several structures which receive striatal efferents (GP, SNr) were abolished. The asymmetries of labeling remained in the OT, NAS and EN.

These findings suggest that after damage to the ascending DA system, cellular changes can occur in the striatum and its output pathways (i.e., strionigral and striopallidal systems) which compensate for the decreased DAergic innervation and therefore may underly behavioral recovery.

124.9 DEVELOPMENT OF SUPERSENSITIVE GABA RECEPTORS IN SUBSTANTIA NIGRA AFTER EITHER CHRONIC BLOCKADE OF DOPAMINE RECEPTORS OR DESTRUCTION OF NIGROSTRIATAL DOPAMINE NEURONS. Karen Gale and Holli Bernstein*, Dept. of Pharmacology, Georgetown Univ., Schools of Medicine and Dentistry, Washington, D.C. 20007.

After 8 weeks of daily treatment with chlorpromazine or haloperidol, the density of binding sites for GABA in substantia nigra (SN) was increased by 35%, as previously reported (Gale, Nature 283: 569, 1980). This increase in nigral GABA receptors probably occurs in response to a loss of striatonigral GABAergic tone which is normally maintained by nigrostriatal dopaminergic activity. In order to determine whether the increased GABA binding could have a functional impact, the behavioral effects of intranigral muscimol were examined in rats which had been treated with chlorpromazine (20mg/kg) daily for 8 weeks. When infused into the SN bilaterally, muscimol, a potent GABA receptor agonist, is known to cause stereotyped sniffing and gnawing behavior. Rats which had been chronically treated with chlorpromazine showed a significant increase in sensitivity to the intranigral muscimol treatment; in these animals, a dose of 2.0ng produced marked stereotyped behavior, equivalent to that observed in control rats which had received 5.0ng. Similarly, supersensitivity of nigral GABA receptors was observed following virtually total destruction (>85%) of nigrostriatal dopaminergic neurons after 6-hydroxydopamine injections into either caudate-putamen or medial forebrain bundle. The data suggest that GABA receptors, located on non-dopaminergic neurons in SN, become supersensitive in response to the sustained loss of nigrostriatal dopaminergic function; these receptors may participate in the behavioral supersensitivity to dopamine agonists that develops after chronic interference with dopamine transmission.

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125.1 ONE EYE CAN INCREASE THE SENSITIVITY OF ANOTHER IN LIMULUS. R. B. Barlow, Jr., S. C. Chamberlain,* S. J. Bolanowski, Jr., L. A. Galway, Jr.,* and D. P. Joseph.* Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

Illumination of the median eyes (ocelli) can increase lateral-eye sensitivity by increasing efferent optic nerve activity. At night a circadian clock in the brain generates the efferent activity (Barlow et al., Science 197:86, 1977) which can be enhanced by illumination of the ocelli. During the day when no efferent activity is generated by the clock, ocellar illumination is ineffective. Since the efferent input to the lateral eyes increases their sensitivity, ocellar illumination causes an additional increase in sensitivity.

No reciprocal effect has been detected, that is, lateral-eye illumination does not increase the sensitivity of the median ocelli. On the other hand, lateral-eye illumination can shift the phase of the circadian clock, but ocellar illumination cannot. As a result of endogenous clock activity, both sets of eyes exhibit circadian rhythms in response to visible stimuli (400-700 nm). The median ocelli are also sensitive to ultraviolet radiation (λ_{max} = 370 nm), but the UV responses do not exhibit a prominent circadian rhythm.

The ability of the circadian clock to gate the ocellar influence on lateral-eye sensitivity requires a brain circuitry involving the neuronal processes of all three structures. Cobalt impregnation of the major afferent pathways shows that both sets of eyes innervate neighboring loci in the brain, but thus far tracing the neural pathways has not revealed the location of the circadian clock.

The Limulus visual system is not simple. Neural interactions among widely separated visual structures and a centrally located circadian clock point to a complex system. Although the functional organization of the system is not yet understood, one consequence of the neural interactions is that at night maximal lateral-eye sensitivity cannot be achieved in complete darkness. Maximal visual sensitivity appears to require ambient illumination--a case of negative dark adaptation.

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125.2 SPECTRAL SENSITIVITY AND PHYSIOLOGICALLY ADAPTIVE ASPECTS OF THE LATERAL AND PARIETAL EYES OF A WESTERN FENCE LIZARD SCELOPORUS OCCIDENTALIS. T.G. Hedberg, and D.J. Kimeldorf*. Dept. of Physiol. Biophys., Univ. of Illinois, Urbana, Illinois 61801 and Radiation Center, Oregon State University, Corvallis, Oregon 97331.

To clarify and compare functional and adaptive aspects of the lateral and parietal eyes of a lizard in which the parietal was well developed, electroretinograms were obtained from both eyes of Sceloporus occidentalis. The animals were collected in the field, dark adapted and anesthetized by oral administration of sodium pentobarbital. Under varying conditions of exposure to white light and monochromatic radiation the lateral eye exhibited extremely rapid dark adaptation, a linear stimulus-response correspondance and showed peak photosensitivity at 575 nm. Responses to monochromatic radiation were seen only between 425 and 700 nm. Responses in all tests were measured at an irradiance of 0.69 mW/cm^2 and as the amplitude of the b-wave component. Maximum amplitude at this irradiance was 40 μV, obtained from the lateral eye under white light. The parietal eye, situated dorsally on the cranium, after removal of the interparietal scale, showed an ERG in which b-wave amplitude was reduced to 20% of the lateral eye response under white light of identical irradiance. Relative to the lateral eye, the parietal displayed a greater spectral sensitivity (350-770 nm), a peak response at 570 nm and a secondary sensitivity peak at 375 nm. The isolated interparietal scale transmitted monochromatic radiation with a 90% attenuation of middle and longer wavelengths but a 40% attenuation of wavelengths between 360-385 nm. While the spectral sensitivity peaks of both eyes compare favorably with the colors of the surrounding environment and thus may be useful to contrast colors of potential prey or predators, the secondary sensitivity peak in the parietal eye at 375 nm. may indicate a greater biological involvement of UV in parietal function than previously suspected. Heightened UV sensitivity coupled with the bright sunlight and open, unshaded environment in which this species commonly occurs supports speculation that the parietal utilizes a ratio of variable UV intensity to more stable mid-range wavelengths in the zenith sky as a zeitgeber, to aid in its established function as a circadian rhythm synchronizer and thermoregulation mechanism.

125.3 NEURONAL AND PHOTORECEPTOR CELL TYPES IN THE FRONTAL ORGAN OF RANA PIPIENS: A GOLGI STUDY. W. D. Eldred, Dept. of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, N.Y. 11794.

The frontal organ, part of the anuran pineal complex, is a photoreceptive system capable of distinguishing different wavelengths and intensities of illumination. Little is known concerning the cellular elements underlying these physiological responses. The present Golgi study was undertaken to determine the detailed morphology of the cells present, and on the basis of their morphology several types of photoreceptors and neurons in the frontal organ were identified. Frontal organs from adult Rana pipiens were impregnated using procedures similar to those of Stell and Lightfoot (J. Comp. Neur. 159:473-502, 1975).

It is possible to divide the photoreceptors into two classes based on differences in their size and morphology. One class has a small, rounded cell body, averaging 13μm long by 9μm wide. The second class is larger, with a more elongated cell body averaging 24μm in length and 10μm in width. Both photoreceptor classes can send long (up to 50μm), branching processes from the bottom or side of their cell body to adjacent regions of neuropil.

In addition to these two classes of photoreceptors, impregnated neurons can be found with three types of dendritic arrays. The first type has a multipolar arrangement with long dendritic processes (up to 80μm) radiating uniformly from the cell body. These dendrites can exhibit small spines and varicosities along their length. The second neuronal type has a large elongated cell body with dendrites projecting primarily from the narrow ends of the cell body; thus producing a bipolar dendritic array. The third neuronal type has long dendrites projecting primarily from one side of its cell body which creates a unipolar dendritic pattern.

In some preparations, cutaneous nerve fibers in the region of the frontal organ were also impregnated. These small cutaneous sensory axons ramified primarily in the dermis and epidermis, but in several cases, they also sent numerous processes with varicosities into the frontal organ as well.

The existence of different morphological classes of photoreceptors and neurons suggests that the frontal organ is capable of complex neural processing of light stimulus information.

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125.4 MELATONIN EFFECTS ON CIRCADIAN ROD OUTER SEGMENT SHEDDING. M. P. White and L. J. Fisher. Departments of Psychology and Anatomy, Univ. of Mich., Ann Arbor, MI 48109.

The hypothesis that melatonin regulates circadian rod outer segment (ROS) shedding was tested by determining the effect of exogenous melatonin on ROS disposal.

Albino rats were adapted to a 14hr:10hr light-dark cycle for 2 weeks (light onset at 4 am). At 4:30 pm melatonin was administered as a subcutaneous implant or a single 100 ug injection. Injected rats and paired controls were sacrificed throughout the night and during the time of expected ROS shedding the next morning (5 - 8 am). Implanted rats were sacrificed at the time of shedding on the following 2 days, or 7 weeks later. To measure ROS shedding, phagosomes were counted and phagosome diameters were measured in electron micrographs of retinal pigment epithelium. Tissue samples from 130 eyes were analyzed. These counts were converted to size-frequency distributions, corrected for the sampling biases due to section thickness and eccentric sectioning of phagosomes.

The daily morning peak in large phagosomes (those with diameters greater than .375 um) was not abolished by melatonin treatment, nor did injected melatonin induce abnormal shedding. However, implanted melatonin increased the frequency of large phagosomes seen during the shedding peak to .233/um^2 retinal planimetric area, compared to .176/um^2 in controls ($p < .01$). Long-term melatonin implants resulted in higher frequencies of small phagosomes as well.

These data support the hypothesis that melatonin is involved in the control of ROS shedding. The failure of injected melatonin to affect shedding suggests that duration of melatonin exposure was an important factor.

In control eyes, the volume of phagosome material present during the shedding peak was .215 um^3/um^2 retinal planimetric area, 55% of which was due to large phagosomes. As this does not appear to account for the total daily volume of ROS disposal, small phagosomes may constitute a significant amount of the daily shedding. Alternatively, very many large phagosomes may be shed during the daily burst, but become reduced in volume so quickly that only a few are observed histologically.

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125.5 THE EFFECT OF A GLIOTOXIN, L-ALPHA-AMINOADIPIC ACID, ON THE FROG ELECTRORETINOGRAM. Roger P. Zimmerman. Departments of Neurological Sciences and Physiology, Rush University, Chicago, IL 60612.

The vertebrate electroretinogram (ERG) is the extracellularly recorded mass electrical response of the retina to light. Two major components of the ERG, the b-wave and the slow PIII component, are believed to be generated by the retinal glial cells (Mueller cells) in response to changes in extracellular potassium ion concentration caused by electrical activity of postsynaptic neurons and photoreceptors, respectively. L-alpha-aminoadipic acid (a structural analog of L-aspartate, a putative photoreceptor synaptic transmitter) abolishes the b-wave and slow PIII components while leaving that produced by the photoreceptors, the fast PIII, relatively unchanged. Histological examination of retinas treated with L-alpha-aminoadipic acid reveals that the retinal glial cells show signs of necrosis: dense cytoplasm and extensive vacuolization. These effects are reversible and are found after either in vivo or in vitro treatment with L-alpha-aminoadipic acid.

L-alpha-aminoadipic acid is a relatively ineffective blocker at the aspartate synaptic receptor when compared to D-alpha-aminoadipic acid. This point, and the reduction of the slow PIII component, argues for a direct effect on the Mueller glial cells. One possible hypothesis is that L-alpha-aminoadipic acid interacts with the high affinity glutamate uptake system, a glial mechanism for inactivation of amino acid neurotransmitters. Toxic but reversible consequences produce observable cytological changes and an inability to respond to changes in extracellular potassium ion concentration.

125.6 USE OF KAINIC ACID AND AMINOADIPIC ACID AS TOOLS FOR THE LOCALIZATION OF NEURONAL AND GLIAL NEUROTRANSMITTER RECEPTORS IN THE RETINA. P.F. Spano, M. Memo and M. Trabucchi (SPON: L. Beani) Dept.s of Pharmacology, Universities of Milan and Cagliari, Italy.

The search and the use of specific neurotoxins have become of particular significance in the last few years. The finding, in fact, that chemical compounds, such as kainic acid or aminoadipic acid, are capable to selectively affect neuronal or glial components, respectively, has provided the bases for new tools in neurochemical pharmacology. In the present report we show that the above mentioned compounds, injected intravitreally in adult rats, differentially affect retinal radioreceptor binding of various neurotransmitters and some enzymes considered specific markers of neuronal population or glial cells.

Adult Sprague-Dawley rats wheighing about 150 g, were administred intravitreally through the sclera either kainic acid (50 nmoles in 5 µl) or aminoadipic acid (100 µl in 10 µl) and then killed at various times. Radioreceptor binding and enzyme activities were measured in retina homogenates. Intravitreal injection of kainic acid elicited a rapid reduction in the activities of the enzymes in the biosynthetic pathways for ACh and GABA (CAT and GAD) suggesting, as previously described for chick retina, the ablation of retinal cholinergic and gabaergic neurons. In contrast, the administration of aminoadipic did not elicit any change in the activities of CAT and GAD, as previously found. However we observed that aminoadipic acid, contrary to kainic acid, elicited a remarkable reduction of glutamine-synthetase activity, which is considered to be selectively localized in the glial compartment of the retina. In addition the retinas of aminoadipic acid treated rats exhibited severe alterations of ^{3}H-GABA and ^{3}H-spiroperidol binding. Kinetic analysis of the results indicated that the observed alterations are due to a decrease in the number of binding sites (Bmax) with no change in the affinity constant. The entity of Bmax reduction for ^{3}H-GABA and ^{3}H-spiroperidol binding closely paralleled the reduction of retinal glial Muller cells (as observed by light microscopy) elicited by aminoadipic intravitreal administration. On the other hand the kinetics of other neurotransmitter receptors are not affected by aminoadipic acid. These results may overall indicate that glial cells, at least in rat retina, possess specific binding sites for GABA and dopamine.

125.7 THE EFFECT OF INTRAOCULAR KAINIC ACID ON CHICKEN RETINA. I.G. Morgan, C. Ingham*, F. Guldner* and D. Ehrlich* Department of Behavioural Biology, Research School of Biological Sciences, Australian National University, Canberra, 2600, Australia.

Intraocular kainic acid has been reported to destroy the amacrine cells of chicken retina (Schwarcz and Coyle, *Invest.Opthalmol.vis.Sci.* 16 (1977) 141-148). Using similar doses (60 nmol in 10µl adjusted to pH 7) we have found that other cell types are destroyed. One week after the injection, a population of small neurons in the ganglion cell layer was destroyed. They probably correspond to the displaced amacrine cells, since they were resistant to section of the optic nerve.

The horizontal cell layers were also eliminated. One day after injection, necrotic cells could be seen along the border between the outer plexiform and the inner synaptic layer. One week after injection the outer plexiform layer could no longer be seen by light microscopy. Under the electron microscope no trace of the complex synaptic arrangements characteristic of the outer synaptic layer, including the cone and rod terminals and their synaptic ribbons, could be seen. Nor could the complex array of horizontal cell and bipolar cell processes be detected. Instead the photoreceptor cell bodies appeared to sit directly upon layers of bipolar cell bodies. Although there were only slight changes in the numbers of photoreceptors, bipolar cells and ganglion cells there were changes in their morphology which suggested that their functioning had been severely disturbed.

Biochemical estimations confirmed that there had been extensive loss of cells, since one week after injection there was a marked decrease in the amount of DNA per retina. Other general parameters, including glutamine synthetase, a marker of the Muller glial cells, were less severely disturbed.

In contrast, neuronal transmitter systems were extensively lesioned. Taurine levels were normal despite the loss of the photoreceptor terminals, which suggests that taurine is not a photoreceptor transmitter, and that it is concentrated in non-terminal parts of the photoreceptor. The levels of aspartic acid were slightly decreased, consistent with a role for the amino acid as a transmitter in the photoreceptors. In addition, the activities of components of the GABA, glycine and acetylcholine neurotransmitter systems associated with horizontal and amacrine cells were markedly decreased.

The pattern of cell death, coupled with the biochemical changes, can be interpreted by assuming that the horizontal and amacrine cells are innervated by photoreceptor and bipolar cell terminals which use aspartic and/or gluatmic acid as transmitters. However, this assumption raises the question of why the bipolar and ganglion cells, which are innervated by photoreceptor and bipolar respectively, are not killed.

125.8 DEPLETION OF RETINAL TAURINE CONTENT BY GUANIDINOETHYL SULFONATE. Norma Lake. Department of Research in Anaesthesia, McGill Univ., Montreal, Canada.

Taurine is the most abundant free amino acid of the vertebrate retina, and over half is located in the photoreceptor cell layer where its concentration has been estimated to be 30-50 mM (Voaden, Lake, Marshall & Nathwani, Exp. Eye Res. (1977) 25, 249-257). There is evidence that one source of retinal taurine is the blood, via a blood-retinal transport system located in part in the pigment epithelium (frog: Lake, Marshall & Voaden, Brain Res. (1977) 128, 497-503; mouse: Voaden, Oreadu, Marshall & Lake, in press).

The source of blood taurine is either directly from the diet or from biosynthesis by peripheral organs such as the liver. As yet the functions of retinal taurine are unknown, however the importance of taurine for normal retinal function is indicated by the finding of specific photoreceptor degeneration and ERG abnormalities associated with reduced plasma and retinal taurine in cats placed on taurine-free diets (Schmidt, Berson & Hayes, Invest. Ophthal. (1976) 15, 47-51). The peripheral biosynthesis of taurine is virtually non-existent in cats, however taurine-free diets are ineffective in reducing retinal taurine levels in other species such as guinea pig, rat or rabbit probably because liver biosynthesis provides sufficient taurine to maintain retinal levels (Lake, Voaden & Morjaria, unpublished).

Recently Huxtable and colleagues have shown that treatment of rats with guanidinoethyl sulfonate (GES), a competitor with taurine for transport into the heart, leads to a rapid depletion of the taurine content of peripheral organs and some CNS sites (Huxtable, Laird & Lippincott, J. Pharmacol. Exp. Ther. (1979) 211, 465-471). The present study used a protocol similar to Huxtable et al. Male Sprague-Dawley rats were treated by adding 1% GES to their drinking water, while control rats consumed water with no additives. After 11 or 21 days their eyes were enucleated, the retina dissected and its free amino acids extracted into ethanol. Dual label microdansylation was used to estimate the levels of taurine, γ-aminobutyric acid, glycine, glutamate, aspartate and glutamine in the retinal extracts. Treatment with GES led to a reduction of retinal taurine to 73% of control after 11 days ($n = 6$; $p < .02$) and to 27% of control levels after 21 days ($n = 3$; $p < .001$). There were no significant changes in any of the other free amino acid levels.

These findings indicate that transport of taurine across the blood-retinal barrier is required to maintain retinal taurine levels in the rat, and that GES may be a useful tool to study the involvement of taurine in retinal function and degenerations.

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125.9 IDENTIFICATION OF SYNAPSES FOR Y-AMINOBUTYRIC ACID IN CHICK RETINA USING ^{3}H-MUSCIMOL. S. Yazulla and N. Brecha. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Long Island, NY 11794.

In the vertebrate retina several classes of neurons transport the neurotransmitter ^{3}H-Y aminobutyric acid (^{3}H-GABA). However, the identity of neurons which actually use GABA as their neurotransmitter and the distribution of their synaptic contacts are unknown. We attempted to answer these questions by localizing the binding sites for ^{3}H-muscimol, a GABA analogue, in the synaptic layers of the chicken retina by light microscopic (LM) and electron microscopic autoradiography (EM-ARG).

LM-ARGs of cryostat sections incubated in ^{3}H-muscimol or ^{3}H-GABA displayed identical binding patterns: a band over the outer plexiform layer (OPL) and a uniform band over the inner plexiform layer (IPL). This binding pattern differs from the transport pattern for ^{3}H-GABA which shows labeling over horizontal, amacrine and ganglion cell bodies as well as very intense labeling over lamina 5 in the proximal IPL. For further analysis, isolated retina were incubated in ^{3}H-muscimol and processed through EPON for EM-ARG. In the IPL, statistical analysis indicates that only amacrine synapses bind ^{3}H-muscimol (i.e. make GABAergic synapses). Processes of amacrine, bipolar or ganglion cells can be post synaptic to these amacrine synapses. The highest concentration of synapses binding ^{3}H-muscimol occurred in laminae 2 and 4 of the IPL and not in lamina 5 as might be expected from the density of ^{3}H-GABA transport. In the OPL, ^{3}H-muscimol binding occurs over specialized junctions proximal to photoreceptor terminals. The identity of these junctions is unknown, but they behave as integrated point sources of radioactivity. In cone receptor terminals ^{3}H-muscimol binding is suspected near horizontal cell dendrite/receptor terminal membranes lateral to the synaptic ribbon. This supports the hypothesis that horizontal cells are involved in a GABAergic feedback loop with cone terminals.

We conclude that the synaptic binding pattern provides a more accurate conception of GABAergic synaptic interaction than the transport pattern for ^{3}H-GABA because, in the IPL, the two patterns are unrelated. ^{3}H-muscimol can be an effective probe for GABA synapses when cautions are taken to account for neuronal transport of ^{3}H-muscimol. (Supported by NIH grant EY01682 and EYO 2146.)

125.10 LOCALIZATION OF NEUROPEPTIDES, INCLUDING VASOACTIVE INTESTINAL POLYPEPTIDE AND GLUCAGON, WITHIN THE ADULT AND DEVELOPING RETINA. N. Brecha, H. J. Karten and B. Davis*. Dept. of Neurobiology, State University of New York at Stony Brook, Stony Brook, N.Y., 11794.

The localization of several different neuropeptides within the pigeon and chicken retina was studied using immunohistochemistry. Pigeon and chicken retinas were fixed in 4% paraformaldehyde, 0.1M D,L lysine-HCl and 0.01M Na periodate in 0.1M phosphate buffer, cryoprotected in 30% sucrose buffer and sectioned at 10µM perpendicular to the vertical axis of the eyecup. Retinal sections were incubated in either enkephalin, neurotensin, somatostatin, vasoactive intestinal polypeptide or glucagon antiserum and processed according to standard immunohistochemical techniques Specificity to the antiserum was established by preabsorption of the antiserum with 10µM synthetic peptide prior to incubation of the retinal sections.

Previously, we reported the localization of specific enkephalin-like, substance P-like, neurotensin-like and somatostatin-like immunoreactivity to amacrine cells in the pigeon retina (Brecha, Karten and Laverack, '79; Karten and Brecha, '80; Brecha, Karten and Schenker, '80). This localization pattern was also observed in the chicken retina; enkephalin-like, neurotensin-like and somatostatin-like immunoreactive amacrine cells were characterized by multistratified processes which ramify within the distal (lamina 1) and proximal (laminae 3 and 4) laminae of the inner plexiform layer (IPL). In pigeon retina, specific vasoactive intestinal polypeptide-like containing amacrine cells are characterized by medium sized somata located at the border of the inner nuclear layer (INL) and IPL and bistratified processes which ramify within laminae 3 and 5 of the IPL. In pigeon and chicken retina, glucagon-like containing amacrine cells are also characterized by medium sized somata located at the border of the INL and IPL and bistratified processes which ramify within laminae 1 and 3 of the IPL.

The appearance of specific enkephalin-like immunoreactivity within the developing chicken retina was also studied using immuno histochemistry. Retinas from staged chick embryos and 1, 2 and 3 day posthatched chicks were processed for immunohistochemistry as described above. Within the embryonic chick retina, very sparse enkephalin-like immunoreactivity was detected within somata located in the proximal INL at stage 39. At later stages, (st.42-44), sparse enkephalin-like immunoreactivity was observed within both somata in the proximal INL and processes in the IPL. 24-48 hours after hatching the intensity of enkephalin-like immunoreactive staining within amacrine cells was similar to that observed in the adult retina. These studies have demonstrated a variety of peptidergic amacrine cell populations within the bird retina.

125.11 CHANGES IN RETINAL MORPHOLOGY AND LOCAL GLUCOSE UTILIZATION (LGU) IN 1 TO 34 MONTH OLD FISCHER-344 RATS. N.L. Shinowara*, S.I. Rapoport, M.J. Hoover* and E.D. London. Lab. of Neurosciences, Nat'l. Inst. on Aging, Gerontol. Res. Ctr, Balto. City Hospitals, Baltimore, MD 21224.

Local glucose utilization (LGU) and morphological changes were characterized and compared in retinas of 1-, 3-, 12-, 24- and 34-mo old albino male Fischer-344 rats reared in a 12 hr light/dark cycle. LGU was measured by the method of Sokoloff et al. (1977, J. Neurochem. 28:897) in light adapted retinas, optic nerves and superior colliculi from rats injected with ^{14}C-2-deoxy-D-glucose (2DG). Timed arterial samples were taken to measure radioactivity and free glucose and rats were killed after 45 min. Single retinas, dissected under saline, optic nerves and superior colliculi were assayed for total radioactivity by liquid scintillation spectroscopy. Morphological observations were made by light microscopy on plastic embedded 1 µ sections of retina strips. Measurements were made at peripheral, medial and central regions of the retinal strip and included the thickness of different retinal layers and the number of photoreceptor and inner layer nuclei. Whole retinal LGU significantly decreased between 3 and 12 mo from 59±8 to 37±3 µmoles/100g/min, plateaued at 24 mo with 34±5 µmoles/100g/min and further decreased by 34 mo to 15±3 µmoles/100g/min. Compared to the measured changes in retinal morphology from 3 and 34 mo, total decrease of 76% in LGU most closely paralled the continuous 86% decrease in mean photoreceptor nuclei for the 3 measured regions. During the developmental period from 1 to 3 mo there was a negative association between a significant increase in LGU and a 32% loss in mean photoreceptor nuclei which decreased in all 3 regions. Total retinal thickness decreased peripherally, remained unchanged medially and increased slightly centrally. Progressive retinal degeneration with photoreceptor cell loss occurred throughout the retina, but was most severe in the periphery, in agreement with Lai et al. (1978, Invest. Ophthalmol. 17:634). From 1 to 34 mo, 91% of the mean photoreceptor nuclei degenerated, while the mean inner layer nuclei decreased by only 39% with the greatest cell loss of 23% from 1 and 3 mo and a plateau from 12 to 34 mo. LGU in the superior colliculus, which receives retinal ganglion cell input, significantly increased by 29% from 1 to 3 mo, decreased by 23% from 3 to 12 mo and then plateaued with a slight decrease from 24 and 34 mo. Optic nerves showed an insignificant decrease in LGU from 3 to 34 mo. The results suggest that in the immature rats (1 to 3 mo) LGU does not decrease as a function of cell loss in the retina, but does increase in the superior colliculus. As retinal degeneration progresses in the aging adult, light-exposed eye, photoreceptor cell loss appears correlated with a decline in whole retinal LGU.

125.12 DISSOCIATED TISSUE CULTURE OF CAT RETINAL NEURONS. John K. Stevens, J. Martin Wojtowicz, John F. MacDonald, Playfair Neuroscience Unit, University of Toronto, and Toronto Western Hospital, 399 Bathurst Street, Toronto, Ontario, Canada, M5T 2S8.

Retinas from four week fetal cats were removed, and processed using conventional neural tissue culture methods. Connective tissue was enzymatically digested, remaining cells were mechanically dissociated and plated on collagen coated petrie dishes in a medium of MEM and 10% fetal calf serum. After three days the mitotic division of fibroblast cells was blocked and the medium was changed to a maintenance mixture of 10% horse serum and 90% MEM.

The cultures in early stages consisted of many nondistinctive spherical cells on a fibroblast base. At the end of one week, dendrites appeared, forming populations of monopolar, bipolar, and multipolar cells. A small number of cells also appeared to have axons. At the end of the second week the cultures were further differentiated and could be placed into four basic groups: 1) spherical small somas (SSS), approximately 10 microns in diameter with monopolar branching dendrites 2) SSS bipolar non-branching dendrites 3) SSS bipolar branching dendrites 4) irregular (square, triangular) shaped somas 10 to 50 microns in diameter with multipolar branching dendrites. Many of the class 4 cells appeared to have axons. At the end of the two week period individual cells migrated into tightly packed groups containing all four cell types. It appeared at the light level that dendrites were interconnecting within these groups to form a neural network. No photoreceptor-like cells were found in these cultures.

Intracellular recordings demonstrated that many of the cells in group 4 had synaptic potentials and clear action potentials (80 mv). Other class 4 cells displayed a small active all or non potential (2-5 mv) in response to depolarization. All other cell types had either no action potential at all, or a small potential similar to that described above. Resting potentials in all cell types ranged from -20 to -55 mv. Comparisons with our cell types and those found in the normal intact retina must await additional physiological and electron microscopic studies. (Supported by MRC grants MA-7345 and MA-7216).

125.13 VELOCITY TUNING OF RETINAL GANGLION CELLS IN TURTLE (PSEUDEMYS). J.E. Fulbrook, A.M. Granda, and J.H. Maxwell. Inst. for Neuroscience, Univ. of Delaware, Newark, DE 19711.

Receptive field (RF) properties of retinal ganglion cells (RGC) were investigated with flashing and moving light spots. We examined some of the spatial, temporal, chromatic and movement-sensitive properties of these cells. Here, we report on the velocity-tuning properties of a few specific RGC types.

Single units were isolated from optic nerves with tungsten microelectrodes. Spot stimuli were swept across RF centers at velocities ranging from 0.2 - 200°sec^{-1}. Velocity-tuning plots were made as a function of spike count or response frequency.

Most cells were sensitive to moving stimuli and were on the average twice as sensitive and more responsive to moving than to stationary, flashed stimuli. They responded as classic, transient on, off and on-off units, on-off being most common. About 5% of the cells gave sustained responses. 40% of the units were directionally selective (DS).

Some cells showed very broad velocity-tuning curves, responding over the entire velocity range. Other units showed narrower curves giving peak responses to either slow (4.5°sec^{-1}), moderate, or fast (45°sec^{-1}) stimulus velocities.

Units with preferences for slow stimulus velocities responded differentially within this range. Non-directional, center-surround, opponent-color cells failed to respond to stimuli moving faster than 4.5°sec^{-1}. Some DS units did not respond to stationary stimuli, but responded to velocities below 9° sec^{-1}, preferentially at 0.2° sec^{-1}. Most DS units, however, showed response peaks at higher velocities, had broader tuning curves, and responded to stationary stimuli.

One cell type responded optimally to fast-moving targets. These units gave on-off responses to stationary flashes but usually demonstrated only a single component response to moving stimuli. Peak responses were obtained between 90 - 160° sec^{-1}. These cells were non-directional with circular RF centers, 6-9° in diameter. The same velocity-tuning curve was obtained with both spike-count and spike-frequency criteria. Velocity preferences were essentially the same for different stimulus intensities, spot sizes and adaptation conditions for most of the units tested.

These results support the notion that a high degree of visual information is processed at the retinal level in turtle.

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125.14 BETA GANGLION CELLS RECEIVE CONVERGENT INPUT FROM 2 TYPES OF CONE BIPOLARS. Barbara A. McGuire*, John K. Stevens, Peter Sterling (SPON.: P. Sterling). Anatomy Dept., Sch. of Med., Univ. of Penn., Phila., PA 19174.

The inner plexiform layer of cat retina is stratified into sublamina a, which contains the dendrites of off-center ganglion cells, and sublamina b, which contains the dendrites of on-center ganglion cells. We have identified two types of cone bipolars in each sublamina by reconstructing adjacent cells from electron micrographs of 188 serial sections taken near the area centralis. Both members of a bipolar pair converged onto the same ganglion cell.

In sublamina a, the first type of cone bipolar had pale processes with smooth contours and made many reciprocal connections with the lobular appendages of AII amacrines. The second type had dark processes with scalloped contours and made only rare contacts with AII cells. This type has been determined by electron microscope autoradiography to accumulate ^{3}H-glycine.

In sublamina b, the first type of cone bipolar had pale processes which branched and made synapses (ribbon contacts and gap junctions with AII cells) throughout the sublamina. The second type had darker cytoplasm, branched only in the outer half of the sublamina, and made fewer gap junctions with AII cells. This cell type also accumulated ^{3}H-glycine.

Both members of a pair of cone bipolars converged strongly onto the dendrites of the same medium-sized (β) ganglion cell. For example, a β cell arborizing in sublamina a received 23 ribbon contacts from a single pale bipolar and 14 contacts from a dark bipolar. Similarly, a β cell arborizing in sublamina b received 49 ribbon contacts from a single pale bipolar and 33 contacts from a darker bipolar.

The convergence onto β cells (presumed X-cells) of two types of cone bipolars might suggest that one cone bipolar in each sublamina is excitatory, and the other, possibly the cell that accumulates ^{3}H-glycine, is inhibitory. An on-center X-cell's excitation might then result from the excitatory input of a depolarizing cone bipolar, and its inhibition might result from the inhibitory input of a hyperpolarizing cone bipolar. X-off cells would have the complementary arrangement. Convergence of excitatory and inhibitory cone bipolars may therefore underlie the anatagonistic properties of X-cells' receptive fields.

125.15 GLUCOSE USE CHANGES IN THE SUPERIOR COLLICULUS AFTER CHOLINERGIC ACTIVATION AND INHIBITION OF RAT RETINA. S.R. Nelson, T.L. Pazdernik,* R.S. Cross* and F.E. Samson. Dept. Pharm. and R.L. Smith Research Center, Univ. of Kansas Med. Ctr., Kansas City, Ks. 66103.

The 2-deoxyglucose functional mapping procedure developed by Sokoloff et al. was used to measure changes in glucose regional metabolism induced by the acetylcholine esterase inhibitor, diisopropyl fluorophosphate (DFP). Thirty minutes after DFP injection (i.m., 2.2 mg/kg) glucose use in many gray and white areas is depressed. One exception is a striking increase in the stratum griseum superficial (SGS) of the superior colliculus. The rate of glucose use of the SGS was 61 μmoles/100g/min in 8 control rats, whereas after the DFP injection, the rate of glucose use in the SGS rose 36% to an average of 83 μmole/100g/min (3 rats). Pretreatment with the nicotinic cholinergic blocker, mecamylamine (i.m., 75 mg/kg) prevented the increase from DFP and decreased the rate in the SGS to 36 μmole/100g/min. The muscarinic blockers atropine or benactazine did not prevent the DFP activation of the SGS.

Since the retina sends fibers to the SGS, the question was raised that the increased metabolism in SGS with DFP might be a result of increased activity coming in from the retina. DFP (0.26 μg) injected into one eye of anesthetized rats induced an increased rate of glucose use in the contralateral SGS (3 rats). However, when mecamylamine (7.5 μg) was injected into the eye 30 min. before DFP (2 rats), the response of the SGS was prevented. These results support the view that cholinergic fibers are involved in the activation of retinal ganglion cells and that these fibers are largely nicotinic.

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125.16 GANGLION CELL PROJECTIONS TO THE SUPERIOR COLLICULUS, LATERAL GENICULATE, PRETECTUM AND VENTRAL LATERAL GENICULATE IN THE CAT. K. Itoh*, M. Conley* and I.T. Diamond. Dept. of Psychology, Duke University, Durham, NC 27706.

The goal of this study was to identify retinal ganglion cells after restricting HRP to single layers of the lateral geniculate, to single layers of the superior colliculus or to other known targets of the optic nerve. After each injection we tried to identify the labeled cells in terms of the morphological classification proposed by Boycott and Wässle (1974), α, β, and γ cells. Electrophysiological evidence has led many researchers to believe that these morphological classes correspond to physiological classes usually designated as Y, X, and W. Since electrophysiological studies of the lateral geniculate and other targets of the optic tract also reveal the same physiological distinctions, one could predict that the α or β cell might project to some, but not to other, layers of the lateral geniculate nucleus. The present results support the idea of a close correspondence between the cytoarchitectonic features of the various visual centers and the types of ganglion cells that project to them. For example, when HRP is injected into those layers of the lateral geniculate which contain the largest neurons, in particular, A, AI, C, and MIN, then a large proportion of the labeled cells in the retina appear to be of the α class. Similarly, a large number of α class cells were labeled after injections of lower SGS of the superior colliculus and the nucleus of the optic tract in the pretectum. On the other hand, very small ganglion cells, presumably of the γ class, were labeled after injections in the upper SGS of the superior colliculus, the olivary pretectum, the nucleus of the optic tract, the parvocellular C layers of the lateral geniculate, and the ventral lateral geniculate body. Cells of the β class are not so easy to distinguish with the HRP method, but the evidence suggests that the cells of this type project to layers A and AI of the lateral geniculate body.

The long run goal of this research is to shed light on the functional significance of parallel visual pathways. It is now possible to identify separate pathways in terms of cell size and fiber size from the retina to the cortex. For example, the smallest cells of the retina project to the small celled C layers of the lateral geniculate as well as upper SGS. The small celled C layers also receive fibers from upper SGS and project to the entire visual field from the middle suprasylvian sulcus to the caudal extremity of the striate cortex. Where these pathways overlap, e.g. in the striate area, they terminate in separate layers. Thus, the small celled small-fiber pathway terminates in layer I, while the large celled, large-fiber pathways terminate in the deeper layers, primarily III and IV.

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125.17 MORPHOLOGICAL DIFFERENCES IN NASAL AND TEMPORAL RETINA OF THE CAT. V. L. Wise*, E. J. DeBruyn* and V. A. Casagrande, Depts. of Anatomy and Psychology, Vanderbilt Univ., Nashville, TN 37232

Previous investigations of the cat visual system suggest that nasal and temporal retina may be differentially specialized. For example, isodensity maps of the cat retina show that for a given eccentricity, the temporal retina exhibits a lower cell density than nasal retina (Stone, '78). Furthermore, studies of lateral geniculate laminae receiving from the two retinal divisions suggest that contralaterally innervated layer A contains proportionally smaller cells and fewer Y cells than its ipsilaterally innervated counterpart (Hickey et al., '77; Sireteanu and Hoffman, '79). To determine if morphological differences exist for the two retinal divisions, we have compared cell sizes along the horizontal meridian in the nasal and temporal retina of normal cats using samples matched for either density or eccentricity. Density matched samples were obtained from the area centralis (AC) and areas of 4000, 3000, 2000, and 1000 cells/mm^2. Eccentricity matched samples were taken at 1.0, 1.5, 4.0, 5.0, 5.5, and 6.0 mm from the center of AC. At each sample point, every cell in 1-6 0.01 mm^2 fields was drawn at 1000X. Two sets of comparisons were made. The first set included all cells within a sample while the second group included only geniculate relay cells as determined by retrograde horseradish peroxidase (HRP) labelling. For these experiments, each cat received 2-20 injections of .2-.5 µl of 30% HRP in layers A and A_1 of the lateral geniculate nucleus (LGN). Retinal whole mounts were reacted with Hanker-Yates reagent and counterstained with cresyl violet. Results indicate two major differences between the nasal and temporal divisions. First, although no differences exist for eccentricity matched samples in the AC, the mean cell size of temporal ganglion cells is as much as 46% larger at greater eccentricities. Second, no significant size differences exist for density matched samples; however, the size-frequency histograms indicate that temporal retina contains a greater percentage of alpha (Wässle et al., '75) and large beta cells. A separate analysis fo the HRP cases indicates similar trends for cells of the retino-geniculate pathway. These results support the hypothesis that nasal and temporal retina are morphologically and therefore possibly functionally distinct.

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125.18 TREE SHREW RETINAL GANGLION CELLS: DIFFERENCES IN NASAL AND TEMPORAL RETINA. E. J. DeBruyn* and V. A. Casagrande (SPON: A. M. Burt). Depts. of Anatomy and Psychology, Vanderbilt Univ., Nashville, TN 37232

Recent investigations of the organization of the retina have suggested that information processing in the nasal and temporal retinae may differ. Our own work and that of others has demonstrated nasal-temporal differences in the topography and sizes of ganglion cells (Wässle et al., '75; DeBruyn and Casagrande, '78; Provis, '79; Wise et al., '80). For example our ganglion cell isodensity maps of tree shrew retina show that at comparable eccentricities the temporal retina exhibits lower cell densities than does nasal retina, suggesting that mean cell area of temporal ganglion cells may be increasing more rapidly with eccentricity. In order to examine the parameters of this difference in the tree shrew, we used cresyl violet-stained whole mounts to compare three specific aspects of the ganglion cell size-density relationship. First, we examined cell density at points of equal eccentricity from the area centralis; second, we compared cell size distributions at these points; and third, we compared cell sizes at points of equal cell density. Samples were taken along the horizontal meridian and at selected points in the upper and lower retina such that matched samples were taken on the same azimuth. At each sample point, every cell in 1-3 0.01 mm^2 fields was drawn under oil immersion at 1000X.

Frequency histograms of cell sizes suggest several differences between nasal and temporal retina. First, the mean cell size is significantly smaller ($p<.001$) in the nasal retina for points of equal eccentricity. Second, although the mean size of temporal cells is larger than that of nasal cells at points of equal density, the difference is not statistically significant and can be attributed to a greater proportion of large (>100 μ^2) ganglion cells in the temporal retina. Third, at points of equal density, the mean size of cells in the superior retina does not differ from that in the inferior retina.

These results demonstrate that 1) at comparable eccentricities temporal ganglion cells are larger than their nasal counterparts, and 2) that at comparable densities, the difference in mean sizes of nasal and temporal cells is not statistically significant, but the percentage of large cells is increased. Taken together these morphological differences support the hypothesis that nasal and temporal retina may process information differently.

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125.19 RETINAL GANGLION CELLS IN THE NORTH AMERICAN OPOSSUM (DIDELPHIS VIRGINIANA): DECUSSATION AND CENTRAL PROJECTIONS OF CELLS OF DIFFERENT SOMA SIZE CLASSES. P. D. Wilson and D. H. Rapaport. Psychol. Dept., Univ. of California, Riverside, CA 92521.

A previous investigation (Rapaport, D. H. et al., Anat. Rec. 190: 158, 1978) indicated that the retinal ganglion cells in the opossum can be divided into four groups on the basis of soma size, and that the size groups differ in their retinal distribution, in that small and small-medium cells are relatively more common in nasal and inferior retina, large-medium cells are more prominent in superior temporal retina, and large cells are fairly evenly distributed. In order to study the central projections of these size groups, horseradish peroxidase (HRP) was injected into the optic tract (OT) and superior colliculus (SC) by pressure injection and into the lateral geniculate nucleus (LGN) by iontophoresis. After 3-4 days survival, the animal was perfused with 1.25% gluteraldehyde: 1% paraformaldehyde, and the retinae were reacted with Hanker-Yates reagent, whole-mounted on slides, and counter-stained with cresyl violet.

Following HRP injection into one OT, nasal retina was labelled only contralaterally and the distribution of labelled cells closely matched the size distribution of the total population of ganglion cells in nasal retina. Temporal retina was labelled both contra- and ipsilaterally, showing that a significant proportion of ganglion cells in temporal retina project contralaterally. Labelled cells in contralateral temporal retina included small (8-13 µm), small-medium (14-18 µm), and large (25 µm dia.) ganglion cells, while those in ipsilateral temporal retina were predominantly large-medium (19-24 µm) and large cells, though some small-medium cells were labelled.

HRP injected into the SC labelled cells in all size classes with a predominance of small-medium cells labelled in a contralateral retina and large cells in ipsilateral temporal retina. HRP injected into the dorsal LGN labelled small-medium and large-medium cells in both contra- and ipsilateral retinae, and large cells in only the ipsilateral retina. The differences in the central projections of ganglion cells of different size groups suggest that these groups may differ in function. (Supported by Biomedical Support Grant UCR and Predoctoral Fellowship MH07204 to D.H.R.)

125.20 COMPARISON OF PRIMARY RETINAL PROJECTIONS AND RETINAL GANGLION CELL SIZE BETWEEN PIGMENTED AND ALBINO RABBITS. Ellen S. Takahashi and Clyde W. Oyster. School of Optometry/The Medical Center, University of Alabama in Birmingham, Birmingham, AL 35294.

Albino animals have abnormal primary retinal projections characterized by reduced ipsilateral input. These projections have been previously described in the rabbit and the patterns of abnormal projection in the retinogeniculate pathways have been compared in some detail in rabbits of the albino allelomorphic series.

In the present study, an autoradiographic technique used to compare the primary projections between pigmented and albino rabbits confirmed and extended the results of previous studies, but also revealed some differences. In the pigmented rabbit, the retina projects bilaterally to the dorsal and ventral lateral geniculate nuclei and an intergeniculate leaflet, the olivary and posterior pretectal nuclei, the superior colliculus, and the medial terminal nucleus of the accessory optic septem (AOS). The anterior pretectal nucleus and the nucleus of the optic tract, and the dorsal and lateral terminal nuclei of the AOS receive contralateral inputs only. In the albino rabbit, all of the ipsilateral inputs were substantially reduced and/or their patterns of projection were different from those of pigmented rabbit. The ipsilateral projection to the intergeniculate leaflet, the posterior pretectal nucleus and medial terminal nucleus were not observed in the albino rabbit.

In certain respects, the retinas of pigmented and albino rabbits were similar when studied as flat mounted cresyl violet stained preparations. Both had a visual streak in which the peak ganglion cell density was about 5000 cells/mm^2, and the decline in cell density away from the visual streak was comparable. The retinas differed, however, in the size (cross-sectional area) of the ganglion cell somata. On average, ganglion cells were larger in the albino retina, and this difference was more pronounced in the nasal hemiretina in and near the visual streak. The superior retina in pigmented rabbit had more large cells than the inferior retina and was not distinguishable from the albino superior retina.

These results may be related to a different representation of functional ganglion cell classes (and therefore different soma sizes) in the albino retina and may also be reflected in the specific projections to central nuclei. (Supported in part by NIH Grant #EY02207.)

125.21 CONDUCTION VELOCITY GROUPS AND CENTRAL PROJECTIONS OF RABBITS' OPTIC TRACT. I. Lessard* and S. Molotchnikoff. (SPON: F. Leporé). Dépt. Sciences biologiques, Université de Montréal, Qué. Canada.

Axons of retinal ganglion cells can be classified in three groups in most mammals studied to date. The common criteria for this tripartite division are the conduction velocity and central projections. Since these classes of ganglion cells have not yet been detected in rabbits, this study was undertaken. In anesthetized and paralyzed rabbits, action potentials were elicited antidromically following electrical stimulation of optic tract terminals at the geniculate level (LGN). Peak latencies were converted to conduction velocities in order to account for distance variations from animal to animal. Over 150 axons were studied. The conduction velocity spectrum extended from 7 m.s. $^{-1}$ to 34 m.s $^{-1}$. The distribution of conduction velocities indicated 4 major modes at 10, 18, 22 and 26 m.s $^{-1}$. Antidromic compound action potentials exhibited a good correlation between the conduction latency (measured at the peak of 4 negative waves) and the major modes of the distribution histogram. These results suggest that the rabbit's optic tract contains four classes according to their conduction velocity.

The central projections of retinofugal axons were studied with electrical stimulations of the Superior Colliculus (CS) and the LGN while recording from the same optic tract fiber. Antidromic spikes could be elicited from all conduction velocity groups and 74% (N = 31) of axons responded to both sites of stimulation. This indicates that most retinofugal fibers branch off and innervate the CS and the LGN. In at least one fiber the response latency to a geniculate pulse was about equal to the response latency to a collicular stimulation (< .1 msec) in spite of the longer distance (5 mm) travelled by the spike originating at the collicular terminal. This result suggests that some retino-collicular collaterals have a larger diameter than the retino-geniculate branch. Further, there is a definite tendancy for fast conducting axons to have their receptive field located eccentrically relative to the optic axis of the eye.

Supp. DGES and CRSNG to SM.

125.22 RETINAL INPUTS TO LIMBIC, AUDITORY, AND MOTOR AREAS IN THE RAT. S.K. Itaya, Dept. of Anatomy, Univ. of Iowa, Iowa City, IA 52242.

Visual pathways in mammals are characterized by a constancy found throughout this class of vertebrates. With minor variations, retinal input is known to seven areas: (1) dorsal lateral geniculate nucleus (LGN), (2) ventral LGN, (3) suprachiasmatic nucleus, (4) pretectum, (5) pulvinar/lateral posterior complex, (6) superior colliculus, and (7) accessory optic system. Following intraocular injections of HRP and using the TMB incubation (Mesulam, 1978), we have followed anterogradely transported HRP from the eye to apparent terminals in limbic, auditory, and motor areas of the rat CNS.

A small number of retinal fibers leave the dorsal LGN and brachium of the superior colliculus to travel rostrally. Some fibers travel with the stria terminalis along its dorsomedial aspect, and other fibers can be followed over the dorsal surface of the lateral-posterior, lateral, and anterior-dorsal thalamic nuclei. The terminals appear to be located mainly in the bed nucleus of the stria terminalis. A somewhat similar pathway has been reported to the anterior-dorsal nucleus in the tree shrew (Conrad and Stumpf, 1975).

A small bundle of retinal ganglion cell axons can be traced caudally from the superior colliculus into the inferior colliculus. They travel mainly just beneath the surface of the inferior colliculus along its superior to caudal extent. Occasionally, fibers alter their course and travel toward the center of the inferior colliculus where they appear to terminate. In addition, in longitudinal sections containing the caudal-most portion of the optic tract as it enters the dorsal LGN, fibers of the optic tract deviate around the medial geniculate nucleus, and scattered fibers can be followed throughout the medial geniculate nucleus.

The medial terminal nucleus (MTN) (or nucleus tractus optici basalis) of the accessory optic system receives a very heavy projection from the retina in the rat. The MTN is a compact nucleus situated just medial to the cerebral peduncle and the substantia nigra. With dark field optics, scattered fibers can be observed which appear to leave the MTN and travel into adjacent substantia nigra. These fibers may terminate within the substantia nigra since they do not appear to continue as axons of passage. Several reports in the literature, all employing degeneration techniques, disagree as to the existence of a retino-nigral pathway.

Retinal information conveyed to several areas of the brain not involved in vision suggests that visual stimuli directly influence other sensory and motor systems.

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125.23 THE LATERAL VISUAL PATHWAY OF THE GIANT BARNACLE. K.A.French, J.H.Hayashi*, L.A.Oland*, and A.E.Stuart. Depts. of Physiology & Ophthalmology, Univ. of North Carolina, Chapel Hill, N.C. 27514

The visual system of the giant barnacle, Balanus nubilus, comprises 10 photoreceptor (PR) cells divided among three eyes, one median and two lateral, that mediate a withdrawal reflex to shadows. The PRs, second-, and third-order cells of the median visual pathway are known (Stuart,A.E. & Oertel,D., Nature 275:287, 1978). We now describe the PRs of the lateral eyes and the effect on higher-order cells of shadowing them.

Each lateral eye consists of two large and one small PR whose axons are 15-30mm long. Cobalt backfills of the lateral ocellar nerve show that these axons enter the supraesophageal ganglion (SEG) and end near the median PR terminals. The light responses of the lateral PRs resemble those of median PRs. Dim illumination depolarizes the cells and increases the amplitude of their voltage fluctuations above that measured in the dark. Brighter light elicits a transient peak that decays to a steady plateau. A hyperpolarization follows the offset of light.

Simultaneous impalements of two lateral PRs show that these cells are not electrically coupled, unlike their equivalents in B. eburneus and B. amphitrite. First, neither depolarizing nor hyperpolarizing current pulses passed into one cell were detected in the other. If the cells are coupled, the coupling ratio must be less than 1:150. Second, Ca-dependent action potentials generated in one PR by bathing the preparation in tetraethylammonium ion caused no voltage change in the other cell. Third, there was no correlation between the voltage fluctuations seen in the two cells in dim lights.

Shadowing a lateral eye produces a burst of spikes in both ipsi- and contralateral circumesophageal connectives from the SEG, as does shadowing the median eye. While bursts mediated by the median eye are similar in the two connectives, the lateral eye produces a stronger burst in its ipsilateral connective. Both eyes drive some, but not all, of the same units in a connective. The system is sensitive to the direction of shadow movement across the eyes. Shadowing first the lateral eye and then the median elicits a burst in each connective as each eye is darkened; however, moving the shadow in the opposite direction elicits a burst only when the median eye is darkened. The lateral eye output is silenced unless more than 500msec separate the shadows.

The spatial separation of the eyes in the animal, and our observations of the interaction of their signals in the ganglion, indicate that the three eyes confer upon the barnacle a spatial sensing capability.

We thank Leslie C. Timpe for participation in initial experiments. Supported by NIH grant EY03347.

126.1 IMMUNOHISTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN RABBIT FOREBRAIN. K.-S.K. Kan, L.-P. Chao and R.A. Giolli. Dept. Neurol. Sch. Med. UCLA, Los Angeles, CA 90024 and Dept. Anatomy, UC Irvine, CA 92717

Choline acetyltransferase (ChAc) catalyzes the synthesis of the neurotransmitter acetylcholine and is considered to be the specific enzymatic marker for cholinergic neurons. ChAc has been purified from bovine caudate nuclei and the homogeneity of the purified preparation has been demonstrated with electrophoresis, specific chemical analyses, as well as degradations, immunological criteria and direct visualization at the neuromuscular junctions (Muscle & Nerve, in press; J. Neurosci. Res. A Review, in press). Antibody to the purified bovine ChAc was produced in guinea pig and it cross-reacted with ChAc from rabbit brain. Immunohistochemical localization of ChAc in rabbit brain (Brain Res. 146: 221, 1978) was carried out with formalin-fixed, paraffin-embedded serially sectioned forebrain oriented in transverse, horizontal and sagittal planes. Some of the cholinergic structures in the forebrain from adult albino rabbits will be briefly described. The distribution of the cholinergic structure, as demonstrated by the immunohistochemical method, in the olfactory bulb is consistent with the biochemical measurements of the activity of ChAc done by others. Cholinergic axons in passage are found leaving the corpus callosum and in fasciculi of the internal capsule. Only some, not all, fibers in the fasciculi of the internal capsule are cholinergic and show the specificity of our localization. Cholinergic fibers and axons in passage are observed in the septal regions, olfactory tubercle including the islands of Calleja, neocortex, basal forebrain area, lateral hypothalamus, neostriatum, globus pallidus and other regions. The above anatomical findings correlate well with the existing biochemical determinations of ChAc activity in these areas as measured by others. This research is supported by NIH grant NS-11087.

126.2 LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN THE RAT OLFACTORY TUBERCLE. Carin R. Gordon* (SPON: G. B. Koelle)
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We are reporting the localization of choline acetyltransferase (CAT) in the laminae of the rat olfactory tubercle. The levels of CAT activity within this area of the cortex are amongst the highest of any region in the mammalian brain. The tubercle contains three parallel laminae within its posterior medial portion. These can be separated by cutting consecutive tangential sections from frozen tissue (Brain Res. 131: 303, 1977). Six consecutive 16μm sections were pooled, homogenized and assayed. CAT was measured according to the procedures of Schrier and Shuster (J. Neurochem. 14: 977, 1967). Every seventh section was stained to confirm the accuracy of the depth and plane of sectioning. The distribution of CAT activity as a function of depth showed a broad peak centered at 500μm from the ventral surface of the tubercle. Enzyme activity measured at this depth (85pmol acetylcholine formed/mg protein/h) was 2 1/2 times greater than that measured in the outermost plexiform layer.

Stereotaxic injections of kainic acid (1μg/1μl in artificial CSF) directly into the tubercle were used to alter the tissue composition within the laminae (Brain Res. 118: 356, 1976; 127: 235, 1977). Three days after injection, histological examination revealed the absence of cell bodies and the proliferation of glial cells. The reduction of enzyme activity, determined as a function of tubercle depth, ranges from 0-55% of non-injected controls. Within the acellular plexiform layer activity was decreased to 80% of control values. In the pyramidal layer enzyme activity was decreased to 50-70% of control values. The reduction of enzyme activity was most obvious at depths deep in the pyramidal layer and into the polymorphic layer (300-600μm); here 55% of the activity was lost. CAT activity was decreased to 70% of control values at depths greater than 600μm within the polymorphic layer.

The data are consistent with the presence of more than one cholinergic population in the olfactory tubercle. Approximately fifty percent of the CAT activity was sensitive to kainic acid injection, which suggests a neuronal derivation from cells intrinsic to the tubercle. The remaining portion of the CAT activity was insensitive to kainic acid suggesting an axonal derivation from neurons extrinsic to the tubercle.

At present we are unable to assign a specific cellular localization of CAT within the tubercle, although we have accomplished a laminar localization.

126.3 DO STRIATO-NIGRAL EFFERENTS CONTAIN ACETYLCHOLINESTERASE (AChE, EC 3.1.1.7)? Nancy J. Woolf[1], Barry Fass[2], and Larry L. Butcher[1,2]. Brain Research Institute[1] and Department of Psychology[2], University of California; Los Angeles, CA, 90024; U.S.A.

Application of the pharmaco-histochemical procedure of Butcher et al. (J. neural Transm., 1975, 37, 127-153) to the study of the rat caudate-putamen complex has revealed the existence of two major categories of AChE neurons: (1) Type A cells that stain lightly for the enzyme; have maximum cell body extents within the range 7-20 μm; are oval, fusiform, triangular, or spherical but most often oval; and comprise approximately 2.2% of the total population of striatal neurons and (2) Type B cells that stain intensely for AChE; have maximum cell body extents of 20-44 μm; possess fusiform, oval, triangular, or complex-shaped somata; and comprise roughly 1.2% of the total number of neurons in the rat caudate-putamen nucleus. The Type B AChE cells are probably the intrinsically organized cholinergic neurons whose existence has been deduced from biochemical studies (Butcher and Butcher, Brain Res., 1974, 71, 167-171; McGeer et al., Brain Res., 1971, 35, 308-314). The Type A neurons are conceivably cholinoceptive, and the enzyme could be contained within striatal efferents. Indeed, recent cytochemical evidence suggests that medium-sized, oval or triangularly-shaped striato-nigral efferents contain AChE (Kaiya et al., Neurosci. Let., 1979, 14, 7-11).

In an attempt to determine whether or not either Type A or Type B AChE neurons of the striatum contribute to the striato-nigral efferent pool, we injected Evans Blue dye into the substantia nigra and examined the caudate-putamen complex for neurons containing both the retrogradely transported, red-fluorescent dye and AChE. A total of 2950 striato-nigral neurons containing Evans Blue were studied. On the same tissue sections that contained these labelled neurons, 76 Type A and 33 Type B AChE cells were present. Of these, only one Type A and possibly one Type B neuron possessed Evans Blue label, suggesting that they might be striato-nigral efferents.

These preliminary results suggest that the vast majority of striato-nigral efferents do not contain appreciable quantities, if any, of AChE. Furthermore, our findings support the suggestions that AChE-containing striatal neurons may be intrinsically organized and the Type B cells represent the cholinergic, local-circuit neurons deduced from previous biochemical and histochemical studies (see Butcher, Ed., Cholinergic-monoaminergic Interactions in the Brain, 1978). [Support: USPHS NS 10928 to L.L.B.]

126.4 SEQUENTIAL HISTOCHEMICAL IDENTIFICATION OF ACETYLCHOLINESTERASE IN CATECHOLAMINERGIC NEURONS OF THE RAT HINDBRAIN. D. Bieger and C. Harley. Basic Sciences Division and Dept. of Psychology, Memorial University of Nfld., St. John's, Nfld. A1B 3V6.

Estimates of cholinesterasic reactivity of catecholamine (CA) neurons have been based on a comparison of histochemical maps obtained separately with the Falck-Hillarp technique for fluorescence and the Shute and Lewis technique for acetylcholinesterase (AChE). Using this approach Palkowits and Jacobowitz (J. Comp. Neurol. 157:29,1974) showed an overlap of cholinesterasic reactivity and specific CA fluorescence in cell groups A2, A5, and A6, while overlap was not seen in A1, A4, or A7. Only in A6 did they suggest perikarya contained both the enzyme and the amine.

The present investigation is based on the observation that the specific CA fluorophor produced by 30 minutes of double aldehyde perfusion (Furness et al., Histochemistry 57:285,1978) is not lost during subsequent washout in situ with phosphate buffer (50 ml) followed by vibratome-sectioning, mounting and air-drying in PO_4 buffer. Processing of specimens in PO_4 buffer subsequent to initial aldehyde fixation was critical for the demonstration of esterasic activity. Horizontal sections (30 μ), coverslipped in oil, were photographed under UV illumination then refloated in PO_4 buffer and reacted for AChE according to either Karnovsky/Roots or Shute/Lewis. Both normal and DFP-pretreated rats were used. To ensure optimal results all steps were carried out with minimal delay.

Our observations unequivocally established the presence of the enzyme and CA fluorescence in the same perikarya. Both specific fluorescence and esterasic reactivity were seen in the neurons of A6, A5, A1 and A7. The A4 group appeared to have a nonspecific ChE reaction while, as yet, we have not been able to confirm esterasic activity in A2. Inasmuch as cholinesterasic reactivity would seem to indicate the cholinoceptivity of these central CA neurons, only a minority of the pontomedullary cell population appears to lack cholinergic control.

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126.5 TOPOGRAPHICAL DISTRIBUTION OF CHOLINERGIC PARAMETERS IN THE RAT CAUDATE NUCLEUS. M.A. Rea and J.R. Simon. Institute of Psychiatric Research and Departments of Psychiatry and Pharmacology, Indiana University School of Medicine, Indianapolis, IN 46223

Several cholinergic parameters were estimated in microdissected regions of rat caudate-putamen (CP) obtained from 500 µm thick serial sections along the rostro-caudal extent of the nucleus (corresponding approximately to Konig and Klippel coordinates A9650-A4620). Except for sections A9650 and A4620, the CP from each coronal section was dissected into lateral and medial halves and, when possible (sections A8620-A6690), dissected further into dorso-lateral, dorso-medial, ventro-lateral and ventro-medial quadrants. The microregions of CP were pooled bilaterally and assayed for high affinity choline uptake (HAChU), choline acetyltransferase activity (ChAT) and muscarinic receptor binding (MRB), using [^{3}H] quinuclidinyl benzilate as a ligand. A total of 26 regions of CP (3-5 mg. wet wt.) were assayed. All three cholinergic parameters were found to be unevenly distributed within the CP and were consistently higher in the lateral aspect of the nucleus. HAChU varied approximately threefold (12 + 3 to 30 + 6 pmol/2 min/mg protein) while ChAT (0.46 + 0.07 to 1.05 + 0.07 nmol/min/mg protein) and MRB (0.9 + 0.1 to 1.6 + 0.2 pmol/mg protein) each exhibited about a twofold variation. In a given coronal section, as much as 70% of the HAChU, 65% of ChAT and 75% of MRB was localized to the lateral half of the CP. Furthermore, all three cholinergic markers were found to decrease along the rostro-caudal extent of the CP in dorso-lateral, dorso-medial and ventro-medial regions of the nucleus. In contrast, no rostro-caudal variations were apparent in the ventro-lateral region, where the highest density of cholinergic parameters was found. No statistically significant differences were noted along the dorso-ventral aspect of the CP. These data suggest that the lateral half of the rat CP may contain a relatively high density of cholinergic components, probably cholinergic interneurons. Therefore, studies of CP neurochemistry involving experimental manipulation of cholinergic parameters may require a consideration of regional effects within the CP, as opposed to treatment of the nucleus as a neurochemically homogeneous tissue. (Supported by USPHS Grant NS 15951).

126.6 THE ONTOGENY OF THE ASCENDING SEROTONERGIC SYSTEM IN THE RAT: AN IMMUNOHISTOCHEMICAL STUDY. Hart G.W. Lidov and Mark E. Molliver, Departments of Cell Biology/Anatomy and Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Recently (Neurosci. 5, 207, 1980) we have reported an extensive neocortical innervation by serotonergic brainstem neurons using an antibody direct against 5-hydroxytryptamine as an immunocytochemical marker. With this technique, which is significantly more sensitive than any previously available, we have now analyzed the development of serotonergic cell bodies and their ascending projections in fetal and perinatal rats.

Serotonin positive perikarya are present in rat embryos at 13 days of gestation. Initially they form extensive paramedian nuclei with mesencephalic and medullary subdivisions. Several features characterize their subsequent development: A) The multiplicity of serotonergic cell groups seen in the adult involves the partitioning of two embryonic serotonergic nuclei by subsequent events in brainstem ontogeny. B) At least two migratory paths may be involved in the formation of the serotonergic cell groups as seen in the adult rat brain. C) Extensive early dendritic development is in striking proximity to major early developing tracts, the pyramidal tract and medial longitudinal fasciculus.

Ascending serotonergic projections penetrate the forebrain on embryologic day 15. They appear to be subject to epiphytic guidance in that they grow along well developed non-serotonergic tracts. In the period of development of major axonal projections there is little development of terminal arborizations.

The first serotonergic axons enter the neocortex on embryonic day 19. Their mode of entry has a number of novel features. There are both medial and lateral components to the neocortical projection: the former traveling in the diagonal band, septum and supracallosal stria, and the latter crossing the ganglionic eminence and external capsule and entering the lateral neocortex. In contrast to the radial model that has been proposed for the growth of subcortical afferents into neocortex, the serotonergic axons clearly enter tangentially from the antero-medial and lateral margin of the cortex and enter the marginal and subplate zones as two independent strata. This is embryologic evidence for the existence of long tangential pathways confined to the molecular layer.

Terminal arborizations develop in the neocortex perinatally and achieve adult density by 3 weeks of age. The development lags in dorsal and parietal cortex as expected from the axonal pathways. During the development of serotonergic neocortical afferents, there is also innervation of the hippocampus and cerebellum. (Support: USPHS NS 08153; H.G.W.L. supported by GM-7309).

126.7 ULTRASTRUCTURAL CHARACTERISTICS AND RELATIONSHIPS OF SEROTONERGIC PERIKARYA IN NUCLEI RAPHE DORSALIS AND OBSCURUS OF THE RABBIT BRAIN. D.L. Felten, S.C. Rightor* and R.G. Peterson, Department of Anatomy, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

The raphe nuclei of the brain stem contain serotonergic neurons which give rise to widespread projections. These systems have been implicated in the regulation of a wide range of motor, sensory, autonomic, and forebrain functions. Within these raphe nuclei are dendrite bundles containing both serotonergic cell bodies and dendrites. This ultrastructural study was undertaken to further examine the local relationships of the raphe cell bodies in the dendrite bundles. The nuclei raphe dorsalis and obscurus were examined with TEM as principle regions where serotonergic dendrite bundles have been previously described in this laboratory.

The raphe perikarya and proximal dendrites within these nuclei contained dense membrane-bound particles which were similar in appearance to lysosomal particles. They demonstrated a regular array of overlapping linear ultrastructural profiles within the particles at high magnification. These particles were depleted of their dense contents by pretreatment with reserpine in both the perikarya and proximal dendrites. The distribution of dense particles overlapped the distribution of yellow fluorescence with the Falck-Hillarp method. We suggest that these particles are the storage sites for serotonin in neurons of the raphe nuclei, and represent an ultrastructural marker for the qualitative identification of serotonergic neurons.

The cell bodies of the serotonergic neurons contained abundant profiles of rough endoplasmic reticulum, heavily accumulated in peripheral zones of the perikarya, and Golgi apparatus within more central regions of the perikarya. The nucleus was highly infolded and convoluted, with the infoldings sometimes cleaving a cytoplasmic channel through the nuclear region, thus expanding the surface area of the nuclear envelope. Within the dorsal raphe nucleus, more than 90% of the cell bodies had less than 5% of their surface area occupied by incoming axon terminal synapses. Most of the surface was in contact with astrocytic processes, abutted oligodendroglial cell bodies, or abutted the basement membrane of capillaries. In nucleus raphe obscurus, a similar paucity of incoming axonal synapses was noted on the somas. A much higher number of abutments with oligodendroglial cell bodies was also noted. We suggest that the glial relationships with the serotonergic cell bodies provide both an insulating and a nutritive function, and that most of the modulation of the excitability of serotonergic neurons takes place at the dendritic level.

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126.8 CORTICAL MONOAMINE LEVELS FOLLOWING FRONTAL CORTEX ABLATION. A BIOCHEMICAL ANALYSIS USING HPLC. J.H. Morrison and M.E. Molliver Departments of Cell Biology/Anatomy and Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

In a previous report based upon histochemical analyses following cortical lesions we proposed that the noradrenergic (NA) projection from the locus coeruleus reaches most areas of neocortex primarily via tangential, longitudinal axons which run from rostral to caudal, traveling within the cortical grey matter. A large group of NA fibers enters cortex at the ventral aspect of the frontal pole, then turn dorsally and caudally passing through the frontal cortex; these same fibers continue caudally through the longitudinal extent of the dorsal and lateral cortex, supplying the cortical innervation throughout their trajectory. We have now attempted to supplement the morphologic data with the measurement of biochemical markers of monoamine neurons.

High Pressure Liquid Chromatography (HPLC) with electrochemical detection was used to measure the levels of norepinephrine (NE), serotonin (5-HT), and their principal metabolites in specific cortical areas at various survival times following cortical lesions. A mobile phase was developed that allowed for simultaneous measurement of MHPG, NE, DA, E, 5HT, HIAA and DHBA (an internal standard) in each sample injection. Dorsolateral frontal decortication resulted in a dramatic decrease in levels of NE and 5-HT throughout the remaining dorsolateral cortex of that hemisphere. The decrease in metabolites, although present, is less marked.

The present biochemical data independently confirm the proposition that the NA projection to cortex is organized tangentially and that caudal regions of neocortex are innervated by NA fibers that pass through the frontal pole. In addition, it appears that the serotonergic projection to lateral cortex has a similar organization, such that a small cortical lesion may have far reaching effects on both transmitter systems. The biochemical and morphologic data taken together suggest that the monoaminergic cortical projections from the brain stem engage the cortex in a manner fundamentally different from most other afferents, which terminate topographically and radially. The tangential, intragriseal trajectory of these non-thalamic cortical afferents (NE and 5-HT) indicates that the neocortex is organized as a three-dimensional matrix in which tangential systems are superimposed upon the columnar organization of cortical circuitry. (Supported by USPHS: MH 15330, NS 15199 and NS 08153).

126.9 THE DISTRIBUTION AND INCIDENCE OF SYNAPTIC CONTACTS OF NORADRENERGIC VARICOSITIES IN THE RAT NEOCORTEX: AN IMMUNOCYTOCHEMICAL STUDY. John A. Olschowka, Reinhard Grzanna, and Mark E. Molliver
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Several laboratories have proposed that monoaminergic varicosities typically do not form synapses, but release transmitter diffusely into the extracell space. Histofluorescence and tritiated-norepinephrine autoradiographic studies have reported abundant noradrenergic (NA) varicosities in layer I of rat neocortex, with a rapid decline in numbers of varicosities in layers II-VI. In contrast immunofluorescence studies have shown that the NA projection ramifies densely through all layers of cortex and may directly influence neurons in all cortical layers. In this study a homologous antiserum directed against rat dopamine-β-hydroxylase (DBH) was visualized by an HRP-Fab conjugate to describe the distribution and ultrastructural relationships of NA axons in rat somatosensory cortex. DBH is the terminal enzyme in the synthesis of norepinephrine and is a sensitive marker for NA neurons.

Vibratome sections of rat neocortex were processed for DBH immunocytochemistry, embedded in plastic, thin sectioned, and the tissue was systematically analyzed. Every DBH-positive profile was identified, classified as either a varicosity or an intervaricose segment, and its position within the cortical laminae recorded. DBH-immunoreactive varicosities and intervaricose segments were observed in all cortical layers interspersed between unlabeled dendrites. DBH-positive profiles were not evenly distributed, but were concentrated in particular cortical layers. Over two-thirds of all DBH-positive profiles (>70% of labeled varicosities) were found in layers IV-V, followed in order of decreasing density by layers I, II-III, and VI. Overall, 64% of all DBH-positive profiles were intervaricose segments, 36% varicosities. The vast majority (80%) of labeled profiles in layer VI were intervaricose segments, suggesting that these NA fibers were axons of passage. A large proportion (>40%) of DBH-labeled varicosities, most of which are in layers IV-V, formed synaptic contacts with specialized membrane appositions. Synaptic junctions, predominantly asymmetric, were observed between DBH-positive varicosities and unlabeled dendrites and dendritic spines. No axo-axonic or axo-somatic contacts were observed. The results suggest that: NA axons may influence neurons throughout the cortex, with a major influence on neurons in layers IV and V; NA fibers in layer VI appear to be axons of passage; NA varicosities exert their influence on cortical neurons via conventional synaptic contacts. (Support: USPHS NS 06117 and NS 15199).

126.10 Visualization and Characterization of Serotonergic Perikarya and Hippocampal Terminal Fibers by Specific Reuptake and Retrograde Transport of ^{3}H-5HT. E.C.Azmitia and P.J.Gannon, Department of Anatomy, Mount Sinai Medical Center, New York, New York 10029.

Hippocampal serotonergic fibers possess a high-affinity reuptake mechanism for ^{3}H-5HT which is both specific and saturable (Azmitia and Marovitz, J.Histochem.Cytochem., 1980). We have utilized this process for the in vivo and in vitro localization and characterization of the perikarya and terminal axons of the midbrain serotonergic neurons efferent to the dorsal hippocampus in the rat. Pharmacological and biochemical in vitro studies were performed on hippocampal slices (0.3 mm) incubated (37°) in Ringer solution with pargyline (10^{-4}M) dextrose (10^{-2}M) and ascorbic acid (10^{-3}M) for 15 min. Studies with unlabeled 5-HT (10^{-5}M), NE (10^{-3}-10^{-5}M) or tryptophan (10^{-5}M) and with fluoxetine (10^{-4}-10^{-7}M), DMI (10^{-4}-10^{-7}M), metergoline (10^{-4}M), or 5,7-DHT (intracerebral injection into fornix-cingulum, 5ugm, 400nl, 6 day survival) showed a specific to non-specific reuptake ratio of 70/30 at a ^{3}H-5HT molarity of 5×10^{-8}M.

Radioautography (NTB-2, 2-5 week exposure, D-170 developer) of the hippocampus after in vitro or in vivo administration of ^{3}H-5HT revealed an extensive distribution pattern of the specific reuptake sites (dense silver grain aggregates) suggesting innervation of both pyramidal and granule neurons. Electron microscopic radioautography is currently being processed to obtain ultrastructural identification of these serotonergic axons.

Furthermore, specific retrograde transport to raphe serotonergic perikarya was seen within 6 hours after in vivo ^{3}H-5HT injection into the dorsal hippocampus (10uci, 80nl Ringer). Combination of this radioautographic method with the HRP-DAB technique was performed after ^{3}H-5HT and HRP (Sigma VI, 10%, 50nl Ringer) injections into opposing hippocampi. Sequential processing of the brains showed an equal number of ipsilateral and contralateral projecting neurons in both the MR and interfasicular part of the DR. Most important, a subpopulation of serotonergic neurons (10%) was found to have bilateral projections to this limbic center. Research supported by NSF 79-06474 and Hirschl Career Development Award.

126.11 EFFERENT ORGANIZATION OF LOCUS COERULEUS: 3-DIMENSIONAL QUANTITATIVE ANALYSIS. S.E. Loughlin, S.L. Foote, and F.E. Bloom. A.V. Davis Center for Behavioral Neurobiology, The Salk Institute, La Jolla, CA 92037.

We have previously reported a method for 3-D reconstruction of the locus coeruleus (LC) based on Nissl-stained LC's. Similar reconstructions can be created for the subsets of LC cells labelled following injection of HRP into selected terminal fields of LC. Quantitative comparisons of the distribution of cells as well as highly accurate visual representations of complete nuclei are possible. Such series of LC's ($N \geq 5$ LC's for each) have been analyzed following HRP injections into pre-frontal cortex, septal hippocampus, temporal hippocampus, hypothalamus, cerebellum, and thoracic spinal cord. Labelled cells in each section through LC were digitized, aligned and registered to a standard coordinate system. As has been reported elsewhere, cells which were labelled following injections into septal pole of hippocampus or spinal cord were distributed differently from the control distribution such that hippocampal injections labelled dorsal LC and spinal cord labelled ventral LC. Both of these differences were readily visible and have been confirmed by various statistical analyses. In addition, analysis of variance has revealed a difference in the anterior-posterior distribution of cells projecting to hippocampus which was not obvious to visual examination. The distribution of labelled cells following injection into the temporal hippocampus appears very similar to that following septal hippocampal injection, though in some cases there are differences in anterior-posterior distribution. The distributions of labelled cells following injection into prefrontal cortex, cerebellum, and hypothalamus appear very similar to each other and to the control. These three patterns of labelling, however, appear qualitatively different when carefully examined. For example, cerebellar injections tend to label large, multipolar cells especially in ventral LC, while prefrontal cortex injections label medium, round cells throughout LC. Precise analysis of these distributions by the more sensitive analysis of variance is required to confirm these qualitative differences. To attempt to answer the question of whether those populations of cells with coextensive distributions represent intersecting or distinct subsets of LC cells, we have simultaneously injected these 2 fluorescent retrograde tracers into different terminal fields. Preliminary results with the tracers true blue and nuclear yellow indicate that cells labelled with each are interdigitated within LC, but that double-labelled cells, which are evident in other brain areas, are rare in LC. The results of further experiments will be reported. Supported by Grants AA 03504 and NS 16209.

126.12 COMBINED IMMUNOCYTOCHEMICAL AND RADIOAUTOGRAPHIC STUDY OF DOPAMINERGIC TERMINALS IN THE NEOSTRIATUM. V.M. Pickel, A. Beaudet, S. Beckley, T.H. Joh, D.J. Reis, and M. Cuenod. Lab. of Neurobiol., Dept. of Neurol. Cornell Univ. Med. Coll. New York, NY and Brain Research Inst., Univ. of Zurich, Zurich, Switzerland.

The topographic relations and ultrastructure of dopaminergic terminals in the neostriatum of rat brain were studied by the immunocytochemical localization of tyrosine hydroxylase (TH) alone or in combination with radioautographic localization of ^{3}H-dopamine. Animals used only for immunocytochemistry were fixed by aortic arch perfusion with 4% paraformaldehyde and 0.2% glutaraldehyde. Animals used for the combined study were given pargyline (75 mg/kg i.p.) 1 h prior to the 2 h intraventricular infusion of ^{3}H-dopamine (100 ul of 10^{-5} M solution). In order to retain the ^{3}H-dopamine in axon terminals, the animals were perfused first with the aldehyde mixture then with 0.5% OsO_4 immediately following the intraventricular infusion (Descarries et al., Nature, in press). The neostriatum from both groups of animals were sectioned on a Vibratome, and processed for the immunocytochemical localization of TH by the peroxidase-antiperoxidase method of Sternberger. Sections used for the combined studies were subsequently processed for radioautography.

By light microscopy using only immunocytochemistry, the peroxidase reaction product was restricted to rosettes of varicose terminals which surrounded unlabeled neuronal perikarya throughout the neostriatum. In the combined study, the ventricular region which had greatest access to ^{3}H-dopamine showed rosettes of peroxidase labeled axon terminals which were superimposed with reduced silver grains. At least one other dopaminergic region, the nucleus accumbens, had a comparable dual label.

Electron microscopic examination of the sections processed only for the localization of TH allowed a more extensive sampling of the dopaminergic terminals throughout the rostrocaudal and dorsoventral extent of the neostriatum. A similar population of TH-containing axon terminals was present in all regions examined. The labeled terminals ranged in diameter from 0.2 to 1.5 μm and contained numerous small (40-60 nm) clear vesicles which were rimmed with the peroxidase reaction product. The smaller terminals, 0.2 to 0.5 mμ, showed symmetric synaptic densities with unlabeled dendrites. However, the larger axon terminals rarely showed morphologically identifiable synaptic densities in single or serial sections. The ability to combine the radioautographic localization of ^{3}H-dopamine with immunocytochemistry has considerable potential for the examination of cellular relationships between the dopaminergic terminals and other neurons having immunocytochemically detectable markers. Supported by NIH grants MH24285, HL18974, and SNF grant 3.506.79

126.13 SIMULTANEOUS DETECTION OF SEROTONIN AND TYROSINE HYDROXYLASE OR ENKEPHALIN CONTAINING NEURONS BY COMBINED RADIOAUTOGRAPHY AND IMMUNOCYTOCHEMISTRY IN THE CENTRAL NERVOUS SYSTEM OF THE RAT: A. Beaudet*, V.M. Pickel, T.H. Joh, R.J. Miller and M. Cuénod, Brain Res. Inst., Univ. of Zurich, Switzerland and Lab. of Neurobiol., Dept. of Neurol., Cornell Univ. Med. Coll., New York, N.Y.

Numerous biochemical, pharmacological and electrophysiological studies have suggested functional relationships between serotonine (5-HT), catecholamine and enkephalin (Enk) containing neurons in the CNS. In order to determine the morphological basis for such interactions, 5-HT and either catecholamine or Enk neurons were simultaneously visualized in rat brain by combining radioautography with immunocytochemistry. Adult rats were pretreated with a monoamine oxidase inhibitor and subjected to a 3h intraventricular infusion of 50 nM [^{3}H]-5-HT. The brains were fixed by intraaortic perfusion of a mixture of aldehydes (0.5-1% glutaraldehyde/1-4% paraformaldehyde) followed or not by 0.25% OsO_4. Thirty micron-thick vibratome sections from the mesencephalon and the caudal medulla were incubated with specific antisera against either tyrosine hydroxylase (TH) or leucine(Leu^5)-Enk, immunocytochemically labeled by the peroxydase-antiperoxydase method of Sternberger (Prentice Hall, 1974), and processed for light microscope radioautography.

In immuno-radioautographs of the lower brainstem, numerous [^{3}H]-5-HT labeled axonal varicosities are visible in the lateral reticular nucleus and the medial nucleus of the solitary tract, in between and often adjacent to TH immunoreactive nerve cell bodies of groups A1 and A2 respectively. Dense plexuses of 5-HT fibers are also detected in the spinal trigeminal nucleus, together with numerous terminals and occasional perikarya showing Enk-like immunoreactivity. Conversely, in the mesencephalon, scattered TH or Enk immunoreactive fibers are detected amidst the intensely labeled 5-HT nerve cell bodies of nucleus raphe dorsalis. The latter are interspersed among non-radiolabeled TH reactive perikarya, thus confirming that both belong to distinct neuronal populations.

These results demonstrate the feasibility of combining immunocytochemistry and radioautography for simultaneous light microscopic identification of different chemically specific neurons. The same approach should be applicable at the electron microscopic level and provide a powerful tool for elucidating their cellular relationships.

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126.14 IMMUNOCYTOCHEMICAL AND DEGENERATIVE STUDY OF ENKEPHALIN AND SUBSTANCE P IN THE SPINAL TRIGEMINAL NUCLEUS. K.K. Sumal*, V.M. Pickel, R.J. Miller and D.J. Reis, Laboratory of Neurobiology, Dept. of Neurology, Cornell Univ. Med. Coll., New York, NY and Dept. of Pharmacol., Univ. of Chicago, Chicago, IL.

The substantia gelantinosa of the spinal trigeminal nucleus (SGV) is a site of termination of sensory afferents from the Gasserian (V) and nodose (X) ganglia. Light microscopic immunocytochemistry has shown the presence of enkephalin (E) in perikarya and processes and substance P (SP) in axon terminals in the SGV. Furthermore, the SP immunoreactivity in the SGV is reduced by lesions of the Gasserian ganglion (Cuello et al., Brain Res. 152, 1978). In this study, light and electron microscopic immunocytochemistry is combined with lesions of sensory afferents in order to determine: (1) whether any of the SP and/or E containing terminals in the SGV are derived from the nodose as well as the Gasserian ganglia and (2) the ultrastructure and synaptic relations of these peptidergic neurons in the SGV. Four days following unilateral electrolytic lesions of the Gasserian ganglion or removal of the nodose ganglion, the brains were fixed by vascular perfusion with 4% paraformaldehyde and 0.2% glutaraldehyde. Vibratome sections taken throughout the rostrocaudal extent of the SGV were processed for the immunocytochemical localization of SP and E using the peroxidase-antiperoxidase technique of Sternberger.

By light microscopy, electrolytic lesions of the Gasserian ganglia reduced the immunocytochemical reaction for SP, but not E, in the more caudal portions of the SGV. Whereas removal of the nodose ganglia reduced the immunoreactivity only for SP in the rostral portions of the nucleus. These light microscopic changes in SP immunoreactivity were accompanied by evidence of degenerating boutons in the SGV by electron microscopy.

The ultrastructural localization of SP was restricted to lightly myelinated axons and axon terminals. The SP-containing axon terminals contained numerous small (40-60 nm) clear vesicles and 1-4 large (100 nm) dense vesicles, and formed primarily axodendritic synapses. E was localized by electron microscopic immunocytochemistry to perikarya, dendrites, and myelinated and unmyelinated axons and axon terminals. The terminals showing E immunoreactivity also contained a similar vesicle population and formed primarily axodendritic synapses. We conclude that SP terminals in the SGV are derived from both the nodose and Gasserian ganglia and that SP- and E-containing terminals form primarily axodendritic synapses in the substantia gelatinosa.

(Supported by NIH grants MH24285, HL18974, and NSF grant 3.506.79)(SP antiserum generously donated by S. E. Leeman)

126.15 ULTRASTRUCTURAL FEATURES OF ENKEPHALIN NEURONS IN THE MONKEY SPINAL CORD. N. Aronin*, M. DiFiglia, A. Liotta* and J.B. Martin. Dept. of Neurology, Mass. General Hospital, Boston, MA and the Endocrine Section, Mount Sinai Sch. of Med., New York, N.Y.

Immunoreactive leu-enkephalin (leu-enk) containing neuronal elements and their synaptic relationships were identified in the monkey spinal cord with use of the immunoperoxidase technique. The leu-enk antiserum employed crossreacts less than 0.1% with met-enkephalin on a molar basis and not at all with β-endorphin or β-lipotropin in radioimmunoassay. Immunoprecipitation of spinal cord extract with excess leu-enk antiserum, followed by separation on high pressure liquid chromatography and gel filtration, reveals authentic leu-enk and 2 larger peptides (approx. M.W. 4620 and 31,000). Preliminary results show that the 4620 M.W. peptide yields intact leu-enk upon trypsinization. It is possible that any or all of these various peptides are immunostained with the titer of leu-enk antiserum used (1:500).

Light microscopy reveals positively stained cell bodies distributed throughout laminae I and outer II (IIo). Most somata are about 10 μm and have a bipolar shape. Emerging dendrites extend long distances from the cell body and are oriented in the marginal zone parallel to the plane of the lamina. A dense distribution of fibers is present in the zone of Lissauer, laminae I, II and X. Processes also appear throughout the remaining dorsal horn and laminae VII and IX. Electron microscopy shows leu-enk neurons to have relatively large indented nuclei, little surrounding cytoplasm, some small spines and few axosomatic synapses. Peroxidase reaction product is localized within proximal and distal dendrites on numerous microtubules and in large granular vesicles (LGV, 100 nm), which are occasionally present. Dendrites and emerging spines are contacted by various types of unlabeled axon terminals. Within laminae I and IIo leu-enk is found in myelinated axons having a diameter of 0.3-0.6 μm. Numerous stained axon terminals contain small round agranular vesicles (40 nm) and some LGV. Reaction product is present primarily around the membranes of small vesicles and within LGV. The boutons have a maximum size of 1.5 μm and form asymmetric synapses with unlabeled dendritic shafts and spines. Less fequently, smaller leu-enk terminals (0.5 μm) are presynaptic to cell bodies.

The present results extend to monkey previous light microscope observations of enkephalin localization in rat and cat spinal cord. Furthermore, ultrastructural data indicate that at least in laminae I and IIo numerous immunoreactive leu-enk containing axons participate in conventional axodendritic and axosomatic synapses. It is therefore possible that in the dorsal horn enkephalin has functions in addition to its proposed role in direct inhibition of primary sensory afferents. Supported by grants # 1-RO1-AM-26 252-01 and # 1-F32-AM06143-01.

126.16 LIGHT AND EM LOCALIZATION OF SUBSTANCE P, MET-ENKEPHALIN AND 5-HT IN HUMAN AND MONKEY SPINAL CORD. N.C.de Lanerolle and C. LaMotte, Sections of Neurosurgery & Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Substance P (SP), met-enkephalin (ENK) and 5-hydroxytryptamine (5HT) have been localized, in recent years, in axons and terminals of the spinal cord of lower mammals. This study describes the distribution of these substances in human and monkey spinal cord as visualized by immunohistochemistry using the peroxidase anti-peroxidase technique. The following table summarizes the distribution of SP and ENK in human mid-thoracic & lumbar cord:

Area	SP	E	Area	SP	E
Lissauer's tract	+++	+	N. intermediomedialis	+	+
Marginal zone	++++	++	N. cornucommiss. post.	++	+
Substantia gelatinosa	+++	++	N. cornucommiss. ant.	-	-
N. proprius	+	+	Lateral motor nucleus	++	+
N. reticularis	+	+	Medial motor nucleus	+	-
N. dorsalis of Clarke	-	-	Central canal zone	-	-
N. intermediolateralis	++	++			

In addition, both SP and ENK positive fibers extended from the marginal zone and Lissauer's tract into the lateral white matter and into the dorsal columns. Several presumed motor neurons were heavily outlined along their soma and dendrites with SP terminal-like processes.

As part of an analysis of the relationships between SP and ENK innervation of spinal cells, serial sections of the human cord were made, and alternate sections were stained for SP and ENK. Both SP and ENK positive terminals were found in close apposition to the soma and dendrites of some neurons in the marginal zone, the reticular, intermediomedial and intermediolateral nuclei, and of motor neurons. The dual innervation of preganglionic sympathetic neurons and motor neurons in addition to neurons in laminae I & V, indicates that these peptides may be involved not only in pain modulation but also in other spinal functions.

In the monkey, the distribution of peptides was similar to that in humans. EM of laminae I to III revealed SP to be contained in fine axons and terminals which most commonly make junctions with dendrites (proximal & distal). Large dense-core vesicles are often found within the labelled terminals.

In the monkey, much of the 5HT was localized in fibers and terminals in the marginal zone, laminae III & IV, and the nucleus intermediolateralis. Less 5HT was observed in the substantia gelatinosa, nucleus intermediomedialis and nucleus dorsalis of Clarke; in the ventral horn 5HT was associated with the surface of presumed motor neurons.

(Supported by NIH grant NS 13335)

126.17 NEUROTENSIN (NT) AND SUBSTANCE P (SP) LOCALIZATION IN MONKEY AND RAT SPINAL CORD: LIGHT AND ELECTRON MICROSCOPY. S.E. Leeman, M. DiFiglia and N. Aronin*. Depts. of Physiology, Harvard Medical School and Neurology, Massachusetts General Hospital, Boston, MA.

NT and SP containing axons were identified in the monkey spinal cord. In addition, the distribution of NT in rat spinal cord was examined. Vibratome sections (20 μm thick) were incubated in NT or SP antiserum and then processed by the immunoperoxidase method. Each antiserum crossreacts with its C-terminal hexapeptide and intact peptide. Fresh spinal cord extracts from each species, when submitted to gel filtration and high pressure liquid chromatography and quantified by radioimmunoassay (RIA), show a single peak co-eluting with synthetic NT or SP and SP sulfoxide. It is likely that NT and SP are the principle immunostained peptides with high affinity to the antisera used. However, other low affinity peptides undetectable by RIA may also be immunostained at the high titer of antiserum (1:500) needed in the immunohistochemical method.

Light microscopy reveals NT fibers in the rat and monkey in the zone of Lissauer and within the dorsal horn mainly in laminae I and II. SP fibers in the monkey are present in I, II, III, IV, IX, X, and the zone of Lissauer. Electron microscopy of the monkey dorsal horn shows a similar morphology of labeled elements for both neuropeptides. Immunostained axons are unmyelinated and are about 0.3 μm for NT and 0.3-0.7 μm for SP. Terminal boutons vary in size and have maximum dimensions of 1.5x3 μm for SP and 2.0x2.5 μm for NT. The largest elements contain numerous large granular (LGV, 100nm) and small round agranular (35-40nm) vesicles both of which are scattered throughout the profiles. Peroxidase reaction product is localized primarily within the LGV and tends to be more lightly deposited on membranes of the small vesicles which are also present at presynaptic sites. NT and SP axons synapse with large and small dendrites and dendritic spines. The largest profiles can contact up to three different postsynaptic elements and frequently form more than one contact with the same dendrite. Most synapses appear to be asymmetric and are sometimes associated with subjunctional dense bodies in the postsynaptic elements. The present results show that 1) NT fibers are present in monkey and rat dorsal horn, 2) the localization and ultrastructure of SP axons in the monkey are similar to those previously reported for the rat, 3) NT and SP terminals in the monkey have a similar appearance and synaptic pattern. Moreover, some of the profiles are identical to those described as central axonal elements, a portion of which are considered to be of primary afferent origin (Ralston, H.J. and Ralston, D.D., J. Comp. Neurol. 184: 643, 1979). Supported by grants # AM-16510, 1-R01-AM-26152-01 and 1-F32-AM-06143-01 from NIH.

126.18 ONTOGENESIS OF SOMATOSTATIN (SRIF) IN THE FOREBRAIN OF THE RAT CENTRAL NERVOUS SYSTEM. R.M. Lechan*, J.L.Nestler*, R.J.Robbins*, S.Jacobson, J.Bollinger*. Endocrine Div. and Dept. of Anatomy, Tufts-New England Medical Center, Boston, MA 02111.

In order to determine the ontogenesis of somatostatinergic neurons of the rat forebrain, the time of appearance and distribution of immunoreactive somatostatin within this region was studied immunohistochemically using the indirect peroxidase-antiperoxidase technique in rats ranging from fetal day 17 to postnatal day 14. Antisera to synthetic cyclic-SRIF was raised in NZW rabbits and incubated at a final titre of 1:1000 for 48 hrs at 4°C with free floating 50 micron vibratome-sectioned tissues. Specificity of the reaction product was demonstrated by the absence of staining with preabsorbed antisera.

Immunoreactive SRIF in fetal day 17 animals was present in perikarya of the hypothalamus, subthalamus, and rhinencephalon, although beaded axons were seen only at the base of the brain coursing with the optic tracts. By postnatal day 1, rich concentrations of SRIF-reactive neurons were widely distributed throughout the forebrain, both in perikarya and neuronal processes. SRIF was present in the dorsal arcuate nucleus, lateral hypothalamic area and in a band encircling the ventromedial nucleus, but staining in these areas was no longer apparent by postnatal day 11. In addition, SRIF present at day 1 in the nucleus accumbens, zona incerta, entopeduncular nucleus and lateral habenula diminished between postnatal day 9 and 14. In contrast, SRIF immunoreactive neurons in the caudate-putamen and stratum oriens of the dorsal hippocampus, and in terminals in the median eminence, paraventricular nucleus of the thalamus and corticomedial nucleus of the amygdala present on postnatal day 1, dramatically increased by postnatal day 5. Regions of comparable immunoreactivity in all neonatal groups included cells of the hypothalamic periventricular nucleus, anterior hypothalamic area, pyriform cortex, olfactory tubercle and axons in the ventral premammillary nucleus, organum vasculosum and stria terminalis. SRIF-containing perikarya of the neocortex was present only in the deep lamina on postnatal day 1 extending into the more superficial lamina by day 14.

These results demonstrate the widespread presence of immunoreactive SRIF in immature neurons of the developing rat brain and the dynamic changes which occur in its distribution during maturation. The loss of immunoreactivity with age in some neurons suggests either a decline in cell numbers by attrition or a reduction in their content of SRIF.

126.19 DEVELOPMENT OF ENKEPHALIN-LIKE IMMUNOREACTIVITY WITHIN THE RAT HIPPOCAMPAL FORMATION. C. Gall, N. Brecha, and H.J. Karten, Dept. of Neurol. and Dept. of Neural and Behav. Bio., State Univ. of N.Y., Stony Brook, New York 11794.

Antisera to leucine-enkephalin have been used to study the developing and mature distribution of enkephalin-like immunoreactivity within the hippocampal formation of the rat. At maturity immunoreactivity was localized throughout the mossy fiber system and within a continuous zone including CA1 stratum lacunosum-moleculare and the distal third of the dentate gyrus molecular layer (the latter zone conforming to the distribution of afferent input from the lateral entorhinal cortex). Perikarya exhibiting enkephalin-like immunoreactivity were observed sparsely scattered throughout the hippocampus most notably in stratum granulosum, stratum pyramidale of field CA1, the apical dendritic field of CA1 and the subiculum.

This immunoreactive staining pattern emerges slowly rather late in the development of the hippocampus. The 'lateral entorhinal' band first appears on postnatal day 6 (p6) and fills the field by p8. Immunoreactivity within the mossy fiber system was first detectable by p11 and at this age is only present within the hilus. By p13 labeled mossy fibers are seen along the pyramidal cell layer as far as CA3b and by p14 are present throughout regio inferior. In contrast, immunoreactive axons in the fimbria and along the alveus/stratum oriens are most conspicuous at the earliest time points, prior to p8, and are seen progressively less frequently thereafter.

A few immunoreactive perikarya are present within the hilus and apical field of regio inferior by p8. Between days 11 and 13 there is an abrupt increase in somal staining such that some labeled neurons appear in all fields of the hippocampus proper; however, within the dentate gyrus labeled neurons are still seen within the hilus rather than within stratum granulosum. By p16 immunoreactive perikarya are largely absent from the hilus and are seen to lie along the base of the granule cell layer. At this age labeled neurons appear to be more intensely immunoreactive than in the adult: in some of the subgranular neurons labeling is sufficiently intense to visualize both dendritic and somal spines. Although at p16 cells are seen labeled in all areas that will include reactive somata in the adult, these neurons have clearly not established their adult morphology. By postnatal day 26 the pattern of enkephalin-like immunoreactive staining in the hippocampal formation is the same as that seen in the adult. The relationship of these patterns of peptide ontogeny will be compared to the structural and physiological development of hippocampal afferents and resident neurons.

126.20 DOUBLE IMMUNOCYTOCHEMICAL LABELING DEMONSTRATES DISTINCTIONS AMONG OPIOID PEPTIDERGIC NEURONS. Jacqueline F. McGinty and Floyd E. Bloom. A.V. Davis Ctr. for Behav. Neurobiol., The Salk Institute, La Jolla, CA 92037.

The opioid peptides, β-endorphin and enkephalin, have distinct distributions in the CNS and may arise from different precursor molecules. In contrast, β-endorphin, ACTH, and α-MSH immunoreactivity (ir) has been located in the same pituitary cells and in closely related, medial basal hypothalamic neurons. These three peptides probably arise from the same 31k precursor, proopiocortin, which also contains γ-MSH. Cell bodies containing γ_3-MSH (ir) have been observed only in the medial basal hypothalamus (Bloom et al, in preparation). To compare the cellular localization of these peptides in the brain, we have developed an immunocytochemical technique to detect two antigens on the same tissue section by modifying immunoperoxidase methods (Nakane, J. Histochem. Cytochem. 16: 557, 1968).

Primary rabbit antisera were raised against synthetic leu-enkephalin (Miller and Chang, U. Chicago), β-endorphin, ACTH, and γ_3MSH (Benoit, Shibasaki, and Guillemin, Salk Inst.). Rat brains were prepared for immunocytochemistry as described (Bloom, et al. PNAS 73: 1591, 1978). Tissue sections were incubated with one of the primary antisera, followed by HRP-labeled-IgG, and stained for HRP with diaminobenzidine as substrate. The antibody complex was eluted with 0.2M glycine-HCl (pH 2) without losing the brown reaction product or denaturing the antigen to be localized subsequently. The second primary antiserum was then applied followed by HRP-IgG. The second reaction product was visualized with o-dianisidine diHCl as HRP substrate, essentially as described (Colman and Scalia, Brain Res. 102: 156, 1976). In the medial basal hypothalamus, we observed cell bodies containing either β-endorphin (ir) or enkephalin (ir), but not both. However, potential interactions between these two neuronal populations were revealed by enkephalin (ir) fibers closely surrounding β-endorphin (ir) perikarya and vice versa, suggesting axosomatic contacts. In contrast to the separate cellular localization of enkephalin and β-endorphin, ACTH (ir) and β-endorphin (ir) were invariably found in the same cells. Although β-endorphin (ir) was always found in γ_3-MSH (ir) cells, we have not detected γ_3MSH (ir) in every β-endorphin (ir) cell. Implications for the origin and processing of these endorphin-related peptides will be examined. (Supported by DA 01785 and MH 07901.)

126.21 VARIATIONS IN AMOUNT OF TYROSINE HYDROXYLASE IN INDIVIDUAL NEURONS OF NUCLEUS LOCUS CERULEUS OF RAT DEMONSTRATED BY COMPUTER ASSISTED QUANTITATIVE IMMUNOCYTOCHEMISTRY. R.H. Benno, L.W. Tucker,* T.H. Joh, and D.J. Reis, Laboratory of Neurobiology, Dept. of Neurology, Cornell Univ. Med. College, New York, NY.

We sought to determine by computer assisted quantitative immunocytochemistry using the peroxidase-antiperoxidase (PAP) technique if there is a heterogeneity among individual neurons of the nucleus locus ceruleus (LC) with respect to the amount of tyrosine hydroxylase (TH) within each cell and, if so, whether the amount of TH is related to morphometric characteristics and/or localization of the neuron within the LC.

Rats were perfused with 4% paraformaldehyde, the brains post-fixed in picric acid paraformaldehyde and embedded in paraffin. Sagittal sections of 5 μ were immunostained for TH using reaction conditions necessary to produce linearity of staining intensity with respect to amount of immunoreactive protein (Benno et al., Neurosci. Abstr. 5., 1979). Using image analysis, 1425 cells were analyzed for amount of TH/unit cytoplasm, location within the LC, and morphometric features. Cells were assigned locations within 5 subregions: anterior, posterior, and ventral poles, central core of the LC proper, and nucleus subceruleus (Grzanna and Molliver, Neuroscience 5, 1980). Collectively, the cells of the LC varied over a three-fold range in staining intensity, a three-fold range in longest dimension (average = 33.6 $\pm$ 6.2 μ) and a seven-fold range in cytoplasmic area (100-700 μ^2). The largest and most darkly stained cells were clustered in the ventral LC and subceruleus. Overall, for each neuron there was no correlation between the amount of immunoreactive TH/unit area and total cytoplasmic area (r=.22$\pm$. 06), nuclear/ cytoplasmic ratio (r=.10$\pm$ .08), and longest dimension/breadth ratio (r=.12$\pm$.04). However, there was a significant ($p<.05$) difference between the staining intensity of neurons in the anterior, ventral, and posterior poles and the subceruleus versus those of the central core, which were lighter. Analysis of density in concentric radii from the center of the LC shows that cells farthest from the center are the most darkly stained ($P<.01$).

We conclude that neurons of LC vary with respect to amount of TH, which in turn relates to the location of the cell within the LC and not its morphometric characteristics. Since, in noradrenergic neurons, the amount of TH is directly influenced by firing rate, the results suggest that the biochemical heterogeneity of LC neurons reflects differences in their functional activities. In turn, this may relate to heterogeneity of LC with respect to projection fields.

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126.22 AN IMMUNOFLUORESCENCE STUDY OF THE NUCLEUS TRACTUS SOLITARIUS IN THE CAT. B.E. Maley and R.P. Elde. University of Minnesota Medical School. Minneapolis, Minnesota 55455.

The indirect immunofluorescence method was used to map a number of putative neurotransmitters [substance P (SP), met-enkephalin (M-ENK), somatostatin (SOM), serotonin (5-HT) and neurophysin (NP)] throughout the feline nucleus tractus solitarius (NTS). Antisera were applied to consecutive ten micron sections cut in the transverse plane from normal and colchicine treated (500 μg in the cisterna magna; 48 hour survival) cats. Specificity of staining was determined using antisera pretreated with homologous and heterologous antigens. All sections were subsequently viewed in a microscope using epifluorescence. Differential amounts of immunoreactive fibers and cell bodies were observed in the various nuclei of NTS, which were named according to Loewy and Burton ('78).

In normal cats, SP and 5-HT immunoreactive fibers exhibited the greatest overall density throughout the nucleus. Both were localized to discrete areas of the NTS, with the lateral nucleus and a region just deep to the area postrema demonstrating the highest fluorescence. Fewer SP and 5-HT immunoreactive fibers were observed within the medial and commissural portions of the nucleus, while little immunoreactivity was present in the parvocellular or ventrolateral regions of NTS. A similar distribution could be found for M-ENK within the NTS, however, the amount of fluorescence was not as great as for SP and 5-HT. As before, the lateral nucleus and a region just deep to the area postrema exhibited the greatest amount of immunoreactivity, while the parvocellular and ventrolateral regions possessed the least. Immunoreactive SOM fibers present within the NTS were largely confined to the lateral and commissural regions. Other areas of the NTS exhibited minor SOM fluorescence. In a similar manner, a few immunoreactive fibers for NP were present in the NTS, however, they were restricted to the commissural region of the nucleus.

The NTS of colchicine treated cats exhibited a similar distribution of immunoreactive fibers within the nucleus, with the addition of cell bodies immunoreactive to SP and M-ENK. Most of these cell bodies were localized to discrete regions of the NTS. The differential occurrence of the previously mentioned neurotransmitters reflects not only the specific termination of afferents within the nucleus, but also its intrinsic neuronal and fiber content. Moreover, the distribution of immunoreactivity in the nucleus provides additional insight into the varied functions which have been attributed to the nucleus tractus solitarius.

R.P.E. is a recipient of a Scholar in Neuroscience Award from the McKnight Foundation.

127.1 β-ADRENERGIC RECEPTORS IN CILIARY PROCESS EPITHELIUM. James A. Nathanson, Dept. of Neurology, Massachusetts General Hospital, Boston, Massachusetts 02114.

Although certain studies have suggested that cyclic AMP may play a role in the control of intraocular pressure, the site of this nucleotide's synthesis and its regulation by β-adrenergic agents remains unclear. Previous studies in our laboratory (Science 204: 843) have shown that the CSF-secreting choroid plexus, a tissue with properties similar to those of the aqueous humor-secreting ciliary process, contains a very active β-adrenergic-sensitive adenylate cyclase. We now present evidence that β-adrenergic-stimulated adenylate cyclase is also present in the rabbit and human ciliary process. This enzyme is enriched in the ciliary epithelium and is more active than that found in the iris or ciliary muscle.

Adenylate cyclase activity was measured, in broken cell preparations, as the rate of synthesis of cyclic AMP from ATP under optimal cofactor and substrate conditions. Hormone stimulation was GTP-dependent and was enhanced in the absence of exogenous calcium. The enzyme was activated by low concentrations of isoproterenol (k_a=3 x 10^{-7}M) and norepinephrine (k_a=1.8 x 10^{-5}M) but was only poorly stimulated by dopamine or the α-adrenergic agonist, phenylephrine. Maximal stimulation in the rabbit ciliary process was 250-500% of control and, in the human, 500-1000% of control activity. Isoproterenol activation was inhibited by low concentrations of the β-adrenergic blockers, propranolol (k_i = 1.3 x 10^{-9}M) and timolol (k_i = 2.7 x 10^{-9}M) but only by much higher concentrations of the α-adrenergic blocker, phentolamine (k_i > 9 x 10^{-5}M) or the dopamine blocker, fluphenazine (k_i = 1.8 x 10^{-5}M). Further pharmacological studies with selective β-adrenergic agents indicated that one subtype of β-receptor predominated. Tissue dissociation and separation experiments demonstrated enrichment of hormone sensitivity in cell fractions enriched in ciliary process epithelial cells.

These findings may be relevant to physiological studies of ciliary process secretion and to the development of drugs with which to control increased intraocular pressure.

127.2 CHEMORECEPTORS FOR SEROTONIN (5-HT), ACETYLCHOLINE (ACh), BRADYKININ (BK), AND HISTAMINE (H) ARE PRESENT ON THE SOMA OF UNMYELINATED VISCERAL AFFERENTS IN THE RABBIT NODOSE GANGLION. H. Higashi* (SPON: J.P. Gallagher). Dept. of Physiology, Kurume Univ. Sch. of Med., Kurume, Japan and Dept. of Pharmacology and Toxicology, Univ. of Texas Med. Br., Galveston, TX 77550.

Rabbit nodose ganglia contain soma of both Type A, myelinated visceral afferents (conduction velocity (CV) = 6-9 m/sec), and Type C, unmyelinated fibers (CV = 0.5-1.5 m/sec). Four different algesic substances, 5-HT, ACh, BK, and H were applied to isolated rabbit nodose ganglia while recording their membrane potential and input resistance with standard intracellular reocrding techniques. Each impaled neuron was classified as to cell type, before applying an algesic. Four of 30 different neurons were Type A and 26 were Type C. None of the four algesic substances caused any significant change in membrane potential or resistance of the Type A neurons. On the other hand, 24 of the 26 Type C neurons responded with a depolarization to at least one of these four algesics. All of the algesics depolarized the soma membrane, but differed in their ionic mechanisms. 5-HT depolarized 90% of these neurons. The 5-HT depolarization was due to an increase in sodium (G_{Na}) and potassium (G_K) conductance (Nature, 267: 448, 1977). ACh depolarized these neurons by a mechanism apparently similar to 5-HT. About 30% of the Type C neurons responded to ACh. This ACh response could be blocked with d-tubocurarine (50μM) but not by atropine (50μM). About 50% of the neurons were depolarized by BK. The BK depolarization appeared to be associated with a decrease in G_K. H depolarized 25% of the Type C neurons. The mechanism for this depolarization was similar to BK, namely a decrease in G_K. Among the 26 Type C neurons 3 responded to all four algesics, 2 responded to three algesics, 11 responded to two algesics, 8 neurons were sensitive to one algesic, and 2 were insensitive to all four algesics. This preparation should prove useful for the study at the membrane level of algesics and potential analgesic substances on pain conducting fibers. (Supported by grants from the Ministry of Education, Culture and Science of Japan and USPHS Grants #NS 16228 and #NS 13727.)

127.3 MOUSE STRAIN DIFFERENCES IN STRIATAL CALMODULIN CONTENT. J.A. Severson* and C. E. Finch. Andrus Gerontology Ctr., Univ.S. Cal., Los Angeles, CA 90007

CBA/J, C57BL/6J and BALB/cJ mice differ in substantia nigra dopaminergic cells, nigral tyrosine hydroxylase (TH) activity, striatal TH activity and striatal dopamine (DA) receptors. Additionally, CBA/J mice lack the striatal DA receptor supersensitivity response to chronic haloperidol treatment. Since striatal DA receptor activation of adenyl cyclase is dependent on the interaction of the receptor-cyclase complex with calmodulin (CaM, calcium-dependent regulator protein), we examined how CaM varies with genetic differences in DA receptor number.

CaM was assayed by the activation of rat brain phosphodiesterase. Total striatal CaM was similar in all strains, however, when expressed as per mg wet wt. or per mg protein, CBA/J mice had 30% more striatal CaM than BALB/cJ mice($P< 0.001$), C57BL/6J mice had intermediate CaM levels. The distribution of CaM in soluble and particulate fractions was similar in all strains(60% soluble, 40% particulate), even though the absolute amounts of soluble and particulate CaM followed the same pattern as total CaM.

Since CBA/J mice have 50% fewer DA receptors than BALB/cJ mice, the ratio of CaM/DA receptor is about 2-fold higher in CBA/J mice. Elevated striatal CaM in CBA/J mice may represent a mechanism to compensate for depressed receptor number.

127.4 SPIPERONE BINDING SITES IN RAT BRAIN: LIGHT MICROSCOPIC AUTORADIOGRAPHIC LOCALIZATION OF DOPAMINE AND OTHER RECEPTORS. J.M. Palacios, D.L. Niehoff and M.J. Kuhar. Depts. of Pharmacology and Psychiatry, The Johns Hopkins University School of Medicine, Baltimore, Md. 21205.

Dopamine receptors have been identified in brain and other tissues by the direct binding of radiolabeled antagonists and agonists. Radiolabeled spiperone (SP: spiroperidol) has been utilized in many studies because of its high affinity for the binding sites and its relatively low level of nonspecific binding. A difficulty with SP is that it binds to more than one site; evidence suggests that it binds to a dopamine and a serotonin receptor and possibly to other sites. One must therefore use a number of pharmacological agents in displacement experiments to carefully define the various binding sites. For example, experiments in the literature indicate that, under certain conditions, ADTN can be used to selectively displace SP from dopamine receptors and cinanserin (CN) can be used to displace SP from serotonin receptors. Neuroleptics, e.g. haloperidol (HP), would displace SP from both sites.

Light microscopic autoradiographic localization of SP binding sites provides a highly sensitive measure of receptors with a correspondingly high degree of anatomical resolution. We have determined the conditions for localizing the various components of ^{3}H-SP binding in intact, mounted tissue sections. ^{3}H-SP binding to the mounted tissue sections exhibited all of the characteristics associated with receptor binding. It was saturable, of a high affinity, had the expected regional distribution and pharmacological specificity. For routine autoradiography, tissue sections were incubated with 0.4nM concentrations of ^{3}H-SP for 60 min at room temp in 0.17 Tris-HCl(pH 7.7) containing selected ions. Following two 5 min washes at 0°C, the sections were dried under a stream of cold, dry air. Autoradiograms were generated by the apposition of emulsion-coated coverslips.

Dopamine receptors, defined as ADTN displaceable SP binding, were found in the striatum, olfactory tubercle, cingulate cortex, substantia nigra and the adjacent ventral tegmentum, retina and the dorsal horn of the spinal cord. Serotonin receptors, defined as CN displaceable SP binding were found in several areas such as the cerebral cortex. In addition, there were SP sites not displaced by ADTN, CN, or haloperidol. These sites were however displaced by unlabeled SP itself. These special SP sites were found in the hippocampus, parts of the cortex, in nucleus accumbens, and in olfactory tubercles and could correspond to the "spirodecanone" site described by others.

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127.5 IN VIVO LOCALIZATION OF 77Br-p-Br-SPIROPERIDOL. H. K. Kulmala*, R. J. Dinerstein, C. C. Huang*, and A.M. Friedman*. Dept. of Pharmacol. & Physiol. Sci., The Univ. of Chicago, Chicago, IL 60637 and Chem. Div., Argonne National Lab., Argonne, IL 60439.

The usefulness of external gamma ray imaging techniques in the localization of gamma labeled neuroleptics is dependent on the development of a labeled neuroleptic which displays a high ratio of specific to nonspecific binding. In vitro studies with Br-Spiroperidol (BrSp) showed that this ligand binds with high affinity to the dopamine receptor (Huang, C. C., et al, J. Pharm. Sci., 1980, in press).

The gamma emitter, 77Br ($t_{½}$=57hr), was produced in a cyclotron by bombarding arsenic trioxide with 37 MeV alpha particles. Following distillation, the $77Br_2$ was allowed to react with spiroperidol (Sp) to produce 77Br-p-BrSp, at a specific activity of 6.85 Ci/mmole. Twenty seven male rats were injected with 77Br-p-BrSp (7.5µg/kg,i.v.) and were sacrificed at various time points. Nine of these rats also received 0.63mg/kg of Sp, i.v. Striatum, cerebellum, frontal cortex, and pituitary were dissected out and counted in a sodium iodide well counter. The counts were corrected for wet tissue weight.

The localization of 77Br-p-BrSp was found to parallel that of 3H-Sp in levels that were highest in dopaminergically innervated areas such as striatum, pituitary, and frontal cortex (Laduron, P. M., et al, Biochem. Pharmacol., 27: (1978) 317-321). A maximal striatum:cerebellum (S:C) ratio of 6.8 was found at 8 hours after injection. Unlabeled Sp at the dose used was found to affect levels of 77Br-p-BrSp only in dopaminergically innervated areas and resulted in an S:C ratio of 1.

Three rats received $77Br^-$ (2.2mg/kg, i.v.) and were sacrificed at 2 hrs. $77Br^-$ showed a nonspecific localization with respect to dopaminergic innervation. Levels in the cerebellum were higher than those in the striatum.

The utility of 77Br-p-BrSp is being tested by administration of the gamma emitter to anesthetized cats. The animals are monitored with a seven pinhole (6mm) collimator on a gamma camera. At four hours post injection, striatum is easily distinguishable from cerebellum in the three-dimensional computerized reconstruction from the seven simultaneously obtained images. Counts detected in corresponding regions gave an S:C ratio of 2.8±0.6, in good agreement with the data obtained from rats (3.8±0.3).

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127.6 SELECTIVE CHANGES IN DOPAMINE RECEPTOR BINDING IN STRIATUM FOLLOWING SUBSTANTIA NIGRA LESIONS. Ira D. Hirschhorn, Maynard H. Makman* and Eliot L. Gardner. Depts. of Biochem., Mol. Pharmacol., Psychiatry and Neuroscience, Albert Einstein Coll. Med., Bronx,N.Y.10461

Following lesion of the substantia nigra, the nigro-striatal dopaminergic neurons degenerate and most of the dopamine (DA) content of the striatum is lost. Unilaterally lesioned animals develop a motor asymmetry in which DA agonists, such as apomorphine, cause the animal to turn in a circular pattern (rotational behavior). Ungerstedt (Acta Phys. Scand., Suppl. 367, 69, 1971) originally postulated that rotation was due to supersensitivity of the denervated striatum. Later, Mishra, et al. (PNAS 71, 3893, 1974) found that DA stimulated adenylate cyclase (AC) activity in denervated striatum was doubled. The present experiments examined whether there are also changes in binding of ^{3}H-spiroperidol (^{3}H-SPI), a DA antagonist, and ^{3}H-ADTN (2 amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene), a DA agonist, to DA receptors. Agonist and antagonist ligands label different receptors or different conformations of the same receptor. Destruction of intrinsic striatal neurons with kainic acid causes almost total loss of ^{3}H-ADTN binding with relatively little effect on ^{3}H-SPI binding. Although increases in DA receptor binding in denervated striata have been reported previously, these are considerably less than the behavioral or DA stimulated AC supersensitivity. We examined guanine nucleotide regulation of ^{3}H-ADTN binding to determine whether there is a selective change in that component of binding which is regulated by guanine nucleotides. Striata from rats that rotated at a high rate were individually homogenized in Tris HCl buffer, centrifuged twice, incubated with ^{3}H-SPI or ^{3}H-ADTN with or without 50 µM Gpp(NH)p, and filtered through glass fiber filters. ^{3}H-SPI binding was equal in denervated and control striata (con=176, les=182 fmol/mg prot). Total ^{3}H-ADTN binding was 64% increased (con=63, les=103 fmol/mg prot.), with a greater increase in the Gpp(NH)p regulated component (155%). These data provide further evidence that ^{3}H-SPI and ^{3}H-ADTN label different populations or conformations of receptors and, furthermore, that ^{3}H-ADTN, but not ^{3}H-SPI, labels receptors linked to DA stimulated AC. They are consistent with the hypothesis that it is the guanine nucleotide sensitive ^{3}H-ADTN receptors that are linked to DA stimulated AC and these receptors are selectively supersensitive following substantia nigra lesion. Rotational behavior may involve supersensitivity of the receptors labeled by ^{3}H-ADTN, particularly the guanine nucleotide sensitive component, but not the ^{3}H-SPI receptors.

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127.7 RADIOAUTOGRAPHIC VISUALIZATION OF SEROTONERGIC BINDING SITES IN RAT AND CALF BRAIN. R. C. Meibach, S. Maayani and J. P. Green. Department of Pharmacology, Mount Sinai School of Medicine of the City University of New York, N.Y. 10029.

Rapid filtration methods for binding to homogenates and in vitro autoradiographic techniques (Young and Kuhar, 1979, Brain Res. 179;255) for visualizing binding sites in cryostat cut brain sections were used to characterize and visualize serotonin (5-HT) binding sites in both calf and rat brain. Specific binding was determined with cold LSD (10^{-6}M) added to ^{3}H-5-HT (29.2 Ci/mmole); in alternate sections, specificity was determined with cold 5-HT. Incubations were carried out at room temp. in 50mM Tris maleate, pH 7.4, 0.1% ascorbate and 5 x 10^{-5}M pargyline. ^{3}H-5-HT binding to both the P2 fraction and coronal brain slices were saturable with similar Kd and Bmax (Table). Binding sites were found in all layers of the cerebral cortex, olfactory tubercle and lateral septal nucleus. The thalamus was poorly labeled except for the lateral geniculate n. The superficial layer of the superior colliculus was labeled as was the midline hypothalamus substantia nigra and periaqueductal grey. No label was seen in the cerebellum. Within the hippocampal formation label was seen in the subiculum, dentate gyrus and field CA1 but not in field CA3. P2 fractions from dissected calf hippocampal subfields (Table) confirmed the absence of ^{3}H-5-HT binding to CA3. Cold LSD displaced most of the binding except in the substantia nigra and subiculum which were only displaced by cold 5-HT.

Preparation	Kd (nM) Scatchard	Bmax (fmoles/mg)	Hill Slope
Rat			
Slices	2.9	63	0.98
P2	1.1	30	1.00
Calf			
Slices	1.7	134	0.98
P2 -whole	1.4	67	0.74
-CA1	1.3	100	0.99
-CA3		NON-DETECTABLE	

(Supported in part by NIDA and NSF grants DA-01875 and BNS-7921286)

127.8 HETEROGENEITY OF [^{3}H]-D-LSD BINDING TO CALF HIPPOCAMPUS. S. Maayani, R.C. Meibach and J.P. Green. Dept. Pharmacol., Mt. Sinai Sch. Med., C.U.N.Y., New York, N.Y. 10029

Radioautographic visualization of 5-HT sensitive (1 µM) [^{3}H]-D-LSD binding sites in calf hippocampal slices (16 µm) revealed a distribution, similar to that found in rat brain hippocampus (Meibach et al., submitted). Both CA1 and dentate gyrus (DG) contain dense [^{3}H]-LSD sites while no binding could be visualized in fields CA3/CA4. Direct binding experiments on the P-2 fractions of these three hippocampal regions from calf (25°C, pH = 7.4) showed apparent homogeneous, non-interacting, 5-HT (1 µM) sensitive, [^{3}H]-LSD binding sites in both CA1 and DG but not in CA3/CA4. The binding characteristics of the 5-HT sensitive [^{3}H]-D-LSD binding in the P2 fraction of CA1 and DG were similar: K_d = 4 and 6 nM; B_{max} = 182 and 192 fmoles/mg protein, and like the binding characteristics of [^{3}H]-D-LSD in calf slices (Table). This heterogeneity of [^{3}H]-LSD binding sites, visualized by autoradiography and confirmed by direct labeling experiments, may reflect heterogeneity of in-vivo interactions of LSD in the hippocampus.

Preparation	K_d (nM)	Bmax fmoles/mg protein	Hill Slope
Slices	1.0	130	0.85
P-2			
Whole hippocampus	3	120	1.06
CA1	4	180	0.88
DG	6	190	0.99
CA3/CA4	Not detected		-

(Supported by NIDA and NSF grants DA-01875 and BNS-7921286)

127.9 ACCUMULATION OF COATED VESICLES BEARING α BTX BINDING SITES IN BRAIN TREATED MYOTUBES. S. Bursztajn and G.D. Fischbach. Pharmacology Dept. Harvard Medical School, Boston, Mass. 02115.

Coated vesicles 80 to 120nm in diameter are present in cultured chick myotubes. We have shown that the number of coated vesicles is increased at functional nerve-muscle contacts in spinal cord-muscle co-cultures and that many of these coated vesicles contain α BTX-HRP binding sites. Coated vesicles were investigated further in myotubes treated with saline extracts of embyonic chick brain. Such extracts produce a large increase in surface receptor number and an increase in number of receptor clusters (Jessell et al., PNAS., 76:5397-5401, 1979). We measured the internal pool of ACh receptor with ^{125}I α BTX (5nM) in saponin (0.5%) permeabilized cells after blocking the surface sites with unlabeled α BTX. The number of specific ACh sites revealed by saponin was approximately 30% of the number present on the surface. There was a 3.4 fold increase in intracellular α BTX binding sites in cultures treated with brain extract daily (330μg protein/day) for 3 days as compare to controls, and a five fold increase in the number of coated vesicles as compared to non-brain treated controls. We investigated the intracellular ACh binding sites with α BTX conjugated to HRP. The conjugate blocks ^{125}I α BTX binding to the same extent as the unlabeled toxin, indicating that the conjugate has access to approximately the same number of intracellular ACh receptors. Approximately 50% of coated vesicles beneath "hot spots" in brain extract treated cells were labeled.

The following results suggests that at least some of the coated vesicles are on route to the cell surface: (1) Cultured myotubes labeled from 1 to 6 hr. with α BTX-HRP show only 17% ± 3.1% of the coated vesicles labeled. (2) Cultures whose surface receptors are blocked for 4 hr. with unlabeled toxin showed only a 6% decrease in total coated vesicles subsequently labeled following permeabilization and incubation in α BTX-HRP conjugate. (3) myotubes treated with puromycin for 6 hr. show a 50% decrease in number of coated vesicles beneath "hot spots" and only 23% of this reduced population of coated vesicles was labeled with α BTX-HRP toxin. One possible route of ACh receptor rich coated vesicle insertion into the surface membrane is via the extensive tubulo-vesicular membrane network that communicates with the cell surface. It is significant in this regard that 80% ± 3.8% of the length of the dialated sarco-tubular membrane was labeled near "hot spots" as compared to 8% ± 5.4% at "non hot spots" membrane. The cisternal membrane length that was labeled at "hot spots" decreased 4.7 fold after puromycin treatment. (Supported by NIH Grant #NS 11160 and MDA Fellowship (SB)

127.10 DEVELOPMENT OF α-BUNGAROTOXIN RECEPTORS IN CULTURED CHICK CILIARY GANGLION NEURONS. A. Messing * and S.U. Kim* (SPON: J.Q. Trojanowski). Div. of Neuropathology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

We have maintained embryonic chick ciliary ganglion neurons in dissociated cell culture and studied the progressive appearance of surface receptors for ^{125}I-α-bungarotoxin. Cultures were established from 8-day-old embryos and fed a medium supplemented with 180 μg/ml of a soluble protein extract prepared from the eye, the target organ for the ciliary ganglion. Approximately 8100 neurons survived per ganglion and there was no evident loss of neurons through two weeks in culture. Binding of ^{125}I-α-bungarotoxin was determined at room temperature on intact cells still attached to their coverslips. Non-specific binding was less than 2% of the total. Specific binding of ^{125}I- α- bungarotoxin was saturable with respect to both time of incubation (20-30 minutes) and concentration of toxin (5-10 nM), with an apparent K_D=1.0nM. Binding sites for ^{125}I-α-bungarotoxin increased during the first week in culture from 1.8 fmoles per 10^4 neurons at 1 day in vitro (DIV) to 8.6 fmoles per 10^4 neurons at 7 DIV, after which the number of sites seemed to plateau. Light microscopic autoradiography was performed on cultures at 4 DIV and showed most of the grains associated with the surfaces of neuronal cell bodies, while scattered grains occurred over neuronal processes. When compared with the in vivo development of α-bungarotoxin receptors in chick ciliary ganglia[1], the appearance of receptors in these cultured neurons followed a time course similar to, but at lower levels than, their in vivo counterparts. Nevertheless, this culture system should prove useful for the study of questions concerning the regulation, surface distribution and intracellular pathways of neuronal α-bungarotoxin receptors.

[1]Chiappinelli, V.A. and Giacobini, E., Neurochem. Res., 3 (1978) 465-478.

127.11 LOCALIZATION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN GOLDFISH RETINA. Charles Zucker and Stephen Yazulla. Department of Neurobiology and Behavior, SUNY at Stony Brook, Long Island, NY 11794

In goldfish retina there is evidence for nicotinic-cholinergic transmission in the inner retina. Binding of the nicotinic ligand [^{125}I] -α-bungarotoxin has been localized to three discrete bands in the inner plexiform layer (IPL). We have attempted to localize nicotinic binding sites in the IPL at the electron microscopic (EM) level using a horseradish peroxidase-α-bungarotoxin conjugate (HRP-α- BTx). An active ester method was used to crosslink HRP to α BTx (Vogel et al., J. Histochem. Cytochem. 27:846-851, 1979). Our conjugate has an apparent K_D of 6.1 x 10^{-10}M as measured by competition with native α BTx for solubilized acetylcholine receptors from Torpedo californica.

Retinal slices 150-200μ thick were incubated for 30-60 minutes in oxygenated goldfish Ringer containing either 10^{-6}M HRP-α BTx or 10^{-6}M HRP-α BTx plus 10^{-3}M d-tubocurarine chloride. The tissue was stained for HRP activity with 3,3'-diaminobenzidine and processed for EM. In the IPL, the majority of synapses containing HRP reaction product are located in two distinct bands, one in the most proximal region of sub-lamina A and the other in the proximal half of sub-lamina B. Of the identifiable synapses showing reaction product, amacrine-to-amacrine contacts are seen with the highest frequency while amacrine-to-bipolar synapses and synapses made by bipolar cells are seen less often. Synapses made by bipolar cells ordinarily have two post-synaptic processes (dyad). Reaction product at these synapses usually is restricted to one member of the post-synaptic dyad suggesting that receptors on the other post-synaptic process may be muscarinic. A significant number of small unidentified processes show discrete patches of reaction product which may indicate synaptic junctions. In control slices, prior treatment with curare abolished HRP reaction product at synaptic sites.

These results indicate that there are some classes of cholinergic bipolar and amacrine cells which make nicotinic synapses. Our findings are similar to the HRP-α BTx localization studies in chick retina but they differ from the findings in mouse retina in which HRP reaction product was never found at synapses made by bipolar cells.

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127.12 MUSCARINIC RECEPTORS IN INTACT CHICK HEART CELLS IN CULTURE R.E. Siegel* and G.D. Fischbach. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115

The muscarinic acetylcholine receptor (AChR) has been studied extensively in tissue homogenates but little is known about the receptor in intact cells. Cells dissociated from 8-day old chick embryo hearts were plated at 8x10^4 cells/6mm well. Binding assays were performed 2-3 days later with the muscarinic antagonists 1-quinuclidinyl-[phenyl 4-^{3}H]benzilate ([^{3}H]-1QNB) and [^{3}H]N-methylscopolamine ([^{3}H]-NMS). [^{3}H]-1QNB (S.A.=40 Ci/mmol) bound to a single class of sites with a K_d of 30 pM. Specific binding was defined as that which was inhibited by 10^{-6} M atropine methyl nitrate. At the K_d, nonspecific binding amounted to about 20% of the total. The number of binding sites ranged between 180-300 fmol/mg protein. Muscarinic AChRs were also assayed in separately plated atrial and ventricular cells. Atrial cells bound 200 fmol/mg protein while ventricular cells bound 150 fmol/mg protein. Since a quaternary antagonist was used to determine specific binding, it is likely that most of the specific sites represent cell surface receptors. This is supported by the fact that [^{3}H]-NMS (S.A.=53 Ci/mmol, K_d=300 pM; nonspecific binding <5%) yielded the same estimates of receptor number as [^{3}H]-1QNB.

[^{3}H]-1QNB can be used for autoradiography since it dissociates slowly ($t_{\frac{1}{2}}$>4 hr); few specific counts were lost during processing of the samples. Myocytes were distinguished from fibroblasts on the basis of cell shape and by indirect immunofluorescence using an antiserum against cardiac myosin. Grains were distributed uniformly over all myocytes. The mean receptor density on atrial myocytes was 83±5 sites/μ^2 and the density on ventricular cells was 81±4 sites/μ^2. The grain density over cells in small aggregates was greater than that over isolated myocytes. Thus, if [^{3}H]-1QNB binding is an adequate measure of functional muscarinic receptors, then reported differences in sensitivity of atrial and ventricular cells to cholinergic agonists must not be related to receptor density.

Saline extracts of embryonic spinal cord and brain produce a 4- to 5-fold increase in the number of nicotinic AChRs and a 40-fold increase in the number of receptor clusters on cultured chick skeletal muscle fibers (PNAS 76,5397-5401, 1979). The same extract produced only a 50% increase in the number of myocardial muscarinic receptors. This small increase could be accounted for by mitogenic effects of brain extract, rather than an effect on receptor densitiy. Thus muscarinic and nicotinic AChR density may be regulated in different ways.

127.13

Withdrawn by Author

127.14 QUINACRINE BINDING TO MOUSE BRAIN TISSUE IN VITRO: A POSSIBLE METHOD FOR LABELLING HISTAMINE RECEPTORS AND/OR HISTAMINERGIC NEURONS. E. L. Orr* and R. J. Wordinger* (SPON: I. M. Korr). Dept. of Anatomy, North Texas State University/Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

Quinacrine (QC) has been shown to bind to specific cells in peripheral organs and nerves by Ålund and associates [1,2,3]. After an i.v. injection of QC hydrochloride (5 or 10mg/kg), Ålund and Olson[1] reported QC fluorescence of cell bodies in the median eminence, and the supraoptic and paraventricular nuclei of the hypothalamus. We now report the specific binding of QC to neuronal perikarya and processes (axons) after in vitro exposure of brain slices to low concentrations of QC.

Frozen sections (10 microns) of mouse brains were thaw-mounted to microscope slides and incubated for 5-30 minutes at 37°C in 50 to 100 nM QC. Phosphate-buffered saline (pH 7.4) containing 0.68 mM $CaCl_2$, and 0.49 mM $MgCl_2$ and 2.68 mM KCl (PBS) was used as diluent and incubation buffer. After incubation, the tissues were rinsed in ice-cold PBS followed by distilled water (to remove excess salts) and after air-drying approximately 30 min., covered with mineral oil and a coverslip. The fluorescence was viewed with a Zeiss Photomicroscope III equipped for catecholamine and fluorescein isothiocyanate fluorescence.

The granule cell layer of the cerebellar cortex was found to be highly fluorescent, with little or no fluorescence in the molecular layer. Purkinje cells were less fluorescent than granule cells, while the white matter was slightly fluorescent. Apparent ponto-cerebellar fibers were also fluorescent, and at 50 nM QC (30 min. incubation), these fibers were varicose in appearance. At higher concentrations (100 nM QC), these fibers were brightly and evenly fluorescent. Preliminary studies have shown that 100 µM mepyramine (an H_1-receptor antagonist) will block the aforementioned fluorescence (50 nM QC) whereas 100 µM cimetidine (an H_2-receptor antagonist) had no effect. These results suggest that QC may be binding to histamine receptors and/or histaminergic neurons in the CNS.

[1] Ålund, M. and L. Olson (1979). Cell Tiss. Res. 204:171-186.
[2] Olson, L., M. Ålund and K.-A. Norberg (1976). Cell Tiss. Res. 171:407-423.
[3] Olson, L. and M. Ålund (1979). Med. Biol. 57:182-186.

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127.15 GLYCINE RECEPTOR LOCALIZATION IN RAT BRAIN BY RADIOHISTOCHEMICAL METHODS. M.A. Zarbin*, J.K. Wamsley and M.J. Kuhar. Depts. of Pharmacology and Psychiatry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Glycine receptors have been identified in the CNS by the direct binding of radiolabeled strychnine to homogenates. We have labeled glycine receptors in mounted tissue sections for the purpose of localizing the receptor by light microscopic autoradiography. The binding of radiolabeled strychnine to tissue sections was kinetically and pharmacologically identical to its binding to homogenates: the binding was saturable, of a high affinity, had the appropriate regional distribution, and the binding of strychnine to tissue sections of spinal cord was displaced by glycine but not by serine, proline, or GABA. Autoradiographs were generated by the apposition of emulsion coverslips as described by Young and Kuhar (Brain Res., 179:225, 1979).

Glycine receptors were concentrated in the spinal cord, medulla and pons. The receptor was widely distributed in the gray matter of the spinal cord. Levels were especially high around motoneurons in the ventral horn and in the substantia gelatinosa in the dorsal horn (laminae II and III of Rexed). In the medulla, high levels of glycine receptors were found in the nuclei of the cranial nerves V (trigeminal) and XII (hypoglossal). In addition receptors were concentrated in the gracile and cuneate nuclei; in the lateral reticular nucleus; in the nucleus intercalatus; and in portions of the inferior olivary nucleus. The lateral reticular nucleus of the medulla (pars ventralis and pars dorsalis) and the nucleus reticularis gigantocellularis showed significant but less striking densities of receptors. In the forebrain, the density of glycine receptors was generally very low or undetectable. Some areas of high receptor density, however, were noted: the parafascicular nucleus of the thalamus and the zona incerta. The nucleus of the lateral geniculate body (pars ventralis) contained a moderately dense population of glycine receptors. These studies should be useful for mapping glycinergic pathways in the brain and suggest an association of glycine receptors with sensory as well as motor pathways in the CNS.

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127.16 RELATIONSHIP BETWEEN GABA AND BENZODIAZEPINE RECEPTORS: QUANTITATIVE AUTORADIOGRAPHIC STUDIES. J.R. Unnerstall, J.M. Palacios and M.J. Kuhar. Depts. of Pharmacology and Psychiatry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

The pharmacological effects of the benzodiazepines (BZ) may be mediated through interactions with the GABA receptor. We have localized both the high affinity GABA receptors and the BZ receptor by light microscopic autoradiography in the rat CNS. The autoradiographic procedure involves the in vitro labeling of mounted tissue sections and the generation of autoradiograms by the apposition of emulsion-coated coverslips. By exposing suitable standards along with these labeled sections, one can relate the autoradiographic grain density to receptor density in terms of pmoles/g tissue.

Standards were constructed by mixing known amounts of non-volatile radioactive compounds with brain tissue ground to a paste. Blocks of tissue paste containing layers of different concentrations of radioactivity were frozen onto microtome chucks. Autoradiographic studies of sections of these blocks (4-10 uM in thickness) revealed that grain density increased linearly with time and concentration of radioactivity up to certain limits. Our routine receptor autoradiographic studies are normally within these limits.

GABA and BZ receptors were labeled as previously described (Palacios, J.M., Young, W.S., III and Kuhar M.J., J. PNAS 77:670, 1980; Young, W. S., III and Kuhar, M.J., J. Pharmacol. Exp. Ther. 212:337, 1980). The in vitro labeling of tissue sections for autoradiography allows for the careful control of the binding parameters. It was clear that the distribution of high affinity GABA and BZ receptors was different. For example, in the diencephalon, there were high densities of GABA receptors in the thalamus while BZ receptor density was high in the hypothalamus. Biochemical studies with our mounted tissue sections showed that GABA potentiated BZ receptor binding in agreement with other laboratories. Autoradiographic studies of the sites of this potentiation revealed that not all regions showed the same increase. For example, BZ receptor binding in the external plexiform layer of the olfactory bulb increased 40% upon exposure to 0.1 mM GABA while the increase in the molecular layer of the cerebellum was about 230%. There results suggest that there is no simple fixed-ratio relationship between the GABA and BZ receptors revealed in these studies. The effect may involve subpopulations of the receptors or even different receptors than the ones labeled under the conditions used in these studies.

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127.17 OPIATE RECEPTOR BINDING IN RAT BRAIN SUBCELLULAR FRACTIONS, Bryan L. Roth*, Michael Laskowski and Carmine J. Coscia*, Depts. of Biochemistry and Physiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Recent binding studies involving opiate receptors have utilized crude membrane preparations isolated in hypotonic buffers with little attempt at subsequent purification. Binding phenomena associated with these membranes cannot accurately reflect neuronal processes but are accepted as an approximation. Accordingly, in an effort to contribute to an understanding of the mechanism of action of opiates at the molecular level, we have initiated an investigation aimed at characterizing opiate receptor binding in purified subcellular fractions of rat brain. We have found that two distinct subcellular classes of receptors exist--one associated with a highly purified synaptic plasma membrane (SPM) fraction and the other with a crude microsome preparation (P3). In both fractions, receptor binding was enriched approximately 4-6 fold over crude homogenates. Furthermore, [^{3}H]naloxone and [^{3}H]dihydromorphine displayed high affinity binding with Kd's for naloxone of 1.3 nm (SPM) and 2.9 nm (P3) obtained by Scatchard analysis. The β_{MAX} for SPM and microsomes was 1.08 and .36 pmol/mg protein respectively. Marker enzyme assay revealed opiate binding was highly correlated with acetylcholinesterase activity and desmethylimiprimine-sensitive NE uptake. No correlation was observed with microsomal (NADPH-cytochrome-c-reductase, RNA), mitochondrial (monoamine oxidase), glial (2',3'-cyclic nucleotide phosphohydrolase), or cytosolic (LDH) enzyme activities. Subfractionation of the P3 preparation by continuous sucrose density gradients (18-28%, 200,000 x g, 10 h) revealed 5 distinct protein bands, two containing acetylcholinesterase activity. Additionally, fractions have been monitored by electron microscopy. In conclusion high affinity binding sites for opiate agonists and antagonists have been found to be associated with purified SPM as well as with microsomal preparations. (Supported by NS-12342)

127.18 DIFFERENTIAL DISTRIBUTION OF TYPE I AND TYPE II OPIATE RECEPTORS IN MONKEY CEREBRAL CORTEX. M. E. Lewis,* E. Bragin,* M. Mishkin, C. B. Pert and A. Pert. Biological Psychiatry Branch and Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205

Although opiate receptor density is reportedly low in rat cerebral cortex, some regions of monkey cerebral cortex have been found to contain a relatively high concentration of opiate receptors. Because of the large literature emphasizing the functional and anatomical heterogeneity of monkey cerebral cortex, a detailed study of the cortical distribution of two types of opiate receptors was carried out. Type I and Type II opiate receptors (C.B. Pert and D.P. Taylor, in Endogenous and Exogenous Opiate Agonists and Antagonists, E.L. Way (Ed.), New York, Pergamon, 1980, pp. 87-90) are labeled under appropriate assay conditions by [^{3}H]naloxone and [^{3}H]D-Ala^2,D-Leu^5-enkephalin, respectively.

Rhesus monkeys were anesthetized with sodium pentothal before the brains were removed and dissected over ice into 39 areas which could be ordered into several functional hierarchies (cf. M. Mishkin, Neuropsychologia 17, 139-151, 1979). For example, in the visual system, neuroanatomical, electrophysiological and behavioral evidence indicates that visual information is processed sequentially through a series of cortical loci corresponding to von Bonin and Bailey areas OC (striate cortex), OB (lunate and inferior occipital sulci), OA (prestriate cortex), TEO (posterior inferotemporal cortex), TE (anterior inferotemporal cortex), and ventral TG (temporal pole). The density of stereospecific Type I opiate receptors increased in a gradient along this system from primary sensory cortex (OC) to the temporal pole regions (TG) which relay sensory information to the amygdala. The relative opiate receptor density observed was 1 (OC), 1.3 (OB), 1.7 (OA and TEO), 2.5 (TE) and 4.2 (ventral TG). A similar progression was apparent in the auditory cortical system, with a 3-fold increase in Type I opiate receptor density in the temporal pole (lateral TG) over that in the supratemporal plane (TB and TC). Evidence for receptor gradients was also obtained in the somatosensory and motor systems and in the prefrontal cortex. This pattern of distribution of Type I opiate receptors contrasts markedly with the distribution of Type II receptors labeled by [^{3}H]D-Ala^2,D-Leu^5-enkephalin. The enkephalin-labeled receptors were relatively evenly distributed throughout the cerebral cortex, showing less than a 2-fold variation among the 39 regions. Therefore, the two ligands label clearly different populations of opiate receptors in the monkey cerebral cortex. Discussion will focus on the possible functional implications of the gradients of Type I opiate receptors within hierarchically organized cortical systems.

127.19 OPIATE RECEPTOR DISTRIBUTIONS IN BRAIN SUGGEST "OPIATERGIC" TRACTS. S. Moon Edley,* M. Herkenham, C. B. Pert. Lab. of Neurophysiology and Biological Psychiatry Branch, NIMH, Bethesda, MD 20205

Autoradiographic visualization of specific, high-affinity [^{3}H]naloxone binding to slide-mounted tissue slices shows anatomical localization of opiate receptors in brain. In vitro incubation of serially cut fresh-frozen brain sections in a 4°C medium containing [^{3}H]naloxone in 0.05 M Tris buffer (pH 7.4), 100 mM NaCl and protease inhibitors is followed by rinsing in 4°C phosphate buffer solutions, rapid drying, hot paraformaldehyde vapor fixation and routine autoradiography (Herkenham and Pert, PNAS, in press). The results of this new method compare favorably with previously published techniques, as judged by examination of labeling in the caudate nucleus, where receptor patches stand out in sharp contrast to unlabeled areas. The ease with which in vitro labeling is accomplished suggests that this technique has general applicability for studying receptors in any tissue, including human brain. In rat brain, light microscopic resolution is so striking that close concordances between opiate receptor distributions and patterns of termination of some known tracts can be noticed, suggesting numerous, often sequential "opiatergic" pathways. A previously described five-link neural connection originates in the accessory olfactory bulb and passes to the medial and posterior cortical nuclei of the amygdala and then is sequentially relayed to the bed nucleus of the stria terminalis, the border of the medial and lateral habenular nuclei and, finally, the interpeduncular nucleus and median raphe. This linkage is noteworthy for the close correspondence of patterns of dense opiate receptor labeling with the patterns of termination of these tracts.

Another striking and general phenomenon is that opiate receptors are found within most primary and many secondary sensory nuclei, and are localized to superficial and/or molecular layers in laminated structures. Dense labeling in the superficial laminae of the spinal cord dorsal horn is one example, but localization of receptors in the superficial layer of the superior colliculus and the dorsal cochlear nucleus and in the glomerular and outer plexiform layers of the main and accessory olfactory bulbs are other examples. The "opiatergic" neurons whose axons are presynaptic to these receptors have not been identified but would be expected to functionally affect sensations at early stages of processing. Opiate receptors are also prominent in limbic cortical areas. By their laminated appearance in layers I and III of frontal and cingulate cortices, layer VI of the entorhinal area, and dense localization in the presubiculum, the possiblity of "opiatergic" thalamic or intracortical pathways terminating at these receptor sites can be surmised.

127.20 COMPETITIVE BINDING OF INSULIN TO BOVINE CEREBRAL MICROVESSELS. I. Gozes, Y. Gozes*, T.B. Strom* and M.A. Moskowitz. Lab. of Neural and Endocrine Regulation, MIT, Cambridge, MA 02139, and Immunology Lab. and Section of Neurology, Department of Medicine, Peter Bent Brigham Hospital, Boston, MA 02115.

Insulin receptors have been identified by competitive binding assays within the brain and spinal cord of several mammalian species. A relatively high proportion of these receptors appear by radioautography to be associated with sites on cerebral arterioles and capillaries where they may be important in the regulation of cerebral or cerebrovascular metabolism. In order to examine further the nature of hormone-receptor interaction, we have studied the binding of insulin and its various analogues to a highly purified preparation of isolated arterioles and capillaries from bovine cerebral cortex.

Bovine cerebral microvessels were isolated by isopycnic sucrose gradient centrifugation and molecular sieving and were shown by light and electron microscopy as well as by biochemical criteria to be free of contaminating neuronal and glial cells. The insulin binding to homogenized cerebral microvessels was measured utilizing a competitive ^{125}I-insulin radioreceptor assay. Final separation of microvessel-bound from free insulin was accomplished by centrifugation of the incubation mixture through oil. There was virtually no contamination of the microvessel fraction by free insulin.

Our data show that the amount of ^{125}I-insulin bound by the microvessel homogenate was proportional to the microvessel protein added to the incubation mixture. Between 20-35% of total bound counts was specific, however the displacement of pg amounts of ^{125}I-insulin required the addition of µg amounts of the cold polypeptide. Biologically less potent insulin analogues such as proinsulin or desoctapeptide appeared more active than insulin in their ability to compete with isotopically-bound insulin to the microvessel homogenate. In several preliminary experiments, nerve growth factor (NGF 2.5S and 7S) exhibited the most marked ability to displace insulin from this tissue. Our results suggest that insulin does not bind with high affinity to brain microvessels. However, we cannot rule out the possibility that there are vascular receptors for related molecules such as NGF or somatomedin.

128.1 EVIDENCE FOR ASCENDING ENDORPHINERGIC INHIBITION OF DISTRESS VOCALIZATION. N. Najam*, B.H. Herman* and J. Panksepp (SPON: W. Nelson), Dept. of Psychology, Bowling Green State Univ., Bowling Green, OH 43403.

Electrical brain stimulation (ESB) was employed to map brain distress vocalization (DV) loci in adult guinea pigs, and the inhibitory effects of brain endorphins and serotonin (5-HT) on these DV sites was evaluated.

DVs resembling natural separation DVs were produced by stimulating the dorsomedial thalamus (DMT) and septum/preoptic (POA), whereas stimulation of sites immediately surrounding the mesencephalic periventricular grey (PVG) produced "pain-like" screams. Other areas such as the cortex were free of vocalization loci. Thus, there appear to be specific subcortical emotional vocalization zones in the guinea pig brain.

In other studies, we examined the effects of a variety of drug and ESB mannipulations on DMT and septum/POA DVs. Naloxone increased the frequency of these forebrain derived ESB DVs by about 50% over saline baseline, whereas morphine and quipazine (5-HT agonist) decreased ESB DV frequency. In previous investigations, we found that separation DVs of infant guinea pigs are also increased by naloxone and decreased by morphine (B.H. Herman and J. Panksepp, Pharm. Biochem. Behav., 9: 213, 1978). Therefore, endorphins are inhibitory to natural or brain-elicited DVs.

Analgesic PVG stimulation inhibited DMT and septum/POA DVs by about 50%, and naloxone blocked the effects of analgesic PVG stimulation on DMT DVs. These data provide evidence for ascending endorphinergic inhibition of DMT DV sites, and extend the observations of others indicating that pain as well as brain-elicited DVs are under endorphinergic modulation.

Stimulation of nonanalgesic mesencephalic sites surrounding the PVG produced increases in the frequency of anterior elicited ESB DVs, and these effects were additively potentiated by naloxone pretreatment.

128.2 AFFERENT AND EFFERENT CONNECTIONS OF THE ORGANUM VASCULOSUM OF THE LAMINA TERMINALIS: A HORSERADISH PEROXIDASE (HRP) STUDY. Arturo Camacho* and M. Ian Phillips (SPON: W.J. Steele). Dept. of Physiology, Univ. of Iowa, Iowa City, IA 52242.

The organum vasculosum of the lamina terminalis (OVLT) is a single structure in the anterior ventral part of the brain lying immediately above the optic chiasm and below the anterior commissure. The OVLT has been proposed as a receptor site for the central dipsogenic, blood pressure and antidiuretic effects of angiotensin II (AngII).

In the present report, we have approached the OVLT ventrally through the optic chiasm to eliminate damage to structures dorsal to the OVLT and pressure injected 10-15 nl HRP (20% w/v) using a 1 µl Hamilton syringe with a glass micropipette glued to the needle. Following a 24 hour post-injection period, the animals were transcardially fixed (Karnovsky fixative) and brains removed. The rat brains were cut in 40 µ sections coronally, sagittally, or horizontally, and processed by the tetramethyl benzidine (TMB) method, with alternate sections near the site of injection processed by the diaminobenzidine (DAB) method. The brain sections were counter-stained with neutral red for the TMB sections and with methylene blue for the DAB sections. Table I summarizes the results of 12 male Sprague-Dawley rat brains.

TABLE I

Brain Area	Cell Bodies	Fibers
Subfornical organ	+++	+
Nucleus medianas	++	+++
Medial preoptic	+++	++
Supraoptic	+	++
Ventromedial nucleus	++	
Lateral hypothalamus	++	
Arcuate nucleus	+	
Pericallosal region		+++
Lateral preoptic	+++	+++
Hippocampus		+
Septum	+	+++
Locus coeruleus	+	
Central gray	+	

In conclusion, the HRP-positive perikarya and fibers confirm a neural connection between the OVLT and the subfornical organ (Miselis et al., Science 205:1022, 1979), both of which have been reported to be AngII receptor sites. The retrograde and anterograde transport of HRP revealed hypothalamic as well as extrahypothalamic connections of the OVLT, which may be related to the homeostatic functions of this structure.

128.3 NEONATAL HANDLING STRESS AND ACTH 4-10 ADMINISTRATION AFFECT ADULT ACTH-INDUCED GROOMING BEHAVIOR IN RATS. D.L. Colbern*, S.M. Dray, A.N. Taylor and E.G. Zimmermann. Neuroscience Program, Brain Research Inst. and Depts. of Psychology and Anatomy, UCLA and Brentwood V.A. Med. Ctr., Los Angeles, CA 90024.

Drug or environmental manipulations of neonatal rats alter adult behavioral and physiological responsivity to stressful stimuli. The present study examined effects of neonatal handling stress and exposure to ACTH 4-10 on adult grooming behavior induced by intracerebroventricular (ICV) injection of ACTH 1-24 in male Sprague Dawley rats.

Two types of early handling stress were produced by daily injection of 0.1 ml saline sc on postnatal days 7-9, using either 1) a standard injection procedure where pups are removed from the nest, placed on a heating pad, injected and returned as a group to the dam, or 2) a huddled injection procedure where the dam is removed from the cage, the pups injected in the nest under an external heat source and the dam returned at the end of the procedure. Rats, unhandled neonatally, served as additional controls. At 85 days of age, all animals were implanted with a polyethylene cannula and, 6 days later, injected ICV with 0.3 µg ACTH 1-24 in 2 µl saline or 2 µl saline. Animals were placed individually into novel observation chambers and, beginning 15 min postinjection, grooming was measured for 50 min using a time sampling technique.

Animals given saline neonatally by the standard injection procedure failed to show adult ACTH-induced grooming when compared to controls given ICV saline ($p > .05$) and they exhibited less peptide induced grooming than did unhandled controls ($p < .01$) or animals injected with saline in the huddle ($p < .05$). The huddle-injected animals did not differ from the unhandled controls ($p > .05$), both groups showing marked increases in grooming response to ICV ACTH 1-24 when compared to their ICV saline injected controls ($p < .01$).

In preliminary studies, pups given 10 µg ACTH 4-10 in 0.1 ml saline on days 7-9 showed intact adult excessive grooming to ICV ACTH 1-24 when compared to controls which were either unhandled or injected with saline in the huddle. Thus, in contrast to animals given saline on days 7-9, those given ACTH 4-10 by the standard injection procedure showed unimpaired adult grooming levels.

These findings demonstrate that neonatal handling stress can modify adult peptide-induced behavior and that quality and/or quantity of the early stress is important for the manifestation of this long-lasting peptide induced behavioral effect. Moreover, neonatal exposure to ACTH 4-10 seems to interfere with the action of early stress on the development of such effects. These findings support previous hypotheses that protracted neuroendocrine and behavioral effects of neonatal stress and ACTH 4-10 are mediated by altered functional organization of the brain. (Support: March of Dimes Birth Defects Fdn. and USPHS Grant NIAAA-03513.)

128.4 EFFECT OF BOMBESIN ON BODY TEMPERATURE IN AWAKE AND HIBERNATING GROUND SQUIRRELS. Delphi M. Toth. Dept. of Anat. Sci., Coll. of Med., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK 73190.

There is considerable evidence that numerous brain and gastrointestinal peptides, including the opioid peptides, have potent effects on body temperature (T_b) in non-hibernating species. Bombesin, a tetradecapeptide isolated from anuran skin, has been shown to be one of the most potent substances affecting thermoregulation. At doses as low as 1 ng administered intracisternally or intraventricularly, bombesin produces dramatic reductions in T_b in cold-exposed rats. Bombesin hypothermia is reversed by naloxone. However, the effect of bombesin on hibernation is unknown.

Mammalian hibernation represents a highly effective strategy for prolonged survival under adverse climatic conditions of cold and starvation. From spring through early fall, these mammals are active and euthermic, maintaining a T_b of approximately 38°C. During the winter these same mammals appear to abandon euthermia as T_b drops to near 0°C and they become dormant. Throughout the winter they hibernate in bouts of 7-12 days duration, with a return to a T_b of 38° during the brief arousals between bouts. The ability to rewarm from nearly 0° to about 38° against the thermal gradient at any time (e.g., in response to sensory stimuli or adverse conditions) distinguishes hibernation from poikilothermia or hypothermia.

In order to determine the effect of bombesin on T_b throughout the hibernation cycle, cannulae were implanted into the cisterna magna of adult golden-mantled ground squirrels (*Spermophilus lateralis*). Ground squirrels were then placed into a cold room (5°C) and permitted to hibernate. Thoracic temperature, heart and respiratory rates, and oxygen consumption were monitored continuously, before and after administration of the peptide.

Bombesin produced a sharp and prolonged decrease in T_b when administered to animals during periods of wakefulness between bouts of hibernation. On several occasions, animals re-entered hibernation without regaining euthermia, but the ensuing bout of hibernation did not appear to be unusual. In hibernating animals, bombesin produced only slight decreases in T_b, however these animals continued to hibernate for approximately one week beyond the expected time of re-awakening. These protracted bouts of hibernation ended in death in 1/4th of the cases, apparently due to an inability to arouse against the thermal gradient. Animals rewarmed artificially proceeded to the next bouts of hibernation without ill effect. Further, hibernating ground squirrels receiving bombesin could not be made to arouse by intense sensory stimuli, as can untreated hibernators. This may have been due to the known analgesic effect of bombesin.

128.5 ENDOGENOUS OPIOID PEPTIDES MAY REDUCE SELECTIVE ATTENTION. Amy F.T. Arnsten and David S. Segal. Psychiatry Dept., Sch. of Med., Univ. of Calif., San Diego, La Jolla, CA 92093.

The effects of morphine and naloxone were examined on the behavioral response pattern of rats in a novel environment. After injection of saline, naloxone, or morphine, rats were monitored for locomotion and frequency and duration of contact with stimuli in a multicompartment chamber. In agreement with previous studies, naloxone at doses as low as 0.25 mg/kg increased both the total duration of contact and the average time spent per contact with stimuli in the environment. Conversely, morphine induced a dose-related reduction in both total duration contact and the average time spent per contact with these stimuli. This reduction was apparent at doses of morphine which produce hyperactivity (0.5-2.0 mg/kg) and hypoactivity (5 and 10 mg/kg), thus indicating the effect is independent of locomotor response. The concurrent administration of 0.25 mg/kg naloxone and 10 mg/kg morphine produced a response indistinguishable from saline controls. In addition, the enantiomer (+)-naloxone (0.5 mg/kg), which is devoid of opiate antagonism, had no effect on stimulus interaction. These results indicate a role for endogenous opioid systems in environmental stimulus interaction. Furthermore, these data suggest that opioid peptides may be involved with selective attention. The time spent per contact with stimuli index can be viewed as a model of selective attention; longer contacts, such as those induced by naloxone, are associated with focused activity, while the briefer contacts exhibited by morphine treated rats are affiliated with distraction. This hypothesis is supported by clinical reports that morphine produces an inability to concentrate in humans. The effects of naloxone on human selective attention and the involvement of locus coeruleus neurons in the naloxone response will be discussed. (Supported by USPHS Grants DA-01994-02 and MH-30914-03 and NIMH Research Scientist Award MH-70183-07 to D.S.S.)

128.6 DRUG-INDUCED SELF-BITING IN RODENTS AS MODELS FOR SELF-MUTILATION IN HUMANS. K. Mueller[a]. Dept. of Psychology, Univ. of Arizona, Tucson, AZ. 85721.

Self-mutilation is a behavioral characteristic of two syndromes associated with physiological abnormalities, the de Lange syndrome and the Lesch-Nyhan syndrome. Several drugs have been reported to produce self-biting in rats or mice. If the behavioral characteristics of drug-induced self-biting in rodents are similar to those of self-mutilation in humans, the same neurochemical mechanisms may be involved in both. Self-biting was induced in rats by oral administration of 140 or 220 mg/kg pemoline or by chronic caffeine (185 mg/kg/day) and in mice by 40 mg/kg clonidine. Pemoline reliably induced self-biting in rats; self-biting occasionally became so severe that animals were euthanized before completion of testing. Self-biting was behaviorally indistinguishable from stereotyped grooming and the most common targets were the medial digits or the dorsomedial aspect of the foreleg. Self-biting was accompanied by highly stereotyped behaviors, "hyperactivity", abnormal social behavior, abnormal sensorimotor behavior, and unresponsiveness or avoidance of moderate levels of sensory stimulation. Self-biting was very persistent but was somewhat environmentally modifiable. De Lange patients are also hyperactive, exhibit stereotyped behaviors, and exhibit abnormal social behaviors. Their self-biting can be controlled by punishment. Preliminary data indicated that intracranial hypoxanthine reduced the severity of pemoline-induced self-biting and normalized behavior to some degree, perhaps due to its benzodiazepinergic actions. Lesch-Nyhan patients exhibit high cerebrospinal fluid hypoxanthine levels and their self-biting cannot be controlled by punishment.

14 days of caffeine administration resulted in very mild self-biting of the dorsomedial aspect of the foreleg of 7% of treated rats. Behavior of these animals appeared normal. Clonidine induced relatively brief self-biting of the medial digits of the foreleg of 30% of treated mice. Self-biting only occurred in the absence of other objects to bite. This behavior was accompanied by a severe tremor and by sympathetic arousal.

Caffeine-induced self-biting is a poor animal model for practical reasons. Clonidine-induced self-biting appears to be a poor model because of the importance of biting per se. Pemoline-induced self-biting appears to be a good model for the behavioral aspects of the de Lange syndrome. A combination of pemoline and hypoxanthine may provide a behavioral model for the Lesch-Nyhan syndrome.

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128.7 A MULTIVARIATE APPROACH TO THE CLASSIFICATION OF PSYCHOACTIVE DRUGS USING OPERANT RESPONSE MEASURES. Cynthia H. Walker*, William O. Faustman*, Stephen C. Fowler, and David B. Kazar*. Department of Psychology, University of Mississippi, University, MS 38677.

The aim of the present research was to evaluate the classificatory power afforded by the conjoint measurement of response rate and response duration as opposed to a univariate analysis based exclusively on response rate. Chosen for study in this context were several psychoactive drugs (chlorpromazine, haloperidol, chlordiazepoxide, & pentobarbital) which, despite their pharmacological differences, produce similar dose-related decreases in rate of responding on simple fixed-ratio schedules of reinforcement. The two multivariate techniques of factor analysis and linear discriminant analysis were used to describe the data. Factor analysis was employed to assess the relationships among the two operant variables, their higher-order components, and their nonadditive components regardless of drug class, and linear discriminant analysis was applied to the data to determine the usefulness of these variables in separating the four different drugs from one another.

Twenty-four rats were divided into four groups of six animals each and were administered either chlorpromazine (1.0, 2.0, & 4.0 mg/kg), haloperidol (.06, .125, & .25 mg/kg), chlordiazepoxide (2.5, 5.0, & 10.0 mg/kg), or pentobarbital (2.5, 5.0, & 10.0 mg/kg), with each group receiving only one drug. All drugs were administered intraperitoneally 30 minutes prior to the operant session, and three drug-free days (including one saline control day) separated the drug assessments. The reinforcement schedule which maintained the lever pressing behavior was a simple fixed-ratio 10, with a session length of 10 minutes.

Factor analysis demonstrated that there were no major redundancies between the rate and duration variables--a finding which indicates that useful and nonoverlapping information regarding the drug effect was provided by both response rate and response duration. Further, linear discriminant analysis clearly separated the four drugs into three distinct classes: neuroleptic, chlordiazepoxide, and pentobarbital. Under the conditions of this experiment discriminant analysis was not able to separate chlorpromazine from haloperidol.

These findings demonstrate the importance of considering operant response duration along with response rate in characterizing the differences in behavioral effects produced by various pharmacological agents. Moreover, the present work also points up the appropriateness of applying multivariate statistical techniques to data obtained in behavioral pharmacology experiments.

128.8 EFFECT OF PRENATAL HALOPERIDOL TREATMENT ON THE ONTOGENY OF LOCOMOTOR ACTIVITY IN RAT PUPS. James M. O'Donnell* and Lewis S. Seiden. Department of Pharmacological and Physiol. Sciences, The University of Chicago, Chicago, IL 60637.

Prenatal administration of haloperidol causes alterations in the ontogeny of locomotor activity in rat pups. It has been proposed that the normal development of locomotor activity in the rat depends on the development of dopaminergic pathways in the brain. This suggests that prenatal alteration of the developing dopamine systems could lead to profound changes in the ontogeny of locomotor activity. This prompted us to investigate the effect of prenatal treatment with the dopaminergic receptor antagonist haloperidol on the development of locomotor activity in rat pups. Haloperidol (vehicle, 0.5,1.0,2.0 and 4.0 mg/kg/day) was administered s.c. twice daily to female rats from the fourth day of pregnancy until the birth of the pups. Male pups were selected for experimental use and were fostered with vehicle-treated mothers. Locomotor activity was measured daily in one hour sessions. Pups were temporarily removed from the mother and placed in individual stabilimeter cages. Vehicle-treated pups showed the characteristic pattern of locomotor activity ontogeny. Locomotor activity increased progressively between ages 8 and 15 days, and then gradually decreased with further increases in age. We found that: 1) prenatal treatment with haloperidol from 0.5 to 2.0 mg/kg/day dose-dependently increased the peak level of locomotor activity during its development while 4.0 mg/kg/day caused a slight reduction; 2) prenatal haloperidol administration shifted the locomotor activity peak to later ages in a dose-dependent manner; 3) the within-session distribution of locomotor activity was altered by prenatal haloperidol treatment at those days of age when the experimental pups were hyperactive in that experimental pups had a greater proportion of activity counts in the latter portions of the session than did controls. These results suggest that pharmacological manipulation of the central dopaminergic systems with prenatal haloperidol treatment can alter the ontogenetic pattern of locomotor activity. Such a finding provides support for the hypothesis that dopamine neurons are involved in the ontogeny of locomotor activity. (Supported by USPHS Grants MH-11191; RSA MH-10562).

128.9 ANTICHOLINERGIC AND ANTICHOLINESTERASE EFFECTS ON A REPEATED ACQUISITION BASELINE. D.M. Penetar* (SPON: J. McDonough), USA Biomedical Laboratory, Aberdeen Proving Ground, MD 21010.

The operant learning paradigm of repeated acquisition has been the subject of much psychopharmacological research (Harting & McMillan, Psychopharmacology, 1976, 49, 245; Thompson & Moerschbaecher, Environ. Health Persp., 1978, 26, 77). Representative drugs from the stimulant, sedative-hypnotic, major and minor tranquilizer classes have been systematically studied for their effects on learning and performance behaviors during this paradigm. The anticholinergics and anticholinesterases have received scant attention however.

Cynomolgus monkeys were trained to emit a four member chain of key presses. Three backlit pushbutton keys were simultaneously transluminated with either red, yellow, green or blue stimulus colors (in that order). A correct response advanced the color sequence. Four repetitions of the sequence (16 correct responses) was required for reinforcement to occur. Correct sequences changed daily. Session length was 100 reinforcers. The effects of atropine (0.014, 0.044, 0.14, 0.44 mg/kg) and physostigmine (0.025, 0.050, 0.075 mg/kg) were assessed. Preinjection times were equated for onset of central effects. Atropine was injected 45 min prior to the session while physostigmine was injected 15 min prior.

Four measures of learning and performance were analyzed: 1) Session Time - minutes required to earn 100 reinforcers: 2) Trials to Criterion - the number of 16 response chains (trials) required to emit three consecutive chains at 90% correct or better; 3) Errors to Criterion - the number of incorrect responses emitted until criterion was reached; 4) Performance Errors - the number of incorrect responses emitted during the session after the learning criterion was reached. Results are summarized below:

Atropine	Baseline	0.014	0.044	0.14	0.44 mg/kg
Session Time (minutes)	42 ± 3	44	36	45	203
Trials to Criterion	7 ± 0	6	5	5	15
Errors to Criterion	25 ± 2	33	14	17	78
Performance Errors	23 ± 16	25	16	44	295

Physostigmine	Baseline	0.025	0.050	0.075 mg/kg
Session Time (minutes)	39 ± 7	46	50	111
Trials to Criterion	7 ± 1	10	6	12
Errors to Criterion	26 ± 6	46	49	81
Performance Errors	15 ± 9	20	22	41

The physostigmine results do not support reports of enhanced learning by anticholinesterase compounds. Atropine results indicate that anticholinergic compounds may have a biphasic effect on learning.

128.10 DIFFERENTIAL ACTIONS OF "CLASSICAL" AND "ATYPICAL" ANTIPSYCHOTIC DRUGS ON NEURONS IN THE AMYGDALOID COMPLEX. Kevin D. Alloway, George V. Rebec and Theodore R. Bashore. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Whereas haloperidol and other "classical" antipsychotic drugs produce immobility, rigidity, and other motor dysfunctions, "atypical" antipsychotics like clozapine are devoid of these effects. We have previously shown that despite these behavioral differences haloperidol and clozapine exert comparable effects on neuronal activity in the neostriatum and nucleus accumbens (Rebec et al., Pharmacol. Biochem. Behav., 1979, 11:529; Neuropharmacology, 1980, 19:281). To further elucidate the brain mechanisms underlying the differential behavioral actions of these antipsychotics, we extended our analysis to the amygdaloid complex. Single unit activity, recorded from immobilized, locally anesthetized rats (350-450g), was amplified and displayed by conventional means. Data were obtained from 70 amygdaloid neurons recorded bilaterally from 48 experimental animals. Haloperidol, clozapine, or d-amphetamine was injected via an in-dwelling intraperitoneal catheter.

At a dose known to elicit catalepsy in rats (2.0 mg/kg), haloperidol failed to change the firing rate of 12 of 13 neurons (the activity of 1 unit was increased by the drug). In contrast, 20.0 mg/kg clozapine, which is devoid of extrapyramidal side effects, produced a prolonged increase in activity in 10 of 19 neurons (7 neurons were unresponsive and 2 units were inhibited by clozapine). In a separate series of animals, 1.0 mg/kg d-amphetamine inhibited the firing rate of 28 of 38 neurons in the amygdaloid complex (3 units were accelerated and 7 neurons were unresponsive to the drug). A subsequent injection of 2.0 mg/kg haloperidol failed to block the amphetamine-induced depression in 8 of 9 neurons, whereas 20.0 mg/kg clozapine reversed this response in every case.

Our results indicate that in sharp contrast to the similar effects of haloperidol and clozapine on unit activity in the neostriatum and nucleus accumbens, neurons in the amygdaloid complex are more responsive to "atypical" than to "classical" antipsychotic drugs. It is conceivable, therefore, that the differential behavioral effects of these drugs are mediated, at least in part, by the relative inability of the "classical" antipsychotics to alter neuronal activity in the amygdaloid complex.

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128.11 ACUTE EFFECTS OF NEUROLEPTICS ON LOCOMOTOR ACTIVITY IN RATS. Gerald J. Schaefer and Richard P. Michael, Dept. Psychiatry, School of Medicine, Emory University and Georgia Mental Health Institute, Atlanta, GA. 30322.

We have previously examined (Psychopharmacology, 67, 9-15, 1980) the acute effects in rats of a series of neuroleptic drugs on brain self-stimulation current thresholds. The titration procedure allowed us to distinguish between the effects of neuroleptics on central reinforcement thresholds and on behavioral performance. The present experiments were performed to evaluate further this series of neuroleptics on motor behavior. The locomotor activity of rats was recorded during 10-min sessions in a circular open-field apparatus after the administration of vehicle or drug. Dose-response curves were obtained for five neuroleptic drugs alone and in combination with 1.0 mg/kg of d-amphetamine. Pretreatment times for the neuroleptics were between 45 min and 4 hr as previously described and d-amphetamine was administered 15 min before the start of the test session. Haloperidol (0.01-0.10 mg/kg) produced a dose-dependent decrease in locomotor activity, as well as a dose-dependent blockade of the increase in locomotor activity produced by d-amphetamine. However, the lowest dose of haloperidol in combination with d-amphetamine resulted in a greater increase in locomotor activity than did d-amphetamine alone. Pimozide (0.1-1.75 mg/kg) and loxapine (0.03-0.56 mg/kg) produced marked reductions in locomotor activity over their entire dose-response curve, together with a marked attenuation of d-amphetamine's effects. Over the dose range 0.1-3.0 mg/kg, clozapine did not significantly reduce locomotor activity when administered alone, nor did it attenuate the increase in locomotor activity produced by d-amphetamine. Chlorpromazine (0.1-3.0 mg/kg) produced a dose-dependent decrease in locomotor activity as well as in the d-amphetamine-induced increase in locomotor activity. Thus, neuroleptics differ with respect to their effects on locomotor activity as well as in their effects on reinforcement thresholds. Further, the changes in locomotor activity described here did not necessarily correlate with the response rate measure previously observed in our brain self-stimulation procedure. These data suggest that, while the neuroleptics alter locomotor activity, the changes in central reinforcement thresholds produced by these drugs are not solely a reflection of such changes. Therefore, the data on locomotor activity help us further in interpreting the relation between the chemical and clinical potency of neuroleptics.

128.12 **POSTSYNAPTIC ACTION OF ANTIDEPRESSIVE DRUGS AS MEASURED BY ACUTE TREATMENT EFFECTS ON AN ANIMAL MODEL OF DEPRESSION.** **H. Nagayama*, J.N. Hingtgen* and M.H. Aprison. Institute of Psychiatric Research and Depts. of Psychiatry and Biochemistry, Indiana University School of Medicine, Indianapolis, IN 46223**

In order to reconcile the conflicting animal data and clinical data, i.e. suppression of animal behavior is caused by an increase in 5-HT in the synaptic cleft, whereas human depression is associated with a deficiency of the cerebral serotonin system, a new theory was developed. This theory suggests that in some types of human depression, a hypersensitive postsynaptic receptor develops in these individuals due to a decreased release of 5-HT. Then, at some later date, a stress factor causes an increased release of 5-HT which now interacts with the supersensitive postsynaptic receptors. In the animal model, excess 5-HT from its precursor also increases in the cleft. In both cases, an increased release of 5-HT occurs when compared to the normal state (Aprison et al., In: Neuropharmacology and Behavior, Plenum Press N.Y., 1978). One way to test this theory is to demonstrate that one or more of the currently used antidepressive drugs blocks the 5-HTP induced depression in our animals acting through postsynaptic rather than presynaptic mechanisms. Rats working on a variable interval one minute (VI 1) schedule for milk reinforcement and exhibiting behavioral depression following administration of 50 mg/kg 5-HTP (I.P.) were pretreated (one hr before the 5-HTP injection) with fluoxetine (5 mg/kg I.P.) or methysergide (5 mg/kg I.P.) to establish a behavioral basis for distinguishing between pre- and postsynaptic events, respectively. Fluoxetine, a known specific uptake blocker of 5-HT, potentiated the depressive effect of 5-HTP by about 200%. Methysergide, a postsynaptic blocker of 5-HT, almost completely (93%) abolished the depressive effect of 5-HTP. Pretreatment (a 5 mg/kg dose given one hr before the 5-HTP injection) with amitriptyline, one of the commonly prescribed antidepressive drugs, reduced the behavioral depression following 5-HTP by approximately 50%. These data indicate that amitriptyline can act as an antagonist of 5-HT at the postsynaptic serotonin receptor. The effects of amitriptyline were compared to those of other antidepressive drugs (imipramine, mianserin, and iprindole) when used with the same model of depression. The results of this study, as well as those recently reported from CNS membrane binding studies, suggest that the therapeutic effects of some antidepressive drugs may be explained by their postsynaptic rather than presynaptic properties at central serotonergic receptors. Thus, these studies support a major portion of the hypothesis noted above. (Supported in part by PHS Research Grant MH-03225-20 from NIMH).

128.13 CHRONIC SPIROPERIDOL OR THIORIDAZINE TREATMENT: DIFFERENTIATION IN EFFECTS ON PREFRONTAL CORTEX SELF-STIMULATION. T. B. Wishart. Dept. Psychology, Univ. Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 0W0.

Rats with electrodes implanted in the dopamine-rich area of the prefrontal cortex were trained to self-stimulate in a photo-beam interruption task. Each animal, in addition to controlling the total number of brain stimulations per test by stepping into and out of the photo-beam, regulated its own duration of brain stimulation per response. Following training and the establishment of baseline levels of responding the animals were divided into groups to receive chronic spiroperidol (0.05 mg/kg/day), chronic thioridazine (10 mg/kg/day) or chronic saline. Two self-stimulation tests were given daily, one immediately prior to drug administration, the second test one and one-half hours later.

Spiroperidol administration inhibited self-stimulation rates and durations by comparable amounts (40-60%) throughout the 28 days of chronic drug treatment. Rates and durations of self-stimulation in the daily pre-drug tests were 60-80% of baseline values.

Chronic thioridazine administration resulted in progressive deterioration of self-stimulation. Initial injections (days one and two) reduced rates and durations to 70% of baseline values but, with continued injections, rates and durations fell to below 10% of baseline levels. Daily pre-drug self-stimulation rates and durations also progressively diminished as chronic thioridazine administration continued, but at a slower rate and the depressions obtained in these tests (60-70%) were not as pronounced as those observed immediately after drug injections.

These results provide support for the supposition that atypical neuroleptics, more so than the typical antipsychotics, strongly affect the meso-cortical dopamine system.

128.14 DIFFERENTIAL EFFECTS OF 17β-ESTRADIOL, 5α-DIHYDROTESTOSTERONE, AND TESTOSTERONE ON DRUG-INDUCED LOCOMOTION IN CASTRATED RATS. F.S. Mennitti*, B. Rudorfer* and M.J. Baum. Department of Nutrition and Food Science, MIT, Cambridge, MA 02139.

The ability of testosterone (T), and its neural metabolites, 17β-estradiol (E_2) and 5α-dihydrotestosterone (DHT), to modulate the functional activity of mesolimbic, dopaminergic synapses was assessed in castrated male rats by studying the ability of chronic treatment with each steroid to affect locomotor activity induced by D-amphetamine sulphate or apomorphine sulfate. The stimulatory action of a single injection of D-amphetamine (1.5 mg/kg) on locomotor activity was significantly augmented in castrated rats implanted s.c. with silastic capsules containing E_2, but not in rats implanted with T or DHT. In another experiment castrated rats received bilateral injections of 6-hydroxydopamine into the nucleus accumbens. In subsequent behavioral tests the ability of apomorphine (1 mg/kg) to stimulate locomotion was significantly heightened in rats previously implanted s.c. with silastic capsules containing E_2, as opposed to T or DHT. This action of E_2 could not be duplicated by chronic infusion of 2 hydroxyestradiol from minipumps implanted s.c. in other castrated males. Groups of neurologically intact, castrated rats implanted with T, E_2, or DHT displayed equivalent levels of stereotyped behavior in response to increasing doses (1,2, or 4 mg/kg) of apomorphine, suggesting that these sex steroids had no differential effects on the metabolism of this dopamine receptor agonist. Taken together, the results suggest that estrogenic metabolites of T may facilitate functional activity at mesolimbic, dopaminergic synapses by augmenting postsynaptic neuronal responsiveness.

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128.15 CHOLINERGIC AND SEROTONERGIC MECHANISMS AND PASSIVE AVOIDANCE LEARNING IN YOUNG CHICKS. Bruce A. Mattingly* and James F. Zolman. Physiology and Biophysics, Medical Center, University of Kentucky, Lexington, Ky. 40536.

The main objectives of this research were: a) to determine whether cholinergic and serotonergic mechanisms are involved in passive avoidance (PA) learning of the young chick; and b) to determine whether developmental changes in cholinergic and/or serotonergic functioning could account for the age-dependent changes in PA learning reported previously between 1- and 4-day-old chicks.

In six experiments, Vantress x Arbor Acre chicks were trained to key-peck for heat reinforcement when 4 days old and then administered response-contingent wing-shock during PA testing. Acute intraperitoneal (IP) injections of different doses of scopolamine and atropine were used to study cholinergic mechanisms, and chronic IP injections of different doses of para-chlorophenylalanine (PCPA) were used to study serotonergic mechanisms.

Major findings were as follows: a) chicks injected with .5 mg/kg scopolamine or 1.0 mg/kg atropine were disrupted in PA learning as compared to saline-injected control chicks; b) chicks pre-treated with PCPA (3- or 5-injections of 75- or 150-mg/kg) over the first three posthatch days did not significantly differ from saline control chicks during PA testing when 4 days old; c) PCPA pre-treatments did not significantly affect the scopolamine-induced disruption in PA learning; and d) scopolamine-treated chicks, like saline control chicks, suppressed responding less in PA tests when given delayed, rather than immediate, punishment.

These findings suggest that cholinergic, but not serotonergic, mechanisms are involved in PA learning in the young chick. Moreover, the significant delayed-punishment effect in scopolamine-treated chicks suggests that scopolamine does not interfere with the chick's sensitivity to the stimulus- and/or response-shock contingencies in PA tests, and therefore, the disruption in PA learning may be best explained as a scopolamine-induced decrease in the chick's ability to withhold a prepotent response. Finally, a comparison of the behavior of the scopolamine-treated 4-day-old chick with that of the untreated 1-day-old chick on these tasks suggests that the deficient PA performance of these two groups is related to deficits in different behavioral processes. Consequently, maturational changes in cholinergic mechanisms apparently cannot account for the age-dependent changes in PA learning observed previously in the chick.

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128.16 SELECTIVE EFFECTS OF ACUTE AND CHRONIC CAFFEINE ON SCHEDULE PERFORMANCE IN RATS. P.S. McGuire, S.H. Snyder, and Z. Annau. Division of Toxicology and Department of Pharmacology, The Johns Hopkins University, Baltimore, MD. 21205

Caffeine is a central nervous system stimulant which is widely and chronically consumed. Chronic caffeine use may result in the development of a degree of tolerance, although this, and its other behavioral effects, are poorly understood. The differential-reinforcement-of-low-rates schedule (DRL) allows the determination of response and reinforcement rates, as well as response patterning (interresponse time (IRT) distribution), and is well suited for a behavioral assessment of caffeine.

Adult male Long-Evans rats were water deprived for 23 hr and trained to press a response lever for .05 ml water under a DRL >18-sec schedule. This schedule requires the rat to space its responses at intervals greater than 18-sec. In the dose response phase, rats received caffeine (2.9, 16.6, or 50.0 mg/kg, i.p.) prior to the experimental session, while rats in the chronic phase received daily caffeine (16.6 mg/kg, i.p.) injections prior to testing.

The dose-response experiment showed selective disruption of aspects of DRL performance as a function dose. Caffeine at 2.9 mg/kg had little effect on any behavioral measure, while 50 mg/kg decreased both responses and reinforcements. The 16.6 mg/kg showed selective behavioral disruptions, increasing the number of responses occurring before the end of the 18-sec interval, thereby decreasing the number of reinforcements obtained by the animal.

Rats receiving daily caffeine (16.6 mg/kg, i.p.) injection displayed an initial and selective tolerance to the drug on the second day. The measure of response rate, but not number of reinforcements, was sensitive to this tolerance effect. While response rate returned to normal, the number of reinforcements in the chronic caffeine group stayed below baseline during the 21 day treatment regimen. Thus, different behavioral parameters are uniquely sensitive to the development of tolerance to caffeine.

128.17 XANTHINES ALTER BEHAVIOR MAINTAINED BY INTRACRANIAL ELECTRICAL STIMULATION AND OPERANT SCHEDULES. Z. Annau, J.J. Valdes, P.S. McGuire, and S.H. Snyder. Division of Toxicology, and Department of Pharmacology, The Johns Hopkins University, Baltimore, MD. 21205

The widespread recreational and therapeutic use of caffeine and related xanthines has led to increasing concern regarding the health effects of these chemicals. In behavioral studies both response rate enhancing and attenuating effects have been described, depending on the dose of caffeine and the behavioral measure used. In the present experiments, intracranial self-stimulation (ICSS) and differential-reinforcement-of-low-rates of responding (DRL) for water were chosen because of their demonstrated sensitivity to psychotropic drugs.

Male Long-Evans hooded rats were used in all experiments. Rats in the ICSS experiment were stereotaxically implanted with unilateral bipolar stimulating electrodes in either the substantia nigra (SNC) or the medial forebrain bundle (MFB). They were trained to press a bar for electrical stimulation on a continuous reinforcement schedule for one hour per day. Injections of saline or xanthines (caffeine, theophylline, theobromine, or ethyl-7-theophylline-acetate) at doses of 1.0, 2.9, 16.6, or 50.0 mg/kg, i.p., were given 10 minutes prior to behavioral testing. Between days of drug injections, the rats were tested without drugs to insure that their performance had returned to baseline levels. Rats performing for water reinforcement on a DRL> 18-sec schedule required the rat to space its responses at least 18-sec apart.

Theobromine and ethyl-7-theophylline-acetate were ineffective at any dose, on any behavioral measure. Caffeine and theophylline exhibited dose-dependent potentiation of ICSS, with theophylline being more potent. Rats with implants in the SNC showed a greater enhancement of ICSS as a function of drug treatment than rats with implants in the MFB. Both caffeine and theophylline increased response rates and decreased reinforcements on the DRL>18 schedule although the magnitude of this effect was less than was seen in the ICSS paradigm. The ICSS measure was thus slightly more sensitive to alterations induced by the drug treatments. The different neurochemical substrates of ICSS maintained by stimulation in the SNC and MFB, along with appropriate pharmacological manipulations, have the potential for use in the assessment of the underlying biochemical and behavioral mechanisms of this class of drugs.

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128.18 THE EFFECT OF TAIL PINCH ON THE ACOUSTIC STARTLE RESPONSE IN RATS. N. R. Swerdlow* and C. A. Sorenson, Neuroscience Program, Amherst College, Amherst, MA 01002.

The amplitude of the ASR appears to be modulated by CA neural systems. Previous studies have shown that treatment with apomorphine (Davis & Aghajanian, Psychopharm., 47: 217, 1976) or with amphetamine (Davis et al., Psychopharm., 47: 1, 1975) augments ASR and that this effect is blocked by treatment with haloperidol (Davis & Aghajanian, 1976), pimozide (Kehne & Sorenson, Psychopharm., 58: 137, 1978), or AMPT (Davis et al., 1975). A role for NE neurons is suggested by the observation that phenoxybenzamine lowers startle baseline and blocks the stimulating effects of apomorphine and l-amphetamine (Kehne & Sorenson, 1978). ASR amplitude can also be enhanced by presenting the acoustic stimulus during another stimulus (light for example) that has previously been paired with shock. This "potentiated startle reflex" has been reported to involve noradrenergic influences as well. It is enhanced by piperoxane, which increases LC unit activity, and is attenuated by clonidine, which depresses LC unit activity (Davis et al., Psychopharm., 65: 111, 1979).

It has recently been reported that a 2/sec pinch applied to the tail increases pars compacta activity (York et al., Fed. Proc., 35: 668, 1976) and increases cortical NE release (Antelman et al., Brain Res., 99: 319, 1975). Thus, it would be expected that tail pinch (TP) might also potentiate the magnitude of the ASR.

A test of this hypothesis revealed that TP actually depressed startle amplitude. This depression appears to be distinct from the production of TP-induced "stereotyped behaviors" (gnawing, licking, eating, grooming), since inhibition of DA transmission with 1.0 mg/kg pimozide, which disrupts TP-induced stereotyped behaviors (Antelman et al., 1975), does not prevent TP depression of ASR. Moreover, 2/sec stroking of the tail (tail rub) depresses startle amplitude but does not induce stereotyped behaviors. TP depression of ASR amplitude appears to involve the n. accumbens, since damage to this nucleus reduces the TP depression, and the amount of reduction correlates with the extent of damage. The effect of TP on the n. acc. appears to involve disruption of an inhibitory dopaminergic input into this nucleus, since the potentiation of the ASR normally produced by 8 mg/kg d-amphetamine is blocked by TP. The depression of startle amplitude during TP and tail rub may be a manifestation of a generalized state of decreased sensory reactivity, since TP and tail rub also depress responses to pin prick, foot shock, and air puff. These results are consistent with other observations that the n. acc. plays a role in the modulation of sensory responsivity. These results also suggest that TP is not anxiogenic, despite the increased turnover of cortical NE, which has been associated with species-specific fear display (Redmond et al., Brain Res., 116:502, 1976).

128.19 A SENSITIVE OPEN FIELD MEASURE OF ANXIOLYTIC DRUG ACTIVITY. D. R. Britton and K. Thatcher Britton*. Peptide Biology Lab, The Salk Institute, La Jolla, CA 92037.

Several drugs with demonstrated anxiolytic and anticonflict properties were tested for their ability to alter behavior of fasted animals in an open field. In this paradigm individually housed rats were fasted for 24 hrs prior to receiving a subcutaneous injection of drug or vehicle. Thirty min later, the animals were removed from their home cages and placed in a brightly illuminated open field (30 cm in diameter) which contained a single food pellet secured in the center. Animals were observed for 15 min and records were kept of the number of approaches to the food pedistal, the amount of food eaten, the amount of rearing and grooming and the incidence of urination and defication. Anxiolytic drugs such as diazepam (0.75 mg/Kg - 3.0 mg/Kg), chlordiazepoxide (10.0 - 30.0 mg/Kg), pentabarbitol (5.0 - 20.0 mg/Kg) and others increased the amount of food eaten during the 15 min test and increased the mean g of food eaten per approach to the food pedistal in a dose-related fashion. This latter measure appears to be the more sensitive measure of the anticonflict properties of drugs. As the amount of food eaten by fasted animals in their home cages over a 15 min test is not effected by these drugs, the results do not appear to reflect a hyperphagic action of the drug treatment. Haloperidol which has sedative but not anxiolytic properties, does not increase the amount of food eaten or the mean g eaten/approach. Daily handling of the animals for 8 rather than the standard 3 days prior to testing has the same effect as anxiolytic drugs significantly increasing both measures of food related behavior. This test is currently being used to assess both the anxiolytic and the anxiogenic potencies of a variety of compounds.

128.20 THE ACUTE AND CHRONIC EFFECTS OF IMIPRAMINE ON INTRACRANIAL SELF-STIMULATION. Marinelle Payton*, Howard S. Wheeling* and Conan Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118

Using the rate-independent procedure of Esposito and Kornetsky (Science, 195:189-191, 1977), the effects of single doses of imipramine HCl (2-32 mg/kg, i.p.) on intracranial self-stimulation (ICSS) thresholds were determined. Male albino rats were stereotaxically implanted with stainless steel bipolar electrodes in the medial forebrain bundle (MFB). Significant threshold increases were obtained only at the highest doses (16 and 32 mg/kg). We hypothesized that long-term administration of these high doses might produce tolerance followed by a decrease in ICSS thresholds. Thus, imipramine (either 16 or 32 mg/kg, i.p.) was administered on a daily basis for up to 37 days (mean 19 days). Although most subjects displayed tolerance to the initial threshold elevation, neither post-injection nor pre-injection thresholds were lowered beyond those obtained during preceding and subsequent control periods. These data do not support completely our hypothesis. It appears unlikely that the effect of imipramine on ICSS from the MFB models the therapeutic action of this antidepressant.

(Supported by NIMH Grant MH 12568 and Research Scientist Awardee MH 1759 - CK).

128.21 THE COMPARATIVE EFFECT OF SEVERAL TRYPTAMINE ANALOGUES ON PRIMATE SOCIAL AND SOLITARY BEHAVIOR. W.J. Heinze*, R.F. Schlemmer, Jr., C.B. Tyler, N. Narasimhachari, and J.M. Davis. (Spon: L.F. Eastman). Illinois State Psychiatric Institute, Chicago, IL 60612 and National College, Lombard, IL 60148

Some analogues of tryptamine possess potent psychoactive properties in mammals including psychotomimetic effects in humans. To further examine the behavioral effects of these agents, selected tryptamine analogues were studied in members of primate social colonies and primate dyads of adult Stumptail macaques. The drugs and doses tested were: N,N-dimethyltryptamine (DMT), 2 mg/kg, N,N-diethyltryptamine (DET), 2 mg/kg, 5-methoxydimethyltryptamine (5-MeODMT), 0.25 mg/kg, 5-methoxytryptamine (5-MeOT) 20 mg/kg, 4-hydroxydimethyltryptamine (psilocin), 0.4 mg/kg, d-lysergic acid diethylamide (LSD), 0.01 mg/kg, and 2-bromo-LSD (BOL), 0.10 mg/kg. All drugs were administered i.m. once daily for up to 5 consecutive days. Each experiment was preceeded by a baseline period where saline or vehicle was given. A "blind" observer quantified and recorded the behavior of each monkey in the colony or dyad for 1 hour daily. Upon gross appearance, LSD, psilocin, DET, 5-MeOT, and BOL appeared to sedate treated monkeys, whereas DMT and 5-MeODMT-treated animals appeared hyperalert. Upon acute administration, all agents except BOL induced limb jerks, a myoclonic spasm of an extremity, although 5-MeOT induced considerably less limb jerks than LSD, psilocin, DMT, DET, and 5-MeODMT. Only LSD, 5-MeODMT, 5-MeOT, and to lesser extent DMT induced body shakes (wet shakes). Checking (vigilance) scores were significantly increased by DMT, 5-MeODMT, DET, and psilocin, but not by LSD, BOL, or 5-MeOT. All of the tryptamine analogues studied decreased social interactions initiated by treated monkeys with the exception of submissive gestures. Upon chronic administration, partial tolerance developed to LSD, psilocin DET and 5-MeOT-induced emergent behaviors (limb jerks and body shakes) in monkeys. However, tolerance did not develop to DMT and 5-MeODMT-induced emergent behaviors. From these experiments, the following general structure-activity relationships can be drawn: 1) N-termal methylation of tryptamine greatly enhances psychoactive properties, 2) 4-hydroxylation or additon of a methoxy group at the 5 position increases the potency of dimethyltryptamines, and 3) substituting ethyl groups for the methyl groups of DMT does not alter the potency, but does alter the behavioral changes induced by the drug. Of the agents studied, LSD, 5-MeODMT, psilocin, DMT, and DET all are known hallucinogens in humans and also induce limb jerks in monkeys as an emergent behavior. Moreover, there is a significant correlation between the log hallucinogenic dose in humans and the log dose necessary to induce limb jerks in monkeys for these tryptamine compounds.

128.22 A COMPARATIVE EVALUATION OF MONKEY ECoG AND VI 60 LEVER PRESSING EFFECTS PRODUCED BY SELECTED ANTIPSYCHOTIC AGENTS. A.B. Davidson*, T. Smart* and K.L. Keim (SPON: W. Schallek). Dept. Pharmacology, Research Division, Hoffmann-La Roche Inc., Nutley, N.J. 07110

It was our purpose to comparatively determine the effects of chlorpromazine (CPZ, 0.25-1 mg/kg), haloperidol (HALO, 0.125-0.5 mg/kg), clozapine (CLOZ, 1.25-15 mg/kg), and a new pyrroloisoquinoline derived Roche antipsychotic agent (RO, 0.125-0.5 mg/kg) on the electrocorticogram (ECoG) during an operant procedure. Drugs were administered to food-restricted squirrel monkeys (80% ad lib body wt) on a VI 60 sec schedule of food reinforcement. The ECoG was telemetered from the anterior sigmoid gyrus of the unrestrained animal. A computer quantified and subdivided a range of ECoG frequencies (0-32 Hz) into 8 bands. Each monkey (n= 4 to 8 per drug dose level) was run on two consecutive days; a vehicle was given on Day 1 and a drug on Day 2. Occassionally, a vehicle was administered on the second day (n= 16) to evaluate the placebo effect. Effects on lever pressing rate and total 0-32 Hz ECoG activity are reported as percent change of Day 2 (drug) values compared to Day 1 (vehicle) values. Changes in the relative distribution of ECoG frequencies among the 8 bands within the 0-32 Hz spectra (expressed as percentage of total 0-32 Hz activity) are compared to the previous day vehicle administration. Drugs were administered intragastrically 60 min (CPZ, RO in 1 ml water) or 90 min (HALO, CLOZ in 1 ml 5% tartaric acid) prior to the 90 min VI session. Neither vehicle affected lever response rate or the ECoG.

The doses (mg/kg p.o.) which reduced the VI 60 lever response rate by 50% (ED50) were as follows: RO, 0.43; HALO, 0.51; CPZ, 0.73; CLOZ, 13.55. However, 2.5 and 5 mg/kg CLOZ increased responding rates by 35% ($p<0.01$) and 30% ($p<0.025$), respectively.

Spectral density analysis of the ECoG showed that only CLOZ at 7.5 and 15 mg/kg increased the total 0-32 Hz activity (>30%, $p<0.05$). At the doses tested, the other compounds caused only minor changes in total ECoG activity. However, all drugs altered the relative distribution of frequencies within the 0-32 Hz range. CPZ and HALO had a similar effect on the ECoG. That is, dose dependently, these agents decreased 0-8 Hz and increased 12-24 Hz activity (each $p<0.02$ at highest doses). RO, while reducing lever responding, did not significantly modify the ECoG, although at 0.5 mg/kg it tended to decrease 4-8 Hz and increase 16-30 Hz activity. As with lever pressing, CLOZ differed qualitatively from the other compounds tested. At 7.5 and 15 mg/kg, CLOZ increased 8-16 Hz ($p<0.01$) and decreased 20-30 Hz ($p<0.02$) ECoG activity.

128.23 COMPARATIVE EVALUATION OF FELINE EEG EFFECTS PRODUCED BY PSYCHOTROPIC DRUGS: ANALYSIS BY FREQUENCY SPECTRAL DENSITY ESTIMATES. K.L. Keim and E. Zavatsky*. Dept. Pharmacology, Research Div., Hoffmann-La Roche Inc., Nutley, New Jersey 07110

Frequency spectral analysis of multi-site EEG recorded from 38 acutely prepared cats was used to demonstrate that representatives of clinically therapeutic drug classes could be discriminated from vehicle and distinguished from each other. Diazepam (DZ, 0.04-1.25) and flurazepam (FZ, 0.32-5), chlorpromazine HCl (CPZ, 1-10), amitriptyline HCl (AMI, 0.32-10), d-amphetamine SO_4 (AMPH, 1.25-10), and phenobarbital Na (PHB, 1-20 mg/kg) were administered i.v. in cumulative, doubling doses at 13 min intervals. Monopolar electrodes were located on the anterior sigmoid gyrus (ASG) and in the ventral hippocampus (VH) and the caudate nucleus (CN) under ketamine/methoxyflurane anesthesia; animals were maintained by local anesthetics, decamethonium and artificial respiration. Blood pressure was recorded from the femoral artery. A computer estimated and subdivided the relative distribution of the EEG frequencies into 8 bands over the range of a 0-32 Hz spectrum. The EEG was recorded continuously for 3 hrs.

To varying degrees, cumulative doses of DZ, FZ, CPZ, AMI, and PHB generally increased total 0-32 Hz EEG activity in the ASG and CN by 35%; all, but CPZ, increased 0-32 Hz VH activity. In all brain areas AMPH decreased the 0-32 Hz feline EEG by 15% while saline-acacia vehicle administration reduced activity by 5 ± 3%. However, within certain frequency bands, differential EEG modulation occurred following the drugs. DZ and FZ increased 12-30 Hz activity by as much as 160% in brain areas studied; DZ was more potent than FZ. The 70% increase in 0-12 Hz activity induced by CPZ in the ASG and CN, was limited in the VH to the 0-2 Hz band. In all recordings, AMI caused a marked biphasic frequency shift: 0-2 Hz, +250%; 2-12 Hz, +100%; 16-30 Hz, -50%. While activity >4 Hz was attenuated after AMPH, a 50% increase was registered in 0-2 Hz activity in the ASG, VH, and CN. At all recording sites the predominant action of PHB was an enhancement of 0-2 Hz waves and a marked increase (>250%) of the midrange 8-16 Hz activity. When compared to predrug levels, only high doses of DZ, FZ, AMPH, and PHB significantly reduced mean arterial pressure amounting to a decrease of approximately 20%. CPZ, however, lowered mean arterial pressure by more than 45% at all tested doses; AMI at 5 mg/kg lowered the pressure by 54%.

Although classes of psychoactive compounds could not be differentiated based upon analysis of wide band, total 0-32 Hz integration of EEG activity, we conclude that estimates of selected frequency band densities from the acute feline EEG may provide the resolution for the characterization of different types of psychoactive drugs.

128.24 QUIPAZINE AND METERGOLINE ALTER THRESHOLDS FOR SEPTAL INHIBITION OF MURICIDE. J.L. Gibbons, M. Potegal, A.D. Blau,* S. Ross,* and M. Glusman. Dept. Behavioral Physiology, New York State Psychiatric Inst., New York, N.Y. 10032

Mouse killing behavior (muricide) in rats can be selectively inhibited by drugs which stimulate central serotonergic systems (Gibbons & Glusman, Fed. Proc., 1979, 38, 257) and by electrical stimulation of the septal nuclei (Potegal, Gibbons, & Glusman, Physiol. Behav., in press). The present study was designed to investigate whether these inhibitory effects interact, and specifically whether quipazine, a putative 5-hydroxytryptamine (5-HT) agonist, and metergoline, a putative 5-HT antagonist, alter thresholds for septal inhibition of muricide. 13 male Long-Evans hooded rats were selected for reliable mouse killing behavior within 2 mins. of presentation of a mouse. They were implanted unilaterally under barbiturate anesthesia with bipolar electrodes aimed at the medial and lateral septal nuclei. After a one week recovery period, the rats were food deprived to 80% of ad libitum weight and stimulation tests begun. Using each of the tips as cathode, the motor threshold was established as the minimum current which reliably elicited a motor response. Then the muricide inhibitory stimulation (MIS) threshold was established. Starting at one half the motor threshold, stimulation was incremented by a small amount every second trial until a rat failed to attack or kill a mouse during 2 consecutive 2 min. trials. That current value was designated as the MIS threshold. On subsequent test days, rats were injected with saline, or low doses of quipazine (0.75 or 1.5 mg/kg) or metergoline (.01, .05, 1.0, or 2.0 mg/kg) 15 mins. prior to testing. After the first trial (a non-stimulated control trial) the threshold for MIS was re-established.

The mean current threshold for eliciting a motor behavior was 101 ± 5 μa, and the behavior most frequently elicited was wet-dog shakes. At more than 90% of the tips, mouse killing behavior was inhibited below the motor threshold. Mean MIS was 72 ± 10 μa. In subjects treated with quipazine the MIS threshold was significantly reduced in a dose dependent manner. After injections of 1.5 mg/kg quipazine spontaneous killing was not affected, but MIS threshold was reduced by 50%. In subjects treated with metergoline, the MIS threshold was significantly increased in a dose dependent manner. At 2.0 mg/kg metergoline, spontaneous killing was not affected, but MIS threshold was increased by 148%. These results show that quipazine and metergoline, drugs which are believed to act on central serotonin systems, alter thresholds for septal inhibition of killing.

128.25 BRAIN LEVELS OF ACETYLCHOLINE AND NEOSTIGMINE DURING ANTAGONISM OF METHYLPHENIDATE INDUCED BEHAVIOR. J.J. Freeman, H.E. Ward,Jr.* J.W. Kosh. Div. of Pharmacology, Col. of Pharmacy, Univ. of South Carolina, Columbia, S.C. 29208.

The theory that dopaminergic and cholinergic systems in the brain are functionally antagonistic has been supported by studies in which stereotypy was blocked or reduced in intensity by cholinomimetics. The present study was designed to substantiate the role of acetylcholine (ACh) in the blockade of motor and stereotyped activity induced by methylphenidate. Behavioral evaluations (motor and stereotypy) were made for 3 hrs. following pretreatment with (1) methylphenidate (40 mg/kg i.p.) and (2) neostigmine (30 nmoles i.vt.) plus methylphenidate. Pretreatment with neostigmine (i.vt.) caused a significant blockade of motor and stereotyped behavior induced by methylphenidate. Neostigmine alone caused ataxia and anergia. Brain levels of neostigmine were measured following ion-pair extraction with dipicrylamine and thermal dequaternerization after injection into a gas-chromatograph. Simultaneous determination of neostigmine and ACh levels showed a temporal relationship for 3 hrs. between the blockade of behavioral activation and the increase in ACh levels. The predicted localization of i.vt. neostigmine in brain was verified by: its sustained level (4 nmoles) in the brain, a prolonged elevation of ACh and an extended inhibition of methylphenidate induced behavior. The half-life for disappearance of neostigmine in brain appeared to be biphasic with an initial half-life of 40 minutes. Pretreatment with hemicholinium (10 µg i.vt. 2 hrs) did not potentiate the behavioral effects of methylphenidate but resulted in a behavioral syndrome displaying characteristics of both drugs. This study supports the hypothesis that there is an adrenergic-cholinergic balance in the brain which is functionally antagonistic.

128.26 POST-SLEEP EFFECTS OF FLURAZEPAM, SODIUM PENTOBARBITAL AND TRIAZOLAM ON CEBUS MONKEY OPERANT BEHAVIOR. J.G. Canon*, J.M. Halperin and L.C. Iorio* (Spon: W. Gray). Schering - Plough Corp., Bloomfield, N.J. 07003.

A common side effect following the use of many hypnotics in humans is psychomotor impairment the following morning. This effect is frequently not predictable in new, potential hypnotics, until clinical studies are conducted. This study describes a procedure using an operant discrimination test in cebus monkeys that can detect changes in psychomotor performance at selected times after drug administration, and presents data on three standard hypnotics (flurazepam, sodium pentobarbital, and triazolam) evaluated the morning after treatment.

Water-deprived cebus monkeys, housed under a 16 hr/8 hr light/dark cycle, were shaped to press one of two levers when a cue light over the correct lever was illuminated; a correct response was reinforced with 0.5 ml unsweetened orange juice. Cue lights remained illuminated for either 0.25, 0.5, 1.0, 2.0, or 4.0 sec. Location of cue light and duration of illumination were randomized over 250 trials (50 trials per time duration; 125 trials correct on each lever) with a 20 sec intertrial interval. The percent of correct responses for each duration was determined and the reaction latency (RL50), defined as that duration during which the monkeys responded correctly 50% of the time, was calculated using linear interpolation. On test days, vehicle or drugs were administered orally 30 min prior to the onset of the dark period and testing began 8 hr 45 min later (15 min after the onset of the light period).

Vehicle-treated monkeys responded correctly almost always at the longer and seldom at the shortest time duration; discrimination errors (depressing the wrong lever) rarely occurred. The RL50 for vehicle treated monkeys ranged from 0.5 to 0.8 sec. All three drugs produced dose-dependent increases in RL50's on the following morning. Relative to their hypnotic potencies, triazolam, sodium pentobarbital, and flurazepam caused small, moderate, and large increases in the RL50 respectively. These were characterized by decreased ability to respond prior to the termination of the cue light, rather than discrimination errors.

The post-sleep deficits in performance of cebus monkeys following the administration of these drugs closely parallels those in man. If this correlation holds for other standard hypnotics, this procedure may serve as an index for predicting psychomotor impairment liability of new drugs in man.

128.27 NALOXONE SUPPRESSES BRAIN STIMULATION REWARD IN THE VNB, BUT NOT IN MFB. M.J. Lewis(SPON: L. Hicks) Dept. of Psychology, Howard Univ., Washington D.C. 20059.

The opioid antagonist naloxone was found to suppress the rate of response for brain stimulation reward (BSR) at a ventral tegmental mesencephalic site previously found to contain ascending norepinephrine fibers (Ventral Noradrenergic Bundle, VNB; Ungerstedt, Acta Phys. Scand., Suppl. 367, 1971) but was found to be ineffective on BSR at a hypothalamic medial forebrain bundle (MFB) site. Adult Charles River albino rats were implanted with bipolar platinum electrodes in either the VNB or MFB. All were trained to press a lever for BSR on a continuous reinforcement schedule. After stable response rates were attained, all received a series of 3 saline injections prior to a 15 minute BSR session. The animals then received injections of naloxone (Endo Lab, Garden City, N.Y.). The rate of response for BSR at the VNB site was suppressed by both the 1.0 and 5.0 mg/kg injections of naloxone over the saline baseline values. No suppression was observed in BSR performance at the MFB site with any of the doses of naloxone. These data are consistent with previous data (Lewis, Margules, Costa and Jacobowitz, Brain Res., 107, 156-167, 1976) indicating the involvement of the mesencephalic VNB in opioid effects.

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128.28 STUDIES ON THE MECHANISM AND SITE OF ACTION OF THE DISCRIMINATIVE STIMULUS (DS) EFFECTS OF ARECOLINE AND NICOTINE. L.T.Meltzer* and J.A.Rosecrans, Dept. of Pharmacology, Med.Col.Va, Richmond, Va. 23298

These studies were designed to investigate three issues. The first is whether or not there is a presynaptic cholinergic innervation that impinges on the muscarinic and nicotinic receptors that mediate the DS effects of arecoline (ARE) and nicotine (NIC) respectively. The second issue addressed is, does NIC exert its DS effects through the release of endogenous ACh. Both of these issues were examined by studying the interaction of the cholinesterase inhibitor, physostigmine (PHY), with the DS effects of ARE and NIC. The third issue investigated was, what is the role of the midbrain reticular formation (RF) and dorsal hippocampus (HIP), two sites sensitive to cholinergic stimulation, in mediating the DS effects of ARE and NIC. The experimental subjects in these experiments were 24 male Sprague-Dawley rats. Twelve rats were trained to discriminate ARE (1.14 mg/kg,s.c.) from saline. The other twelve rats were trained to discriminate NIC(0.4 mg/kg,s.c.) from saline. The DS paradigm involved differentially reinforcing with sweetened milk, drug or saline appropriate responding on a VI-12" schedule, in a two-lever operant chamber. PHY(0.125mg/kg) pretreatment shifted the ARE dose-response relationship to the left but did not effect the NIC dose-response relationship. PHY (0.25mg/kg) after different antagonist pretreatments to block some of its peripheral and central effects, produced an approximately 30% generalization to the DS effects of NIC and a 70% generalization to the DS effects of ARE. The PHY effect in NIC trained rats could not be antagonized by mecamylamine, while atropine could block the PHY effect in ARE trained rats. These results suggest that 1) there is a lack of or a negligible cholinergic innervation of the central nicotinic receptors that mediate the DS effects of NIC compared to the cholinergic innervation of muscarinic receptors that mediate the DS effects of ARE, and 2) NIC does not exert its DS effects through the release of endogenous ACh. NIC injected directly into the RF and HIP produced generalization to the DS of peripherally administered NIC. The RF was more sensitive than the HIP. No generalization was observed to the DS effect of ARE when ARE was injected directly into these sites. This study points to a role of the RF and HIP in mediating the DS effects of NIC but not ARE.

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128.29 ANTICHOLINERGIC DRUGS PROMOTE RECOVERY FROM SELF-STIMULATION DEFICITS PRODUCED BY BILATERAL BUT NOT UNILATERAL DOPAMINE LESIONS. R. J. Carey, Psychology Service, VA Medical Center, Syracuse, N. Y. 13210.

Twenty rats with bilateral medial forebrain bundle electrodes which generated comparable rate-intensity functions for self-stimulation were administered either unilateral or bilateral injections of 6-Hydroxydopamine (4 μg of a 2 μg/μl sol.). The nigral injections produced a virtual complete loss of dopamine in the injected hemispheres. The unilateral dopamine depletion was manifested behaviorally by ipsiversive turning and a prolonged partial bilateral deficit in self-stimulation. The bilateral dopamine depletion produced a severe akinesia and severe bilateral loss of self-stimulation. After 1 month postoperative the rats were given injections of the anticholinergic drug scopolamine HCl (0.125, 0.25 and 0.5 mg/kg). In the rats with a unilateral dopamine lesion the drug injections enhanced ipsiversive turning and bilaterally attenuated self-stimulation. In the rats with bilateral dopamine lesions, however, the scopolamine reversed the akinesia and produced recovery of self-stimulation. The results of these studies show that self-stimulation deficits produced by dopamine lesions can be attributed to motor dysfunction.

129.1 A NEWLY IDENTIFIED CLASS OF INTERNEURONS IN THE SPINAL CORD OF LAMPREY. J.T. Buchanan*. (SPON: C.M. Rovainen). Dept. of Physiol. and Biophys., Washington Univ., St. Louis, MO 63110.

Interneurons with axons projecting caudally on the contralateral side of the spinal cord (CC interneurons) were identified electrophysiologically in lampreys. They were tested for pre- and postsynaptic connections with other identified neurons and were stained by injection of horseradish peroxidase. One class of CC interneurons (CC_1) has an inhibitory function.

Intracellular stimulation of a CC_1 interneuron produced inhibitory postsynaptic potentials (IPSPs) in the following cell classes on the opposite side of the cord and within 2 cm caudally: myotomal motoneurons, lateral interneurons (a class of inhibitory neurons with caudally and ipsilaterally projecting axons) and other CC_1 interneurons. These IPSPs were probably monosynaptic as they occurred at a constant latency which was 2 ms longer than the conduction time along the CC_1 interneuron axon at 9°C. CC_1 interneurons were inhibited by rostral and ipsilateral lateral interneurons. CC_1 interneurons also received polysynaptic input from sensory dorsal cells in a pattern similar to that observed in motoneurons and lateral interneurons.

CC_1 interneurons may, therefore, participate in motor coordination in lampreys since they receive sensory input, are active during swimming (Cohen and Buchanan, Neurosci. Abst., '80) and inhibit motoneurons and lateral interneurons. CC_1 interneurons would contribute to the formation and propagation of body waves by being coactive with nearby motoneurons and lateral interneurons on the same side of the spinal cord and by inhibiting those same cell classes in nearby segments on the opposite side. In addition, the activity of ipsilateral cells would be promoted by disinhibition, since CC_1 interneurons inhibit contralateral CC_1 interneurons.

CC_1 interneurons receive monosynaptic EPSPs from the ipsilateral reticulospinal Müller cell, B_1. Tonic stimulation of B_1 at 50 Hz during D-glutamate-induced swimming produced a decrease in the contralateral ventral root burst intensity, consistent with CC_1 interneuron inhibition of contralateral motoneurons. Either increases or decreases in frequency of ventral root bursting were also elicited during B_1 stimulation at 50 Hz. This indicates that B_1 has access to the swimming pattern generator in the spinal cord.

129.2 ACTIVITY OF IDENTIFIED SPINAL NEURONS IN LAMPREY DURING "FICTIVE SWIMMING". A.H. Cohen and J.T. Buchanan*. Dept. of Physiol. and Biophys., Washington Univ., St. Louis, MO 63110.

Bathing the isolated spinal cord of the lamprey in Ringer solution containing D-glutamate elicits episodes of "fictive swimming", i.e., patterns of ventral root (VR) discharges identical to the EMG pattern seen in intact, swimming fish. Such episodes can be long-lasting and stable (Cohen and Wallen, in press) allowing intracellular recording from identified spinal neurons during the "fictive swimming". We present here a description of the activity of previously characterized spinal neurons (cf. Rovainen, Physiol. Rev. 59: 1007, '79) including myotomal motoneurons, fin motoneurons, lateral interneurons, edge cells, dorsal cells and giant interneurons, as well as the newly characterized CC_1 interneurons (Buchanan, Neurosci. Abst., '80).

1) Myotomal motoneurons exhibited oscillating membrane potentials (MPs) which reached a peak depolarization during the middle of the VR burst. 2) Fin motoneurons also showed oscillating MPs, but their depolarizations and hyperpolarizations were opposite, or antiphasic, to those of myotomal motoneurons. Many fin motoneurons simultaneously exhibited a much slower cyclic bursting pattern. 3) The MPs of lateral interneurons oscillated deeply and in-phase with nearby myotomal motoneurons, but most of these cells did not actually fire. 4) CC_1 interneurons had deeply oscillating MPs and often fired. They reached their peak depolarization at the beginning of the VR burst, slightly prior to the peaks in myotomal motoneurons and lateral interneurons. 5) Edge cells have a variety of axonal projections, but behaved similarly during "fictive swimming". Their MPs were not strongly modulated. Their peak depolarization occurred near the end of the VR burst. 6) Dorsal cells, large sensory neurons in the spinal cord, were all silent in the isolated preparation. However, a single action potential produced by intracellular stimulation in one type of dorsal cell was capable of disrupting the ongoing rhythm. 7) Giant interneurons showed no phasic modulation of their MPs, although they did exhibit many synaptic potentials not related to the VR bursting pattern. 8) An assortment of interneurons not otherwise identifiable were recorded during "fictive swimming". Most were active during the first half of the VR burst.

The cellular basis for swimming in lamprey has not yet been determined, but its nervous system offers a convenient preparation in the search for a vertebrate pattern generator.

129.3 ACTIVITY OF THE METATHORACIC AND METACOXAL MUSCLES DURING FLIGHT IN THE COCKROACH, PERIPLANETA AMERICANA. C. R. Fourtner, J. B. Randall*. Dept. Biological Sciences, SUNY/Buffalo, Buffalo, NY 14260.

Recent developments have shown that the flight system of the cockroach may present an excellent opportunity to study the neuromechanisms underlying stereotypic behavior (see Ritzmann, et al. this volume). Although the coxal and thoracic musculature of the cockroach has been well described, data are minimal regarding the activity of these muscles during flight (Pond, J. comp. Physiol. 1972). Using intracellular recording techniques in partially dissected specimens, we have determined some of the temporal relationships of several muscles in the flight cycle. The "wing-beat frequency" in these preparations ranged from 20 c/s to 28 c/s; this is comparable to intact tethered animals. Phase relationships between the FPSP's from muscle fibers and the onset of EMG's from either the elevator or depressor muscles were calculated. Muscles 155, 156, 157, 161, 162, 163, 174, 175, 176 (Notation Carbonell, Smith. misc. Coll. 1947) are active prior to the onset of wing elevation; muscles 153, 154, 164, 165, 167, 169 and 177C are active prior to wing depression. Fibers in each of the above muscles produce large EPSP's or spikes which approached 0mV. A third group of muscles, 159, 160, 170, 171, 172, are active during flight but do not fire synchronously with either the elevators or depressors; fibers in these muscles produce small summating FPSP's. The coxal muscles 181C and 182A-D are femoral flexors and are either continuously active during flight or produce two bursts per cycle. An interesting functional aspect of the activation of the coxal muscles is that 177A, an elevator, and 177C, a depressor, insert on the same apodeme and produce extension of the femur. However, during flight the femur is held in an extreme flexed position; presumably this is due to the continuous activity in 181C and 182A-D. This allows the action of muscles 177A and 177C to be directed on the tergum and episternum respectively to produce their indirect effects during flight. Using the cobalt-backfilling technique we have determined the location of the somata of the motor neurons innervating some of these flight muscles. This information on muscle function and innervation should give a good starting point for future electrophysiological studies on flight motor neurons and on the central control of flight behavior.

129.4 ANALYSIS OF INNERVATION PATTERN IN MESOCOXAL MUSCLES OF PERIPLANETA. Charles R. Morgan*, Paul R. Lennard, and Darrell R. Stokes*. Dept. of Biology, Emory Univ., Atlanta, GA 30322.

This investigation establishes a relation between the innervation, biochemical characteristics, and ultrastructural parameters of muscles in the mesothoracic coxa of the cockroach. The leg muscles used were the posterior coxal depressor (muscle 136) and a coxal branch of the main depressor group (muscle 135e'). Muscle 136 receives fast innervation from a single axon D_f; muscle 135e' receives a slow motor axon D_s, in addition to D_f. Although muscles 136 and 135e' were thought to be homogeneous with respect to fiber composition and innervation, our enzyme histochemical data revealed two separate and distinct populations of fibers within muscle 135e'; muscle 136 was found to be homogeneous (Stokes et al., Cell Tissue Res. 198: 175, 1979). Evidence corroborating the histochemical study was obtained in ultrastructural work upon muscles 136 (Morgan and Stokes, Cell Tissue Res. 201: 305, 1979) and 135e'. Based upon these studies, muscle 136 and the lateral compartment of 135e' appear to be fast contracting and fast fatiguing, whereas the medial compartment of 135e' is considered to be fast contracting and fatigue resistant.

These data suggest a differential innervation for the two compartments of fibers in muscle 135e'. Therefore, multiple intracellular recordings were performed to establish the innervation pattern of each population of fibers within this muscle. Since muscle 136 receives only one axon, D_f, this motor unit serves as a control for our analysis of the distribution of D_f and D_s in muscle 135e'; the time course and amplitude of e.j.p.s in different muscles receiving D_f being very similar. Three features of this system aid in the identification of fibers innervated by these motor axons: 1) axons D_f and D_s have different thresholds and are differentially activated by stimuli of varying duration; 2) the marked antifacilitation of the electrical response in muscles receiving D_f; and 3) the facilitation of the e.j.p.s elicited when D_s activity is invoked.

We will report on the correlation between the distribution of fast and slow electrical responses among the fibers of muscle 135e' and the biochemical and ultrastructural differences previously established.

129.5 SEROTONIN CAUSES OPPOSITE MODULATORY EFFECTS ON DIFFERENT BUCCAL MASS MUSCLES IN APLYSIA. G. Ajimal*, U. Shukla*, and Jeffrey L. Ram. Dept. of Physiology, Wayne State Univ., Detroit, Mi. 48201

Contraction of the accessory radula closer (ARC) muscle of Aplysia buccal mass is enhanced by serotonin (SER) or by excitation of the serotonergic metacerebral neuron (MCN), which has an axon in the nerve root innervating the ARC (Kupfermann, Fed. Proc. 38: 2095, 1979). Since the MCN also sends axons out other motor roots of the buccal ganglia (Weiss & Kupfermann, Br. Res. 117: 33, 1976), and the MCN has been suggested as a common path for behavioral changes associated with a food-arousal state, it seemed reasonable to inquire whether the contraction of other buccal mass muscles was similarly enhanced by SER.

Buccal mass muscles mounted in an aerated chamber contracted in response to injections of acetyl choline (ACh). ACh was washed out after 15 sec and not reapplied for at least 3-5 min.

In addition to the ARC, observations were made on extrinsic muscles E_1 and E_2 (nomenclature of Howells, Q. J. Microsc. Sci. 83: 357, 1942). The responses of E_1 and the ARC were similar: Contraction was produced by a threshold concentration of ACh of 10^{-5} to 10^{-6} M. SER (usually 10^{-8} M) did not cause a contraction by itself, but when applied for one min prior to ACh application, it caused a 2 to >10-fold enhancement of the ACh-elicited contraction. For E_1, the ACh contraction elicited while SER was in the chamber averaged 430% of the magnitude of the preceding ACh contraction ($p<.005$, paired t-test). ACh contractions elicited 5 and 10 min after washing out SER were 240% and 140% of the pre-SER contraction ($p<.005$ and $p<.05$, respectively, on paired t-tests), indicating a modulatory effect that outlasts the presence of SER.

In contrast to the enhancement of the ARC and E_1, SER caused a long-lasting inhibition of E_2. Contractions in E_2 were produced by 10^{-5} to 10^{-6} M ACh. ACh contractions elicited one min after SER (10^{-8} M) was injected into the chamber were reduced to 50% of the preceding ACh response ($p<.005$, paired t-test). ACh contractions elicited 5 and 10 min after washing out SER were 75% and 85% of the pre-SER contraction ($p<.005$ and $p<.05$, respectively, paired t-tests).

Although MCNs send axons out all buccal ganglia motor roots, it is not presently known whether their processes innervate the two extrinsic muscles studied here. If they do, MCNs may produce modulatory changes of opposite direction, as their neurotransmitter, SER, has been shown to do in the present study. This would imply that the role of the MCNs is not only to "arouse" the circuitry involved in feeding but also to change the coordination of muscles controlled by it.

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129.6 IDENTIFIED DUM NEURONS IN THE FIREFLY: MORPHOLOGY, PHYSIOLOGY, AND CONTROL OF THE LANTERN. Thomas A. Christensen, Department of Neurobiology & Behavior, S.U.N.Y. , Stony Brook, N.Y. 11794.

The male firefly (Photuris versicolor) produces a triple - pulsed flash that is recognized by conspecific females during a courtship. The flash is initiated by a complex neural burst from the brain which activates the lantern primarily through the two most posterior abdominal ganglia, A6 and A7. When a male executes a spontaneous flash, all the thousands of photo - cytes in the lantern luminesce and extinguish in synchrony.

Morphological examination of A6 and A7 reveals a small cluster of large (30-75 μm diam.) dorsal unpaired median (DUM) neuron somata in each ganglion. Each soma gives rise to a single neurite which bifurcates into two bilateral axons exiting both sides of the ganglion. Within each ganglion, all the neurites arising from the cluster appear to fuse at a characteristic T - shaped juncture formed by the bifurcation of the neurites. The roots of A6 and A7 branch in the periphery to innervate all areas of the photogenic tissue, which occupies the sternites of the sixth and seventh abdominal segments.

The DUM somata clusters occupy sizable portions of the dorsal surfaces of A6 and A7, and their positions within the ganglia vary only slightly from one preparation to the next. The somata are therefore easily identified visually as well as physiologically. Staining with Neutral Red dye supports the suggestion that these neurons are octopaminergic.

Firefly DUM somata produce spontaneous double - component potentials characteristic of the axon spikes recorded from the soma of DUMETi in the metathoracic ganglion of the grasshopper (Heitler & Goodman, J. Exp. Biol. 76: 63, 1978). These spikes are small suggesting electrotonic spread from the bilateral axons down the neurite to the soma. As in the grasshopper, the compound nature of these potentials indicates separate zones of spike initiation on the axons lateral to the T - juncture formed by the bifurcating neurite.

The firefly DUM neurons with their characteristic bilateral axons are well - suited to the synchronous activation of all the photocytes on both sides of the lantern. The role of multiple spike initiation zones in these neurons however, remains unclear. Experiments intended to correlate these special membrane properties with the control of synchronous flashing are now in progress.

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129.7 IDENTIFICATION AND ASYMMETRY OF LOBSTER CLAW MOTONEURONS. C. K. Govind and Fred Lang (deceased). Scarborough Coll., West Hill, Ont., Canada and Boston Univ. Marine Pgm., Woods Hole, MA 02543.

The paired claws of the lobster Homarus americanus are dimorphic, consisting of a slender, fast-acting cutter claw and a stout, slow-acting crusher claw. Since this asymmetry includes the composition of the paired closer muscles and their neuromuscular synapses, the question arises as to whether the motoneurons are asymmetric. Each muscle is innervated by a fast closer excitor (FCE), a slow closer excitor (SCE) and a closer inhibitor. In order to identify the somata of FCE and SCE in the 1st thoracic ganglion, an isolated claw-ganglion preparation was developed (cf. Wiens, T.J., J. Comp. Physiol., 112:213, 1976). Here the FCE and SCE axons can be positively identified at the periphery by differences in their conduction velocities and in the contractions they elicit. By selectively stimulating each axon at the periphery, a search can be made amongst the soma in the ganglion for an andridomic potential appearing after a suitable latency. Further corroboration as to the identity of the soma can be obtained by depolarizing it and eliciting axon spikes at the periphery. Using such physiological criteria the somata of the FCE and SCE motoneurons have been identified as two of the largest cells on the anterior ventral surface of the 1st thoracic ganglion. The FCE soma is larger (130 μm) than the SCE soma (100 μm) and lies immediately posterior to it. After identifying the soma, their responses to a standard sensory stimulus of the 2nd root with long, intense electrical pulses were compared. The SCE soma produced longer bursts and higher frequencies of spikes than the FCE soma of the same claw. This asymmetry between FCE and SCE somata is not unexpected as their axons conduct at different velocities and cause different contractile behaviours of the closer muscles. Of more interest was the asymmetry between homologous somata. Whereas the FCE soma on the cutter side responded with only an EPSP or a spike to the standard stimulus, its homolog on the crusher side gave a spike barrage lasting up to 1 s. Similarly the cutter SCE soma gave a short burst of low frequency spikes compared to its crusher homolog. Thus the FCE and SCE somata on the crusher side gave longer bursts and higher spike frequencies than their counterparts on the cutter side. Clearly the homologous claw motoneurons are asymmetric in their responsiveness to sensory (extrinsic) stimuli. Whether they are also intrinsically asymmetric remains an interesting question. Supported by grants from NSERCC and MDAC to C.K.G. and by NSF and NIH to F.L.

129.8 ELECTROPHORETIC RESOLUTION OF POSSIBLE FUNCTIONAL AND RECOGNITIONAL PROTEINS IN LOBSTER MUSCLE. D. E. Meiss, Dept. of Biology, Clark Univ., Worcester, MA 01610 and W. J. Costello, Dept. of Biology, Yale Univ., New Haven, CT 06520.

Recent studies have demonstrated histochemical differences in crustacean fast and slow muscle which may at least partially be ascribed to the pattern of fast or slow innervation. This suggests that the muscle fiber may contain proteins characteristic of muscle function as well as proteins which may serve as recognitional molecules for innervation during development and/or regeneration.

The protein composition of the claw and abdominal muscles of the lobster, Homarus americanus, was analyzed by SDS polyacrylamide electrophoresis. The gel protein patterns revealed consistent differences in several major bands that could be correlated with the physiological properties of the muscle fibers. Hence, all fast-type muscle, independent of innervation, showed a common pattern distinct from slow muscles which also had a common pattern of their own. This suggests that functional properties of the muscle fibers are expressed in their protein compositions. Some differences could be observed between different fast muscles and between different slow muscles in minor protein bands. This may be correlated with the specific axonal innervation of the different slow and fast muscles, perhaps serving a recognitional function.

These results add a new dimension to studying muscle fiber properties and innervation. Thus it will be useful for identifying differences among the diverse muscle types found in arthropods and perhaps for determining the genetic and neuronal factors responsible for this diversity.

129.9 PROPRIOCEPTIVE INTERACTIONS WITH A FUNCTIONALLY DIVERSE CRUSTACEAN MOTOR SYSTEM. R.L. Crabtree and W.H. Evoy. Lab for Quantitative Biology, Dept. of Biology, Univ. of Miami, Coral Gables, FL 33124.

The integrative basis for functional interactions between central motor programs and sensory feedback, reflected by resulting movements, has remained elusive in all animals studied. Control of crayfish leg movements for forward and backward walking offers unique opportunities for experimental examination of both motor and sensory components involved in locomotion. Because of its accessibility, we have concentrated on the thoraco-coxal neuromuscular system which underlies promotion of the crayfish limb during the powerstroke in backward walking and the returnstroke of forward walking. In this system, we are also able to isolate coxal proprioceptive elements which may be responsible for modifying motor output during locomotion.

A substantial role of proprioceptive feedback in the operation of a central program for limb movements is indicated by the failure of centrally stimulated commands to provide normal expression of the program in the absence of contact with the substrate (Bowerman and Larimer, 1974, J. Exp. Biol. 60, 119-134; Evoy, 1977, Identified Neurons and Behavior of Arthropods).

The override of inhibitory inputs from proprioceptive pathways and suppression of excitatory inputs to motor neurons of more distal leg segments by stimulation of central descending interneurons suggests that premotor modulation of the pathways is prevalent (Evoy and Crabtree, 1978, Neurosci. Abstr. 4, 192).

The promotor neuromuscular system of the crayfish 5th leg consists of three morphologically and functionally distinct heads. The anterior and posterior promotors share at least 11 axons, are rhythmically active during walking, and are composed predominantly of tonic muscle fibers based on ultrastructural criteria. The phasic lateral head is active during fast escape and defense movements and is innervated by 6 motor neurons, at least two of which are shared with the muscles used in walking.

Several proprioceptors putatively involved in aspects of motor control of these muscles have been examined to determine the nature of this interaction. These include 1) the non-spiking coxal muscle receptor, 2) a chordotonal strand mechanically in parallel with (1), and 3) a population of sensory fibers sensitive to distortion of the interarticular membrane of the thoraco-coxal joint. These three sensory elements each address specific components of the promotor and other systems. Their inputs to relevant motor programs and their contributions to the control of locomotion are being examined.

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129.10 STUDIES ON A PARALLEL ARRAY OF CRAYFISH PROPRIOCEPTORS. B.G. Lindsey and H.K. Brown, Depts. of Physiol. and Anat., Univ. of South Florida Med. Ctr., Tampa, Fl. 33612.

Previous work (Lindsey and Gerstein, J. Neurophysiol. 42:383, 1979) has shown that crayfish (Procambarus clarkii) claw propodite-dactyl (PD) chordotonal organ proprioceptors operate as an ensemble of parallel channels. Individual receptors make divergent connections with two mutually excitatory claw motor neurons, the slow closer exciter (CE) and the opener inhibitor (OI). Collectively the PD receptors play a major role in the generation of complex motor neuron proprioceptive fields. Electron micrograph montages of cross sections of the nerve carrying PD receptor axons now indicate an average of 83±16 S.D.(N=9) axons/nerve. Frequency distributions of axon diameters are positively skewed and bimodally distributed. Diameters ranged from less than 1μ to 22μ. Inspection of the montages reveals that axons with smaller diameters tend to be clustered in one or more groups.

Multiple en passant hook-oil electrodes (Wilkens and Wolfe, Comp. Biochem. Physiol. 48A:217, 1974) placed along the nerve, in conjunction with spike sorting techniques utilizing differences in conduction velocity and waveform, have, to date, permitted analysis of up to 13 simultaneously monitored proprioceptor spike trains. This approach has allowed direct observation of the dynamics of recruitment and changes in firing rates of many proprioceptors as a function of changes in claw dactyl displacement parameters (acceleration, velocity,joint angle, direction) in a single animal and will allow an analysis of issues such as redundancy and variability within the receptor array. When claw motor neurons are also monitored, the relative efficacies of various input channels are measured using cross correlation techniques. In addition, a conditional cross correlation technique is being used to measure the joint influence of action potentials in parallel channels on OI and CE firing probabilities (Lindsey, Fed. Proc. 39:596, 1980). Supported by NIH 5 SO7 RR05749 and NS 14934.

129.11 NEGATIVE AND POSITIVE FORCE FEEDBACK FOR THE REGULATION OF CONTRACTION IN CRAYFISH MUSCLE. J.D. Marrelli* and J.L. Larimer, Dept. of Zoology, University of Texas at Austin, Austin, TX 78712

This report concerns the interaction of motor and sensory elements during early phases of attempted flexion at the meruscarpus (MC) joint. Prior to movement, there is force generated by the flexor muscle (F) against loads placed upon the limb which activates sensitive cuticular receptors (DR). There is parallel activation of the accessory flexor muscle (AF) which drives myochordotonal organ (MCO) receptors embedded in its proximal tendon. In these experiments the receptors of other joints were rendered ineffective by rigid fixation. While the MCO can respond to MC joint movement, this movement was prevented.

Three cases were studied during the first 400-500 msec. of a loaded flexion: 1)Intact animal, 2)All Distal receptors (DR) destroyed, 3)DR and MCO destroyed. Histograms were made of the EMG of the flexor (5 msec. bin, N=21) with reference to the point in time when 60 gm-cm. of joint torque was produced. In each case the development of EMG freq. with time (t) was fitted by a linear regression line. Case 1:Intact; EMG freq.=(40·t)PPS,ρ=0.79. Case 2: Only MCO intact;EMG freq.=(79·t)PPS,ρ=0.94. Case 3: Open loop, no DR or MCO; EMG freq.=(42·t)PPS,ρ=0.75. In case 3, no feedback was possible and the rate of rise of the EMG in this unmodified central program was 42PPS/sec. In case 2 when only the MCO was present this rate of rise doubled to 79PPS/sec.

These data imply that the MCO provides additional excitation to the central program during isometric contractions of the flexor due to positive feedback of force information from the co-contracting receptor muscle, AF , to the working muscle, F. In case 1, the presence of DR reproduces the curve of case 3, that is, the rate of rise of EMG frequency was reduced to 40PPS/sec by their presence.

These findings i.e., positive feedback from the MCO and the modification of this feedback by load sensitive distal receptors have several implications for the control of movement. It seems paradoxical that in the intact animal, increasing the load on the limb should result the reduction of the rate of rise of the EMG frequency. However, enhanced limb stability may be an important consequence of DR-MCO interaction. Reflex lag time tends to destabilize control systems and inertial loads increase this instability. One solution is to move inertial loads more slowly, i.e., reduce the rate of rise of the EMG freq. and thus the play out of the motor program. The observed DR-MCO interaction in the earliest phase of movement may provide temporal rescaling of motor programs for increased stability of movement during load manipulation.

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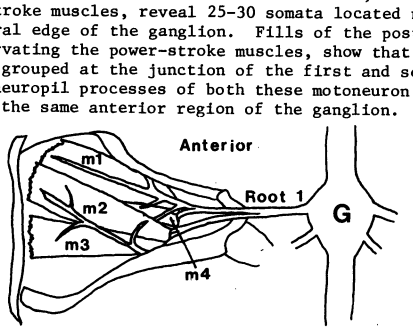
129.12 MOTONEURONS INNERVATING THE PRINCIPAL MUSCLES OF THE SWIMMERETS OF THE CRAYFISH, PACIFASTICUS. V. McDonald, D.H. Paul and B. Mulloney. Dept. of Zoology, University of California, Davis, CA 95616.

The swimmerets, paired appendages of the abdominal segments, are powered principally by four muscles that lie ventrally between the abdominal sternites. There are two power-stroke muscles (m2 and m3) and two return-stroke muscles (m1 and m4). This musculature is innervated by the first root of each ganglion which, in Pacifasticus, is split into anterior and posterior branches. Cobalt backfills of the anterior branch, innervating the return-stroke muscles, reveal 25-30 somata located near the anterio-lateral edge of the ganglion. Fills of the posterior branch, innervating the power-stroke muscles, show that there are 20-25 somata grouped at the junction of the first and second roots. The neuropil processes of both these motoneuron pools project into the same anterior region of the ganglion.

Dorsal view of swimmeret musculature and its innervation



Metachronal beating of the swimmerets is driven by hemisegmental oscillator networks. Previous work has shown that some of the motoneurons are an integral part of these networks (Heitler, 1978); however, the number and identity of these motoneurons is unknown.

Our aim is to identify the motoneurons that are part of the central oscillator. We use dye-filled microelectrodes to impale motoneuron somata in half-segment preparations with the innervation of the swimmeret muscles intact. Motoneurons are described on the basis of the following criteria: 1) their peripheral targets, 2) the sign of junctional potentials they elicit, 3) the dynamic characteristics of the neuromuscular junctions, 4) the effects on swimmeret motor pattern of current injection into the cells, 5) their firing characteristics in response to injected current pulses, and 6) location of soma and structure of neurites.

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129.13 INVESTIGATION OF BURST FORMATION IN A CRAYFISH ABDOMINAL POSTURAL MOTONEURON. Mark D. Kirk and Raymon M. Glantz. Dept. Biol., Rice University, Houston, TX 77001.

The largest motoneuron (F6) innervating the tonic flexor muscle of each hemisegment in the crayfish abdomen patterns its impulses in the form of repeated bursts (Kennedy, D. and Takeda, K., J. Exp. Biol. 43:229-246, 1965b). This centrally generated pattern of activity is well matched to the properties of its peripheral neuromuscular junctions as well as the "faster" muscle fibers which it innervates (Gillary, H. and Kennedy, D., J. Neurophysiol. 32:608-612, 1969b; Velez, S. and Wyman, R., J. Neurophysiol. 41:85-86, 1978b). The present study was undertaken in order to extend a previous extracellular analysis of the mechanism of F6 burst generation (Gillary, H. and Kennedy, D., J. Neurophysiol. 32:595-606, 1969a). Combined intracellular and extracellular investigation of the tonic flexor motoneurons in the third and fourth abdominal ganglia enabled us to make the following observations pertaining to the mechanisms of burst formation in F6: 1) Synaptic coupling of suprathreshold activity within the tonic flexor "motor pool" is not sufficient by itself to produce the bursting output, 2) Depolarizing currents (over a restricted range) injected into the somata or neuropilar processes of F6 results in the clustered discharge characteristic of "command" or sensory induced activation, 3) The frequency-current (f-I) relationship reveals no increase in average sensitivity over this range of injected currents, 4) Both driven and spontaneous F6 bursts are associated with unpatterned synaptic activity, 5) All impulses generated during a burst are seen to arise from a distinct depolarizing afterpotential (DAP) which appears to arise from a passive invasion of dendritic membrane by the preceding impulse, 6) The DAP time course is closely paralleled by an increase in excitability seen by the spike initiating zone following a singlet action potential, 7) the excitability cycle following a rhythmic impulse is a good predictor of the "preferred" intradoublet (intraburst) intervals, 8) Each burst is terminated by a prominent postburst hyperpolarization (PBH), 9) F6 possesses two other features characteristic of many latent endogenous bursters-accommodative adaptation and posthyperpolarization rebound.

As suggested by the earlier extracellular work, our results imply that the characteristic of burst formation is endogenous to the motoneuron and not strongly dependent on patterned presynaptic input. The DAP, repetitive (i.e., tonic) firing nature of the axon, and PBH interact such that during intermediate levels of activation, F6 action potentials become clustered and its endogenous bursting character is revealed.

129.14 SCORPION WALKING LEG MOTONEURONS: PHYSIOLOGY AND MORPHOLOGY. Robert F. Bowerman and Malcolm Burrows. Department of Zoology, Cambridge University, Cambridge, England.

In the arthropods, the central neural mechanisms that underlie locomotion have been studied extensively in only two groups, the decapod crustaceans and orthopteran insects. Arachnid systems have not been described using currently available intracellular stimulating and recording techniques. Consequently, we decided to analyze the physiology and morphology of walking leg motoneurons in the scorpion Paruroctonus mesaensis. The preparation was mounted ventral surface uppermost so that the legs were free to move. The subesophageal ganglion was exposed and perfused with a constant flow of saline. Grass microelectrodes with D.C. resistances of 30-80 MΩ, filled with either 2M potassium acetate or 10% cobaltous chloride, could be driven across the sheath and into the ventrally situated motoneuron cell bodies, without pretreatment by proteolytic enzymes. Once penetrated, the motoneuron was characterized by which muscle it innervated, assessed either by electromyograms or direct observation of leg movement.

Orthodromic spikes have an amplitude of about 2-4 mv and a duration at half-height of 3-5 ms. Occasionally, spikes as large as 8 mv were recorded. The cell body also provides a window onto the synaptic potentials which are generated as a result of inputs from other neurons. Depolarizing and hyperpolarizing potentials, which appear to be unitary events, can have an amplitude as large as 0.5 to 1.0 mv. Resistance reflexes, in response to movements of individual leg joints, can also be recorded.

The cytoarchitecture of cobalt filled-silver intensified motoneurons was studied in whole subesophageal ganglia. Different motoneurons innervating different muscles were indistinguishable from one another. The cell body (30-80 µm diameter) gives rise to a single dorsally running process which has a diameter of 2-9 µm. This process is usually devoid of side branches for the first 150-200 µm of its course through the ventral neuropile areas. The main neurite then turns laterally and becomes somewhat broader, about 7-12 µm. It is from this broader region that most of the dendritic side branches arise. These side branches are distributed in a dorso-ventral orientation Before entering the peripheral nerve, the main neurite tapers to 2-5 µm. Once within the peripheral nerve, the diameter may again increase.

In summary, the scorpion motoneurons are similar to those in insects and crustaceans, both in terms of the electrical events that can be recorded at the cell body and in basic cell morphology. The differences which are noted, primarily in extent and orientation of the dendritic arborizations, reflect the way the motoneurons for all eight legs are packaged into the subesophageal ganglion.

130.1 MORPHOLOGY OF THE REGENERATING EYE OF STROMBUS LUHUANUS, A MARINE GASTROPOD. E.W. Gillary* and H.L. Gillary. Dept. of Physiology, University of Hawaii, Honolulu, HI 96822.

The retina of the mature eye of S. luhuanus exhibits several morphological cell types, arranged to form distinct layers of rhabdome, cell bodies and neuropile (Gillary & Gillary, '79). Following amputation of an eye, another is regenerated in its place. In the present studies, regenerates of animals reared at 28°C (normal ambient Tp) were examined by transmission e.m., to provide a morphological basis for the ontogeny of their electrophysiological responses to light (Gillary, '72). Within a day after amputation, an eyecup (0.2 mm dia) begins to form by epithelial invagination and closes off by 3 days. At this stage the cells (ca. 5 μ dia & 50 μ long) are all similar in appearance. Short microvilli (< 2 μ long) project from their apical ends at the eye's center, and the cytoplasm includes numerous 0.6 μ propigment granules and abundant rough e.r. No neuropile is present at this stage. The cytoplasm of the 4 day eye (0.3 mm dia) contains electron-opaque 0.6 μ pigment granules, as well as much rough e.r. and microtubules. A layer of neuropile comprised of processes 0.3 to > 1 μ dia. has begun to form near the capsule. Some cells possess apical bundles of cilia, but most exhibit apical membranous projections which appear involved in the secretion of lens material. The 5 day regenerate (0.3 - 0.4 mm dia) exhibits a layer of rhabdome (ca. 20 μ thick) comprised of microvilli eminating directly from the apices of cells filled with clear 50 nm vesicles characteristic of mature photoreceptors. Also found are glial cells, with bundles of tightly packed tonofilaments and abundant rough e.r., which is less conspicuous in the other cell types. The layer of neuropile is more like that of the mature eye, exhibiting more numerous and finer processes, and many synaptic-like structures. Retinal thickness, excluding the rhabdome, is ca. 80 μ, as in the mature eye. This stage regenerate can yield simple light-evoked potentials (H. Gillary, unpubl.). The rhabdome of the 7 day eye (0.4 mm dia) is comprised predominantly of photoreceptor distal segments, each with a central shaft (40 μ long) from which emanate closely packed microvilli, as in the mature retina. In the 11 day eye (0.7 mm dia) the distal segments are 70 μ long. The retina of the 14 day eye (0.8 mm dia) is very much like that of the mature eye (1-2 mm dia) in terms of the thickness of the rhabdome (80 μ), the complexity of the neuropile, and the appearance of 3 different cell types, in addition to glial cells, although the latter still display more cytoplasm and rough e.r. than mature glia. This stage of regenerate exhibits light-evoked potentials which are qualitatively similar to those of the mature eye (H. Gillary, unpubl.). (Supported by NIH Grant EY 01531.)

130.2 THE MORPHOLOGY OF NEURONS ARISING DE NOVO DURING METAMORPHOSIS IN THE TOBACCO HAWKMOTH, MANDUCA SEXTA. P.H. Taghert* and J.W. Truman (SPON: M.R. Meyer). Dept. of Zoology, Univ. of Washington Seattle, WA 98195.

During metamorphosis in Manduca, certain central neurons die, some are respecified and some arise de novo. Of the new neurons in the unfused abdominal ganglia, eight have axons in the segmental nerves and are therefore effector cells and not interneurons. However, intracellular depolarization sufficient to cause repetitive firing in these cells does not produce contraction of skeletal muscle. These cells all have somata along the mid-line of the ganglion and are arranged as four pairs. Each pair of neurons appears to arise from a nest of four cells that can be found in late stage larvae. All cells within a nest begin to enlarge two days before pupation; at pupation, however, two cells die and the remaining two continue differentiation. In the adult moth, all eight neurons have bilateral axons, spiking cell bodies and make 'blind' terminations in the neurohaemal transverse nerve. They appear neurosecretory in many respects, but their product is presently unknown. They may be analogous to cells of the Dorsal Unpaired Median group of orthopterans.

Within the ganglion, the new neurons project to discrete areas of the neuropile that are identical to those occupied by neurons previously found to contain the peptide hormone bursicon (Taghert and Truman, 1979, Soc. Neurosci. 5, 263). Bursicon neurons are fully differentiated in larval stages and do not undergo major dendritic reorganization during metamorphosis. The common neurosecretory neuropile is arranged as three dorso-ventral planes along the mid-line and along both lateral edges of the neuropile. Major dendrites connect these planes by way of specific routes along the dorsal-most and ventral-most surfaces of the neuropile. Like the associated bursicon neurons, the new neurons exhibit variabilty in the route and number of major dendritic branches. On the basis of branching number and frequency, we infer the presence of six organizing centers for the growth of these neurons. These centers are situated at the mid-line,just below the cell body layer and along the dorsal and ventral surfaces of the neuropile. All known secretory neurons in the abdominal ganglion - new pupal neurons, bursicon neurons and others not mentioned here - grow towards and branch at these sites. Therefore we conclude that during metamorphosis, neurons differentiating for the first time grow in response to set of morphogenetic signals that was already established during embryonic stages.

130.3 NEURITE GROWTH AND ITS RELATIONSHIP TO SYNAPTOGENESIS IN THE FLY'S VISUAL SYSTEM. A. Fröhlich and I.A. Meinertzhagen, Life Sciences Centre, Dalhousie University, Halifax, N.S. Canada.

The growth of neurites between immature neurons determines the numbers and combinations of elements which may communicate synaptically in the adult. In order to assess the exact extent of this influence we have examined the first optic neuropile, or lamina, of the developing visual system of the fly. Reconstructtions have been made from serial electron micrographs of individual cartridges, the unit synaptic compartments of this neuropile. Cartridges have been studied from animals between 55-100% pupal development. At these stages the positions of the component elements are identifiable with reference to their positions occupied in the adult cartridge, by which each element is uniquely identified. Thus, only the fine grain extension of processes between cartridge elements varies during this period. At the same time synapses emerge (Fröhlich and Meinertzhagen, Soc. Neurosci. Abstr. 5, 245, 1979).

Between stages 55-100%, processes of L1 and L2 extend radially in the plane of the cartridge cross section and simultaneously branch. By 95% all extant processes have spread to the cartridge exterior. Accompanying this lateral extension the processes also spread vertically, continuing to do so after the lateral spread has stopped. At the same time as some processes are extending, however, others are regressing. The total number of processes decreases steadily from 55-95% so that, overall, more neurites are resorbed than extend. After 74% processes which had hitherto not extended to the cartridge exterior are destined not to persist. Consequently, those neurites which fail to start growth early enough or to extend sufficiently by the 74% stage are not represented in the adult. Eventually some branching occurs in most processes but extensive branching is delayed till after 74%.

A further influence upon neurite growth and survival is mediated by the interaction between L1 and L2. Although the attrition of processes seen throughout development is approximately the same for both L1 and L2, the total number of processes is greater for L1 and L2 up till 95%. At this stage the processes of L1 and L2 assume, through the depth of the cartridge, their characteristic adult pattern of interdigitation. Before this time, however, this regular alternating sequence is not found. At 55% the sequence is still random, regular alternation appearing largely between 62% and 74%.

Between 62-74% the fastest extension of neurites to the cartridge exterior is paralleled by the greatest increase in numbers of synapse.

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130.4 VARIABILITY IN THE PROSTERNAL SENSE ORGAN OF DROSOPHILA MELANOGASTER. David G. King. School of Medicine, Southern Illinois University, Carbondale, IL 62901.

The prosternal organ in dipterans is a sensory hair plate located on the anterior prothorax at the articulation with the head. The prosternal organ consists of a bilaterally paired cluster of mechanoreceptor bristles which presumably functions as a proprioceptor, similar to the more familiar hair plates located on the neck and leg joints of other insects (Pringle, J. Exp. Biol. 15:467-473, 1938).

In a wild-type population of Drosophila (Canton-S from R. Wyman, Yale Univ.) the variability in the number of sensilla in each cluster was surprisingly large. This number ranged from 12 to 21 around a median value of 15. (Mean = 15.16; standard deviation = 1.62; n = 166.) This large variation suggests that this hair plate may not provide precise quantitative information on joint position but may simply provide a qualitative indicator for joint movement.

The large variability was reflected not only in differences among individuals but also in differences between the left and right sides of single individuals. Although there was a correlation between the numbers of sensilla in left and right clusters, this correlation was weak (correlation coefficient = 0.40; n = 83). The two sides could differ by as many as 4 sensilla (or 33%); the mean difference between left and right was 1.42 bristles.

The variation between left and right sides indicates that the number of sensilla on each side is not closely coupled to systemic factors (such as body size) which would affect both sides equally and yield a higher correlation. Rather this side to side variability may indicate a developmental process which not only is regulated only within rather broad limits but which also is bilaterally independent.

To isolate the variability arising epigenetically during development from that due to genetic differences, isogenic flies (from W. Doane, Arizona State Univ., and T. Wright, Univ. of Virginia) were examined. Preliminary data from two isogenic lines indicates that in isogenic flies the left-right correlation is absent, while the variability although reduced is still considerable. The mean number of sensilla can differ significantly between strains. (Strain/mean/standard deviation/n: TW-a50/13.02/1.14/45; WD-CS/15.38/1.05/25.) Several more isogenic lines will be examined to determine to what extent the degree of variability in the prosternal organ is under genetic control.

130.5 EMBRYONIC DEVELOPMENT OF AN INSECT ANTENNAL NERVE. Alma Toroian-Raymond* and David Bentley. Neurobiology Group and Zoology Dept., Univ. California, Berkeley, CA 94720. (SPON: G. Westheimer).

In the antennae of grasshopper embryos, the first connection between the periphery and the central nervous system is made by two pairs of specialized cells termed peripheral pioneer neurons (Bate, '76, Nature, 260: 54). We have investigated the development of this afferent system throughout embryogenesis.

Antennae of staged embryos of S. nitens were observed with Nomarski optics in living embryos and stained whole-mounts, and with transmission EM. EM sections were taken at the antennal base at 5-10% intervals from 35% of embryogenesis through hatching. In micrographs, axon profiles were counted, and mean-diameters were plotted in histograms. Profiles ranged from 0.9 to 0.1 um, and histograms were increasingly skewed toward smaller diameters in older nerves. Pioneer axons did not form a group distinguishable by size or appearance.

Pioneer neuron somata were seen in antennae as early as 30%, but axon profiles were not found at the base until after 35%. At 40%, two pairs of 0.9um axons were present on opposite sides of the antennal lumen. They were associated with an a-cellular lamina lining the inner surface of the epithelial layer, and were not ensheathed. In the period from 40%-63%, the number of axon profiles in the two nerves increases steadily(40%=2/2; 45%=14/13; 15/uncounted; 55%=47/67; 58/56; 63%=78/95), and the nerves become ensheathed by glia. After 63%, a large number of axons appear in the nerves, which by 75% contain 4,421 and 4,894. This complement remains relatively unchanged through 87% (5,123/4,672), but rapidly increases again during the last part of embryogenesis. At hatching, the nerves contain 6,628 and 6,593 axons. All axons follow the path of the initial pioneer axon pair.

Embryonic appendages of grasshoppers form three cuticles (each preceded by a pulse of ecdysone; Lagueux, et. al., '79, J. Insect Physiol. 25: 709). The first arises before 40% and completes apolysis(separation from the epithelium) by 50%. The second begins forming after apolysis of the first and completes its apolysis by 80%. The third cuticle begins forming after 80% and is the cuticle of first instar nymphs.

Our data indicate that afferent cells arise concurrently with the formation of each cuticle. The first pioneer neurons appear at the time of formation of the primary cuticle. The second set of afferents (the first sensory neurons) arise during formation of the second cuticle; axons of these cells reach the antennal base before apolysis (their dendrites will innervate sensilla of the third cuticle). The third wave of afferents(second set of sensory neurons) appears concurrently with the formation of the third cuticle.

130.6 UNATTACHED PRE-SYNAPTIC TERMINALS IN A FLIGHTLESS MUTANT OF DROSOPHILA MELANOGASTER. Walter J. Costello. Dept. of Biol., Yale Univ., New Haven, Conn. 06511.

The interaction between nerve and muscle to form neuromuscular systems is much studied. Mutations which affect these systems serve as useful models to analyze their development. The stripe (sr) mutation in Drosophila affects only one set of the indirect flight muscles, the dorsal longitudinal muscles (DLMs). In its homozygous state (sr/sr) the DLMs are reduced to about 50% of their normal volume. The DLMs are completely absent in adult flies with only one copy of the mutant allele (genotype sr/Df(3)sr; one chromosome carries sr, the homologous one has a deletion at the sr locus).

In early stages (the first 30 hrs of pupation @ 25° C) the development of this muscle is no different from that of wild type flies (Costello, 1979, Neurosci. Abstr. 5:243). Subsequently, the DLMs in this mutant degenerate. Serial reconstruction of the thoracic ganglion reveals that the nerve supplying the DLMs, the posterior dorsal mesothoracic nerve (PDMN), persists even without its end target. As in normal flies, the PDMN exits the ganglion and sends a branch to the jump muscle. It then courses dorsally to where the DLMs would be normally located. Here it ends in a tangled mass of tissue, forming a "neuroma-like" structure. The PDMN at this point contains the five motor axons which normally innervate the DLMs. Many small diameter (sensory) axons are also present.

Ultrastructural analysis shows that hundreds of abnormal synapses are located within this structure (cf Hirano and Shin, 1979, Neuropathol. and Appl. Neurobiol. 5:63-70). Normal pre-synaptic elements are present: synaptic vesicles (not misshaped), T-bars, and pre-synaptic membrane thickening. However, no postsynaptic specialization exists. These unattached pre-synaptic terminals are formed onto glial elements as well as other axons.

Small clumps of highly disorganized muscle tissue (DLM remnants?) are also found in this structure. Myofilaments are organized into sarcomeres; the myofibrils look normal, but bundles of myofibrils run through the muscle mass in random orientations. A few neuromuscular synapses with normal postsynaptic specialization are present.

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130.7 AXOTOMY INDUCES INCREASED CHOLINERGIC CHEMOSENSITIVITY. H. Meiri*, M.E. Spira* and I. Parnas* (SPON: J.H. Caldwell). Neurobiology Unit, The Hebrew University, Jerusalem, Israel.

Cholinergic chemosensitivity of intact giant axons of the cockroach Periplaneta americana is very low at the abdominal level (i.e., in ganglia A_2-A_5). This is demonstrated by bath application of 10^{-5}M nicotine, which causes a membrane depolarization of 10±2 mV and an increased membrane conductance of 10±2 percent.

When the axons are cut between ganglia A_2 and A_3, recovery of normal membrane properties (resting potential and input resistance, among others) occurs after about 8 days (Meiri et al.,Neurosci. Abstr. 5:680,1979). Cholinergic chemosensitivity of the axotomized giant axons then begins to increase gradually, reaching maximum values of 30±5 mV membrane depolarization and 85±8 % increased membrane conductance 22 days after the cut. Chemosensitivity remains high for at least 100 days. The effect of nicotine can be blocked by 10 µg/ml alpha-Bungarotoxin.

Local application of nicotine on each of the above ganglia (A_3-A_5) or on the connectives between them reveals that increased cholinergic chemosensitivity appears simultaneously in each of these ganglia despite their differential distances from either the cut end or the cell bodies. However, chemosensitivity in the connectives between the ganglia does not increase.

In order to localize the cholinergic responses more precisely, acetylcholine was applied iontophoretically. After axotomy, increases in responsive membrane surface (where acetylcholine produces direct depolarization) occur in each of the above ganglia, following a time course similar to that of increased chemosensitivity.

When a single axon, or two axons, are cut while the others remain intact, increased chemosensitivity appears only in the axotomized axons. In the neighboring intact giant axons, chemosensitivity remains low. It is therefore concluded that in this case chemosensitivity is increased directly by axotomy.

131.1 LOCALIZATION OF NERVE GROWTH FACTOR BOUND TO CULTURED SENSORY NEURONS. S.T. Carbonetto and R.W. Stach, Depts. of Pharmacology and Biochemistry, S.U.N.Y. Upstate Medical Center, Syracuse, NY 13210.

Neurons were dissociated from the dorsal root ganglia of 9 day chick embryos by trypsinization (0.025%) followed by trituration. The cells were cultured on collagen-coated culture dishes in growth medium consisting of: Eagles Minimal Essential Medium, 10% horse serum, 2% embryo extract and 10 ng/ml β nerve growth factor (βNGF). βNGF was labelled with ^{125}I by the lactoperoxidase procedure to a specific activity of approximately 2000 cpm/fmol.

After 2 days of growth 6 identical sets of cultures (3 experimental sets and 3 control sets) were removed from the incubator, washed 3 times with phosphate buffered Gey's balanced salts solution (PBG) and returned to the incubator for 1 hour in PBG. Experimental cultures were then incubated with ^{125}I-βNGF at 1 ng/ml, 5 ng/ml and 20 ng/ml. To determine the level of "non-specific" binding in experimental cultures, control cultures were incubated with the same concentrations of ^{125}I-βNGF plus 1000-fold concentration of unlabelled βNGF. After incubation for 30 min. at 23°C, all cultures were quickly washed in PBG and fixed in 2% paraformaldehyde/1% glutraldehyde in 150 mM phosphate buffer. The cultures were then dehydrated in alcohol (50%, 70%, 95%, 100%) and coated with Kodak NTB-2 emulsion. After approximately 2 weeks of exposure, the autoradiographs were developed and observed in a Zeiss photomicroscope with phase and bright field optics.

At both 1 and 5 ng/ml the binding of ^{125}I-βNGF in experimental cultures was highly specific and control cultures had little binding above background (background was estimated from cultures not exposed to ^{125}I-βNGF but autoradiographed as described above). Also, at 1 and 5 ng/ml the binding of ^{125}I-βNGF was highly selective for neurons (cells with refractile somata and long processes) with very little binding above background to non-neuronal cells such as fibroblasts. At 20 ng/ml the binding of ^{125}I-βNGF was greatly reduced in its selectivity for neurons, and its specificity.

At 5 ng/ml ^{125}I-βNGF bound extensively to neuronal somata and in the autoradiographs exposed emulsion grains could be found along the length of growing neurites and over growth cones. However, not all neurons bound the ^{125}I-βNGF to the same extent and within small clusters of neuronal somata it was possible to find heavily labelled cells adjacent to cells that had little or no label. Future experiments will be aimed at describing the localization of higher affinity ($K_d \sim 10^{-11}$ M) vs. lower affinity ($K_d \sim 10^{-10}$ M) binding sites for NGF, and whether the amount of NGF bound by neurons reflects their growth rate. This work was supported by NIH grant #NS 12325.

131.2 BINDING AND SEQUESTRATION OF ^{125}I-β NERVE GROWTH FACTOR TO EMBRYONIC SENSORY NEURONS. R.W. STACH, B.J. WAGNER* AND E.J. OLENDER* (SPON: J. Robson). Dept. of Biochemistry S.U.N.Y. Upstate Medical Center, Syracuse, NY 13210.

Nerve growth factor (NGF) is necessary for the growth and development of the sympathetic and embryonic sensory nervous systems. It has been shown that NGF binds to its responsive cells through a cell surface membrane receptor. Recently, it has been shown, for sympathetic neurons, that after NGF binds to the receptor, a time and energy dependent process occurs that makes the bound NGF inaccessible to the external milieu (Stach & Olender, J. Supermolec. Structure, Suppl. 4, 58, 1980). This inaccessible NGF has been termed sequestered NGF. We were interested in determining if a similar process occurred with sensory neurons.

Cell dissociates from 9 day embryonic (E9) chick dorsal root sensory ganglia were used in the studies. The binding of ^{125}I-βNGF was found to be of high affinity and saturable, with apparent dissociation constants of 2.6×10^{-11} M for the higher affinity (type I) sites and 3.6×10^{-10} M for the lower affinity (type II) sites. These dissociation constants are similar to those obtained by Sutter, et al. (J. Biol. Chem 254, 5972, 1979), and are also very similar to the dissociation constants obtained for sympathetic neurons. When E9 sensory nerve cells are incubated with ^{125}I-βNGF (3.8×10^{-10} M) for various times and then incubated with an excess of native βNGF for an hour, or longer, a certain amount of the specifically bound ^{125}I-βNGF can not be displaced. The sequestration process in sensory neurons is also time and energy dependent. The sequestration process is not temperature dependent as is also the sequestration process in sympathetic neurons. The energy dependence can not be explained as an affect on the binding of ^{125}I-βNGF to the sensory neurons; since in the presence of the metabolic inhibitor, sodium fluoride, and the uncoupler of oxidative phosphorylation, dinitrophenol, there is no observable effect on the binding. It also appears that the type I sites are involved to a greater extent in the sequestration process than are the type II sites. At low concentrations of ^{125}I-βNGF a higher percentage of the specifically bound NGF is sequestered than at high concentrations.

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131.3 CAN HUMORAL FACTORS INDUCE ACETYLCHOLINE-RECEPTOR AGGREGATES IN CULTURED MUSCLE CELLS OTHERWISE FREE OF AGGREGATES ? J. Hooisma*, A.J.M. Blom*, T. Magchielse*, H. Muijser* and W.F. Stevens*. (SPON: O.L. Wolthuis) Medical Biological Laboratory TNO, Rijswijk (Z.H.), The Netherlands.

In cultured skeletal muscle cells acetylcholine (Ach) receptors are initially distributed uniformly on the cell membrane. After some time in culture clusters of Ach-receptors, socalled hotspots, are formed. Christian et al. (1978) have presented evidence that the number of hotspots can be increased by humoral factors released from neuronal tissue. For reasons unknown only a negligible number of hotspots are formed in muscle cells in our cultures which were conventionally prepared from leg or breast muscles of 11 day old chick embryo's.

Aggregates of Ach-receptors could be induced in our cultures when muscle cells were grown together with spinal cord neurons or neurons from the ciliary ganglion. No aggregates were formed in mixed cultures with neurons from dorsal root ganglia. Neurons were added as spinal cord explants or as whole ganglia. The density of Ach-receptor aggregates induced by spinal cord neurons decreased gradually with increasing distance from the neuronal tissue. It was observed that contact between muscle cells and neurites was not sufficient to induce aggregation of Ach-receptors. Neurites of the ciliary ganglion visualised by injecting the cellsoma with the fluorescent dye Lucifer Yellow (gift W.W. Stewart, Bethesda, U.S.A.) could be seen coursing over the muscle surface for long stretches apparently without inducing any aggregates. The gradient of cluster density observed in the presence of spinal neurons was lost when the mixed cultures were grown on a slowly rocking table that caused thorough mixing of the culture medium. Clusters then appeared throughout the culture. Conditioned medium obtained from such mixed cultures induced aggregates in cultures of muscle cells deprived of neuronal tissue. The latter observations can only be explained by the assumption that a humoral factor is involved. If such a humoral factor is released from the nerve ending it might be possible to influence the aggregation of Ach-receptors by electrical stimulation of the neurons. In fact a member of our group has shown that such stimulation during one week caused a reduction of the number of endplates (Magchielse et al. 1980). Presently we are investigating this hypothesis.

131.4 EFFECTS OF NEURONAL CELL FACTORS ON KINETICS AND AGGREGATION OF CULTURED RAT MUSCLE ACETYLCHOLINE RECEPTORS. S. Hasegawa*, H. C. Bauer*, and C. N. Christian* (SPON: D. Symmes). Lab. of Devel. Neurobiol., NICHD, NIH, Bethesda, Md. 20205.

Medium conditioned by cells of the neuronal cell line NG108-15(NG108-15 CM) increases the aggregation of the acetylcholine receptors(AChR) of cultured embryonic rat myotubes. We report here that NG108-15 CM also decreases the rate of AChR degradation of AChR and has little effect on the rate of AChR synthesis. Myotube cultures were treated for at least 10 hours with 20-fold concentrated CM, labelled with [^{125} I] alpha-bungarotoxin, and the release of radioactivity measured over 40 hours in the presence of CM. In untreated cultures the average half-time(T 1/2) of AChR is 22.5 hours. In cultures treated with NG108-15 CM, the total number of AChR was increased by 13%, and the T 1/2 of AChR degradation was increased by 33%. Myotubes similarly treated with medium conditioned by C6 glioma cells had no effect on the T 1/2 or the total number of AChR. We tentatively conclude that neuronal cells produce factors which both aggregate AChR and decrease the rate of degradation, and that these factors are not produced by glial cells. It remains to be shown whether the same factor which produces AChR aggregation also increases the metabolic stability of AChR.

The cellular mechanism of the modification of the rate of AChR degradation was studied by treating myotubes with agents known to affect the cross-linking of cell surface receptors or the metabolism of cells. Concanavalin-A treatment increased the T 1/2 of AChR by 100%, whereas succinyl Concanavalin-A was without effect. The cyclic nucleotides dibutyryladenosine cyclic monophosphate and dibutyrylguanosine cyclic monophosphate had no effect on the rate of AChR degradation. The T 1/2 of AChR degradation was increased 16% and 23% by 1mM adenosine 5'-triphosphate(ATP) and 1mM adenosine, respectively. A 24 hour treatment with the cholinergic agonist carbachol at a concentration of 10^{-5} M decreased the total number of AChR to 75% of control values, but had no effect on the subsequent rate of AChR degradation. The presence of neuronal factors and ATP at the neuromuscular junction may contribute to the metabolic stability of junctional AChR.

131.5 SUB-POPULATIONS OF CHICK SYMPATHETIC NEURONS RESPOND DIFFERENTIALLY TO THREE SURVIVAL FACTORS IN CULTURE.

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Quantitative observations on the survival of sensory neurons in culture allowed as to define two classes of survival factors, differing funtionally in terms of the age of maximal neuronal responsiveness. (Barde et al., PNAS, 77:1199-1203, 1980). Thus, brain extracts or glioma conditioned medium (GCM) are distinct from the nerve growth factor (NGF): they support the survival of neurons dissociated from dorsal root ganglia taken from chick embryos older than those responding maximally to NGF.

We now show that such a differential effect is of more general applicability: neurons dissociated from embryonic chick paravertebral sympathetic ganglia become increasingly responsive to GCM throughout embryonic development, whereas NGF becomes less effective after embryonic day 12. It is shown that another commonly used neuronal survival factor present in heart cell conditioned medium (HCM, Collins, Dev. Biol., 65:50-57, 1978) is distinct from both NGF and GCM: the sympathetic neurons become maximally responsive to HCM at embryonic day 14 i.e. after NGF but before GCM.

That the factors are distinct is also supported by the observation that the combination of HCM with either GCM or NGF allows the survival of up to 90 % of the neurons plated, i.e. a greater proportion than those supported by saturating concentrations of any factor acting alone.

The results provided by this experimental approach further indicate that the sympathetic chain ganglia at any given stage of development consist of sub-populations of neurons which differ in their requirements for survival factors in culture.

131.6 NEUROTROPHIC FACTOR: DEPENDENCE OF IN VITRO MYOGENESIS ON A NEUROTROPHIC PROTEIN PRESENT IN CHICK EMBRYO EXTRACT.

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A neurotrophic protein (neurotrophic factor: NTF) purified from nerve tissue has been shown to enhance the morphological development and to promote the maintenance of skeletal muscle cells in vitro (Markelonis, G. J. and Oh, T. H., Proc. Nat. Acad. Sci., USA, 76: 2470-2474, 1979). We have elicited a specific antiserum against purified NTF in rabbits. Using this antiserum, we have examined whether NTF is also required for the initial differentiation of avian myogenic cells in vitro. Dissociated myogenic cells obtained from 12-day chick embryos were grown in the culture medium consisting of Dulbecco's modified Eagle's medium, horse serum and chick embryo extract (EE). NTF was found to be a component of EE, a constituent of culture medium required for myogenesis in vitro, by an immunodiffusion assay and by NaDodSO$_4$-gel electrophoresis. The removal of NTF from EE by immunoprecipitation with anti-NTF serum completely inhibited myogenesis. When myogenic cells were grown in culture medium from which NTF has been removed, the cells failed to differentiate beyond the myoblast stage. However, when NTF (25 µg/ml) was added to the NTF-absorbed culture medium, normal myogenesis ensued. Furthermore, myogenic cells underwent normal myogenesis in the absence of EE if NTF (25 µg/ml) was added to the culture medium. These results demonstrate that NTF is the component of EE required for myogenesis and that it influences the initial differentiation of myogenic cells in vitro.

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131.7 ABSENCE OF ARGININE-ESTEROPEPTIDASE ACTIVITY IN HUMAN NERVE GROWTH FACTOR.

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Nerve Growth Factor (NGF) is one of a family of trophic factors required during the development of the vertebrate peripheral nervous system. The NGF protein has been isolated from the male mouse submaxillary gland, a number of snake venoms, guinea pig prostate and more recently from human placental tissue. In all species studied to date, NGF has been found to exist as a high molecular weight complex. Murine NGF, the most highly characterized form, consists of three different subunits, α, β and γ. This complex is weakly associated by noncovalent forces and can be dissociated either by a simple dilution or a shift in pH. The biological activity resides in the β subunit which has been found to be a basic protein with a molecular weight of ∿26,000 daltons. The γ subunit possesses an arginine-specific esteropeptidase activity. Presently no known biological activity has been associated with the α subunit.

NGF isolated from human placental tissue also appears to be associated in a high molecular weight complex with its biological activity residing in a basic subunit. However, we have failed to demonstrate an arginine-esteropeptidase activity like that demonstrated for the murine γ subunit. It has been reported for murine NGF that the enzyme activity of the γ subunit is present when it is dissociated from the complex and is inactive when bound to the β subunit. Therefore, our inability to detect enzyme activity from human NGF may be more of a reflection of an increase in affinity between the β subunit and a possible γ subunit, rather than the total absence of such a γ-like component.

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131.8 NEUROTROPHIC FACTOR: PURIFICATION AND CHARACTERIZATION OF A NEUROTROPHIC FACTOR FROM CHICKEN SCIATIC NERVES.

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A protein isolated from sciatic nerves of adult chickens promotes the morphological maturation and maintenance of embryonic avian skeletal muscle cells in the absence of innervation and is required for normal myogenesis in vitro. This neurotrophic factor (NTF) has been purified by ion-exchange column chromatography on DEAE cellulose followed by gel filtration on Sephadex G-100 superfine. NTF migrated as a single polypeptide chain of molecular weight (MW) 84,000 on sodium dodecyl sulfate-gel electrophoresis. The native MW of NTF as determined by sedimentation equilibrium centrifugation was 86,400. Amino acid analysis revealed that NTF is relatively deficient in tryptophan, histidine, glycine and arginine, but enriched in cysteine, methionine, alanine and lysine. Carbohydrate determination showed that NTF is composed of 11% sugar by weight with no detectable N-acetylneuraminic acid residues. Sedimentation velocity centrifugation studies revealed an $S^{o}_{20,w}$ of 5.11 with a frictional coefficient of 1.31. NTF had no detectable protease or acetylcholinesterase activity as determined by sensitive assays. The results of the present study provide new biochemical information on a macromolecule with biological activities similar to those of the "maintenance" group of growth factors which includes such proteins as insulin and nerve growth factor (NGF).

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131.9 NEURONAL SURVIVAL PROTEIN (NSP): A SUBSTANCE SUPPORTING SURVIVAL OF PARASYMPATHETIC NEURONS IN CELL CULTURE. J. B. Tuttle*, C. Greene*, G. Pilar and J. Lucas-Lenard*. (SPON: H. Swadlow). Physiol. and Biochem. and Biophys. Sections, Bio. Sci. Grp., Univ. of CT, Storrs, CT 06268.

We have attempted to isolate a substance capable of supporting long-term survival of dissociated ciliary ganglion neurons. Previous work (Tuttle, et al., Brain Res., 183 (1980) 161-180) showed that a saline extract of chick embryo contained survival-promoting activity. Thus, we sought to determine if any single component could substitute for extract in a survival bio-assay. The bio-assay consisted of pre-plating neurons for 24 hrs. at relatively low density (2-4,000 neurons/cm^2) in 80% modified MEM with 10% HS and 10% whole embryo extract. This medium was then replaced with 5% HS-MEM plus test substance. The number of neurons surviving up to three weeks was determined by counting under phase-contrast.

Ultrafiltration of whole extract indicated that a fraction of mol. wt. 10-30,000 supported survival equivalent to control. Further, this fraction was not permissive for non-neural cell growth, thus simplifying the assay. Polyacrylamide gel analysis on a 7% non-denaturing gel revealed a single band, while on a 15% SDS gel four major bands were stained (mol. wt. ≈ 17K, 12K, 10K, 7K). Sephracryl S-100 column chromatography in 0.1M buffer (pH 7.5) of the ultrafiltrate revealed a broad protein peak with a maximum m.w. of about 12K. Bio-assay revealed a corresponding activity peak at 12,000 m.w. The substance thus isolated is sensitive to trypsin, suggesting that it is a protein. It is stable at 4°C in dilute solution and active at nanomolar concentrations. Preliminary specificity tests suggest the substance supports survival and neurite extension from sympathetic neurons also. The substance has been tentatively named "neuronal survival protein" (NSP).

Several other laboratories have recently reported on substances with various "trophic" actions upon cholinergic neurons, and on ciliary ganglion neurons in particular (Helfand, et al., Expt. Cell Res., 113 (1978), 39-45; Collins, PNAS, 75 (1978) 5210; McLennan and Hendry, Neurosci Let. 10 (1978) 269-273; Manthorpe et al., J. Neurochem., 34 (1980) 69-75; Ebendal, et al., Neurosci. Let. 14 (1979) 91-95). The relationship, if any, between NSP and these other factors remains to be determined. However, we believe NSP to be the lowest m.w. substance yet isolated that supports long-term survival of dissociated parasympathetic (and other ?) neurons in culture. Investigations into the chemical nature, physiological activity and developmental significance of NSP are in progress. Supported by NIH NS10338, NS5382, The Univ. of Conn. Research Foundation, and the National Spinal Cord Injury Foundation (J.B.T.).

131.10 DEVELOPMENTAL STUDIES OF CHICK CILIARY GANGLIONIC CELLS IN VITRO. M. Manthorpe, R. Adler and S. Varon. Dept. Biology, Univ. of Calif., San Diego, La Jolla, CA 92093.

In vivo, the survival and normal development of chick embryo ciliary ganglion (CG) neurons depends upon the establishment of connections with their intraocular target tissues, i.e., the choroid and iris musculature. It has been shown that the in vitro survival of 8-day chick CG neurons depends upon the presence of exogenously added ciliary neuronotrophic factor (CNTF), which is highly concentrated in, and has been partially purified from, extracts of choroid-iris tissue (Science 204: 1434, 1979; Dev. Biol. 74: 401, 1980; J. Neurochem. 34: 69, 1980). We have now undertaken the examination of CG neurons with regard to developmental changes in CNTF requirements.

As a first step, adequate CG dissection procedures and a common dissociation method were developed for CG's from 5 to 14 day chick embryos. Total cell yields per ganglion rose from 10^4 at day 5 to 7×10^4 at day 14. Two cell types could be recognized in the dissociate after day 7, but not reliably before: i) small phase dark (SD) cells, possibly non-neurons and ii) large phase bright (LB) cells, presumptive neurons. Dissociates contained 10-11 x 10^3 LB cells per ganglion between days 7 and 9 and this number appeared to decline 50% by day 14. After 24 hours in vitro the number of non-neuronal flat cells increased dramatically in cultures from 9-14 day CG. LB cells were also present in 24 hour cultures from all stages when eye-CNTF was added to the medium. However, neuritic development could be observed in cultures from younger but not older CG. In the absence of added CNTF, no or very few LB cells were present in 24 hr cultures from 5-9 day CG. In contrast, the number of LB cells present at 24 hr in the cultures from older CG increased progressively, reaching for the 14 day CG the same number found in CNTF-supplied ones. Current work is attempting to investigate the basis for these different in vitro behaviors of older CG cells.

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131.11 CHARACTERIZATION OF NEURITIC OUTGROWTH PROMOTING ACTIVITY OF CONDITIONED MEDIUM ON SPINAL CORD EXPLANTS. Lori B. Dribin and John N. Barrett. Dept. Physiol. and Biophys., Univ. of Miami Med. Sch., Miami, Fla. 33101.

Conditioned medium from a variety of nonneuronal primary cultures (rat skeletal muscle, fibroblast and lung) produces a 3-to4-fold increase in the area of neuritic outgrowth from rat spinal cord explants compared to explants grown in fresh, nonconditioned medium [Dribin, L.B. and Barrett, J.N. (1980). Dev. Biol. 74, 184-195]. The neuritic outgrowth promoting factor(s) in conditioned medium is a macromolecule and is stable when heated to 58°C for 30 min. (op cit). We report here attempts to further purify and characterize this factor(s).

Conditioned medium was collected from monolayer cultures of fetal rat lung and concentrated by molecular filtration (nominal molecular weight cutoff of 10,000 daltons). The concentrated conditioned medium was then subjected to column chromatography or trypsin treatment. The outgrowth promoting activity of each treated fraction was assayed by measuring the area of neuritic outgrowth around fetal (15 day) rat spinal cord slices after 7 days in vitro, and comparing this area to the area of outgrowth around matched slices grown in fresh, nonconditioned medium.

When the concentrated conditioned medium was analyzed by molecular gel chromatography with Ultrogel AcA 54 or AcA 34, the eluted fractions containing the highest neuritic outgrowth promoting activity were consistently found in a region corresponding to an apparent molecular weight of approximately 60,000 daltons, although fractions with lower activity were usually found in lower molecular weight regions.

The biologically active portion of the conditioned medium adsorbed to a Concanavalin A-Sepharose column, because the fraction of the conditioned medium that passed through the column displayed no biological activity. The active fraction of the conditioned medium could then be recovered from the column by elution with α-methyl mannoside.

The neuritic outgrowth promoting activity of the conditioned medium was destroyed by trypsin treatment (1 mg/ml, 1 hr., 37°C).

These results suggest that the factor(s) responsible for the increased neuritic outgrowth from spinal cord explants is a glycoprotein.

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131.12 STAGE-DEPENDENT GROWTH INFLUENCES ON FROG TADPOLE DORSAL ROOT GANGLION NEURITES EXERTED BY SPINAL CORD EXPLANTS IN VITRO. E.D. Pollack, V. Liebig* and C.R. Reed*. Inst. for the Study of Developmental Disabilities and Dept. of Biological Sciences, Univ. Illinois, Chicago, IL 60680.

Each of the major components of the spinal reflex arc (spinal cord, dorsal root ganglion, limb muscle) can be represented as part of an interacting nerve-target complex during the growth of nerve fibers. A tissue culture system comprised of combinations of these tissues explanted from Rana pipiens tadpoles at several developmental stages has allowed a determination of stage-dependent influences of target tissues on the growth of neurites from neural tissues normally innervating those targets. An earlier study (Pollack and Liebig, Science 197:899,1977) reported that the appropriate target tissue for peripheral spinal fibers during development is the mesenchymal limb bud rather than differentiated muscle, as evidenced by enhanced and directed nerve fiber growth. We now report that the optimal stage for eliciting neurite growth from dorsal root ganglia (DRG) by its centripetal target, the spinal cord, is that characterized by a moderately differentiated hind limb lacking exteroceptive innervation (stage XI of Taylor and Kollros, Anat. Rec., 94:7, 1946). The maximal response on the part of DRG neurites occurs when both the DRG and explanted cord target are at stage XI. This is the same stage at which the DRG is also most responsive to the presence of limb tissue, also at stage XI (Pollack, et al, J. Cell Biol., 83:135a, 1979). Unlike the cord, which presents maximal neuritic outgrowth in the presence of undifferentiated limb bud tissue, the DRG requires a substantial degree of limb differentiation, i.e., muscle, in order to respond in vitro. The DRG exhibits no outgrowth in the absence of target tissue in the serum- and extract-free medium in these studies (Muhlach and Pollack, TCA Manual 4:875,1978). The percentage of DRG explants showing outgrowth increases as the stage of both DRG and cord advance. However, the extent of outgrowth remains sparse to modest until the DRG has attained stage XI after which the response to different cord stages decreases. It is suggested that the target tissue for nerve fibers, whether neural or non-neural, can exert critical stage-dependent influences on the extension of nerve fibers in vitro with in vivo correlates. The nature of the influence is hypothesized to be nerve growth factor (NGF) of target origin for the DRG, and an as yet uncharacterized factor of limb mesenchyme origin for the cord neurites. (Supported by NIH grant NS 13814 to E.D.P.)

131.13 FACTORS AFFECTING THE APPEARANCE OF JUNCTIONAL AND PERIJUNCTIONAL ACH RECEPTORS IN ORGAN CULTURE. Anthony Olek and Norman Robbins
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A rapid, nerve stump dependent appearance of acetylcholine receptors (AchR) had been previously reported to occur after 42 hours following the introduction of rat phrenic nerve-diaphragm preparations into organ culture (Olek and Robbins, Soc. Neurosci. Abt. 1979, 5,2585). Approximately 2 x 10^6 or more AchR (assayed by I^{125}-alphabungarotoxin binding) appeared in the junctional region between 42-44 hrs. of denervation in organ culture, while only approximately 5 x 10^5 AchR appeared in an equivalent surface area of extrajunctional membrane during the same time. The presence of a long nerve stump prevented the increase of AchR specific to the endplate region, but had no effect on the appearance of extrajunctional AchR.

Further studies have revealed: 1) The increase in junctional region AchR is composed primarily of junctional receptors. Autoradiographic examination of I^{125}-alphabungarotoxin (*BTX) binding indicated that approximately 75% of the increase in binding is confined to the endplate, and approximately 25% to a region within 300μ of the endplate. 2) The increase in junctional region receptors reflects the new appearance of AchR. Prior labelling with cold BTX does not prevent the subsequent appearance of junctional region AchR after 42 hrs. 3) The newly appeared AchR demonstrate pharmacologic specificity. Binding of *BTX to newly appeared AchR (after 42 hrs.) can be blocked with cold BTX. 4) The appearance of AchR is sensitive to block of RNA synthesis. Actinomyosin D prevents the appearance of junctional region AchR if applied before 24 hrs. of culture, but has no effect on the AchR increase after that time. 5) The increase in AchR does not depend on muscle activity or nerve impulse transmission. The nerve stump dependent appearance of AchR persists with the application of tetrodotoxin, cold BTX, and pan-curonium. 6) Stimulation of the nerve stump produces an earlier appearance of junctional region AchR in culture. Stimulating the nerve stump at 10 hz for 1 hr. in the presence of curare, followed by washout of the stimulated media, results in an increase in enplate region AchR found at 24-32 hrs. of culture.

The present findings suggest that junctional and extrajunctional AchR are controlled by different neuronal mechanisms and that junctional receptors are subject to rapid alteration.

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131.14 THE DEVELOPING HIPPOCAMPUS IN CELL CULTURE

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The hippocampus consists of only a few classes of well characterized neurons: the pyramidal neurons CA_1-CA_4, the granular neurons of the dentate gyrus, and the inhibitory basket cells. At a certain developmental stage of the fetal rat brain, only pyramidal neurons will survive and develop in dissociated cell cultures of the hippocampus, together with glial cells and other non-neuronal cells (1). With this culture system we are studying 1) the morphological and biochemical development of pyramidal neurons, 2) the control of possible synapse formation and 3) the trophic interactions between neuronal and glial cells.

We have isolated the hippocampus from fetal rats at day 18 and followed the morphological development of dissociated cultures under various conditions. Identification of cell types was carried out by morphology and by binding of immunocytochemical markers: tetanustoxin for neuronal cells, antiserum against GFAP for astrocytes and antiserum against galactocerebroside for oligodendrocytes. In addition a monoclonal antiserum was used for identification of neurons, and a monoclonal antiserum produced in our laboratory for identification of oligodendrocytes.

After defining optimal conditions for long term neuronal cultures (2-3 months), our goal is to study the development of neurons in a controlled environment in order to evaluate the contributions of genetic programs versus environmental influences such as direct cell-cell interactions or soluble trophic factors. For this purpose we are studying changes in morphology and in the appearance of specific antigens in parallel with some biochemical markers: ganglioside pattern and neurotransmitter uptake (GABA, glutamate).
Trophic effects of glial conditioned media and gangliosides on neuronal cells have been described previously in our laboratory (2,3). We are now investigating such trophic effects in this culture system of the developing hippocampus.

1) Banker, G.A. and W.M. Cowan, Brain Research (1977) 126, 397
2) Seifert, W. (1977) - Hoppe-Seyler's Z.Phys.Chemie 358, 307
3) Morgan, J. and W. Seifert (1979) - J.Supramol.Structure 10,111

131.15 COMPARISON OF THE GROWTH AND DIFFERENTIATION OF SYMPATHETIC NEURONS GROWN IN A BIOCHEMICALLY DEFINED MEDIUM AND IN SERUM-CONTAINING MEDIUM. J. E. Freschi. Neurobiology Department, Armed Forces Radiobiol. Research Inst., Bethesda, MD 20014.

For the past several years we have successfully grown sympathetic neurons in culture over several months using modified Ham's F12 medium supplemented with 5% fetal bovine serum and nerve growth factor (NGF). Recently we have had success in growing these neurons in the biochemically defined medium of Bottenstein and Sato. Beyond cell survival, however, changes in medium composition may have marked effects on differentiated neuronal functions such as electrical membrane properties, neurotransmitter synthesis, chemosensitivity, and synapse formation. In order to compare neurons grown in defined medium (N1) with those grown in serum-containing medium, neurons dissociated from neonatal rat superior cervical ganglia were plated in F12 + 10% fetal bovine serum (FCS) + NGF + antibiotics for less than 24 hrs. They were then fed either N1 + NGF or F12 + 5% FCS + NGF. Neuronal survival appeared to be approximately the same after the first week. In serum, fibroblast proliferation was rapid, and Schwann cells formed overlying aggregates with interconnecting bundles of neurites. In N1, however, fibroblast proliferation was slow. Schwann cells formed a more diffuse meshwork, and neurites showed less tendency to form bundles. In serum, nearly every cell showed many spontaneous EPSPs and action potentials. Recording from neuron pairs showed evidence of widespread complex neuronal networks. In N1, fewer cells showed spontaneous EPSPs, and the amount of synaptic activity on a given cell was less than on neurons in serum. Nevertheless, spontaneous synaptic activity was seen in about half of the neurons studied grown in N1. In both sets of cultures spontaneous synaptic activity was blocked by nicotinic antagonists. Neither serum nor N1 grown neurons showed specific catecholeamine fluorescence between 2 days and a month in culture. The incidences of Ca++-dependent, K+-mediated spike after-hyperpolarizations and iontophoretically evoked muscarinic acetylcholine potentials were similar in the two groups of neurons.

I conclude that sympathetic neurons can be grown in good condition in a biochemically defined medium. This will be of value in studying the influence growth and trophic factors on sympathetic neuronal differentiation.

131.16 SYNERGISTIC EFFECTS OF NERVE GROWTH FACTOR AND CYCLIC AMP IN PC12 CELLS. G.E. Landreth, P.W. Gunning, M.A. Bothwell* and E.M. Shooter. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

A clonal line of rat pheochromocytoma cells (PC12) respond to nerve growth factor (NGF) by cessation of division and development of a number of properties characteristic of mature sympathetic neurons. These cells undergo morphological differentiation and extend neurites over a period of days in response to NGF by a transcriptionally-dependent process. When PC12 cells are exposed to NGF (50 ng/ml) or dibutrylcyclic AMP (dbcAMP, 1mM), 10% and 26% of the cells develop neurites within 24 hrs. respectively. If the cells are exposed to both agents 70% of the cells extend neurites within 24 hrs. The synergistic neurite outgrowth is dependent on the NGF concentration, and is maximal at 0.5 - 1 ng/ml NGF. The dose-dependent stimulation of neurite outgrowth is identical to that observed in a neurite regeneration assay from differentiated PC12 cells.

Potentiation of neurite outgrowth occurs as a function of the simultaneous exposure to both agents, neurites induced by dbcAMP were not maintained after replacement of the dbcAMP by NGF. The initiation of neurite outgrowth in the presence of both dbcAMP and NGF is independent of RNA and protein synthesis, in contrast to that caused by NGF alone.

The response was specific for cAMP, as cholera toxin together with NGF produced the same response as that observed with dbcAMP.

Cellular RNA concentration increased in response to NGF and dbcAMP within 24 hrs, however, in the presence of both agents the increase was additive or greater.

These results are inconsistent with a common mechanism of action for NGF and cAMP, since maximally stimulating concentrations of both produce additive or synergistic effects when added together. The data suggest that cAMP relieves the transcriptional dependency of NGF-mediated neurite outgrowth, permitting rapid reorganization of the PC12 cytoskeleton.

131.17 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF HUMAN NEUROBLASTOMA AND SYMPATHETIC GANGLION CELLS IN SERUM-FREE SUPPLEMENTED MEDIUM. D.W. Burmeister*, G.K. Zeevalk*, J.M. Carroll* and K.M. Lyser. Dept. of Biological Sciences, Hunter College of the City University, New York, NY 10021.

Cells of the clonally derived continuous line SK-N-SH-SY5Y (SY5Y) (Biedler et al., Cancer Res. 38:3751, 1978) have been cultured in Bottenstein and Sato's N2 medium (Proc. Natl. Acad. Sci. 76:514, 1979), in N2 without selenium (N2A), or in medium with 10% fetal calf serum (FCS). As observed by phase, scanning and transmission electron microscopy, SY5Y cells cultured in defined medium showed an altered morphology from cells cultured in FCS. The cells became less adherent to the substrate with only a few areas of the cell periphery making close contact. They developed more surface ruffling and formed more cell clumps at comparable densities. Ten to 20% of the cells developed distinctive processes, which were generally non-adherent to the substrate along their length and adhered preferentially to other cells. The processes were rich in microtubules as well as ribosomes and mitochondria. The differences occurred with or without FCS or fibronectin on the substrate.

The specific activity of dopamine-β-hydroxylase (DBH) in cultures maintained for 1 week in N2, N2A, or N2A plus 50 ng/ml β-nerve growth factor (NGF) was the same as that in the presence of FCS. SY5Y carried in N2 for 90 days maintained this level of DBH activity.

For comparison, human fetal ganglion cells, which are the normal counterparts of the tumor cells, were cultured in N2 plus 10 ng/ml NGF and in serum-supplemented medium containing chick embryo extract and NGF for at least 2 weeks. The human fetal neurons maintained their cytological differentiation in vitro and formed extensive, typical axons with microtubules and neurofilaments. In defined medium, neuronal cells showed good survival and differentiation with extensive neurite outgrowth and formation of fiber bundles. There was a marked reduction in non-neuronal cells, in contrast to cultures in medium with FCS, which were rapidly overgrown with fibroblasts. Exogenous NGF was required for survival of the sympathetic neurons in either medium. The level of DBH in these cells is currently being investigated.

The use of defined medium now allows investigation of the effects on human neuronal cells of a variety of factors (cAMP, NGF, glial factors) without the complications contributed by serum.

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131.18 DORSAL ROOT GANGLION NEURONS STIMULATE DIFFERENTIATION AND MYELIN FORMATION BY OLIGODENDROCYTES DERIVED FROM EMBRYONIC SPINAL CORD. P.M. Wood* and R.P. Bunge. Dept. of Anatomy & Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

We have previously shown that cultured networks of dissociated dorsal root ganglion neurons (DRGN) induce myelin formation by oligodendrocytes (ODC) derived from optic nerve sections taken from 7-14 day postnatal rats (Wood, P., Okada, E., Bunge, R.P., Brain Research, In Press). These optic nerve sections would have already contained mature ODC, which could have accounted for the myelin found. In more recent experiments, spinal cord tissue from 14 day rat embryos has been tested as a source of ODC. ODC differentiation and myelin formation do not occur until after birth in the rat; this embryonic tissue, therefore, contained only ODC precursors.

Neonatal rat dorsal root ganglia were dissociated, cultured for 2 weeks in medium containing fluorodeoxyuridine (10^{-5}M) to suppress Schwann cell ensheathment and fibroblast proliferation, and subsequently maintained for one week in serum rich drug-free medium. At 3 weeks spinal cord tissues from 14 day rat embryos was added to the DRGN networks either as 1) small fragments or as 2) suspensions of mechanically dissociated cord cells. Cultures were fixed at 2 and 4 weeks after cord tissue addition and stained with Sudan Black for light microscopic analysis. ODC type myelin was found at 2 weeks in all cultures of DRGN networks that had received either fragments or cells in suspension but not in control cultures of cord tissue alone. More extensive myelination of axons within DRGN fascicles was observed at 4 weeks in all cultures with both DRGN networks and added spinal cord tissue; in cultures containing just cord fragments, myelin was found only within, or immediately adjacent to the fragments themselves. ODC myelin formation was not observed in dissociated spinal cord cultures in the absence of DRGN networks. These results indicate that both the differentiation of immature ODC precursors into ODC, and myelin formation by ODC were induced (or stimulated) by the presence of stable mature DRGN networks. The proliferation of residual Schwann cells and the formation of Schwann cell myelin was markedly or completely inhibited in areas of the network occupied by glial cells; in contrast DRGN networks not receiving glial cells often became heavily myelinated by Schwann cells by 4 weeks. This culture system demonstrates the importance of a stable neuronal population capable of inducing myelin formation in the study of factors influencing ODC differentiation.

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131.19 RELEASE OF PLASMINOGEN ACTIVATOR FROM CULTURED MUSCLE CELLS. M. Patterson*, K. Romstedt* and B.W. Festoff. (SPON: D.K. Ziegler). Dept. of Neurology, Univ. of Kansas Med. Ctr. and Neurobiology Res. Lab., VA Med. Ctr., Kansas City, MO 64128.

We have been exploring mechanisms of maintenance and development of synaptic connections at the neuromuscular junction (NMJ). One recent model proposed is that muscle cells produce and release proteolytic enzyme (PE) activity augmented by nerve terminal-released acetylcholine (ACh). PE are thought to degrade surface molecules and to be involved in extracellular matrix (ECM) metabolism. Elimination of polyneuronal innervation of muscle fibers in neonatal animals may require PE. This model may be applicable to certain disease states of man such as amyotrophic lateral sclerosis (ALS) and others. Interest in release of plasminogen activators (PA) from cells in various tissues has developed because of involvement of PA's in numerous signal biologic processes. Report of their release from nerve and Schwann cells have appeared this past year. PA release from muscle has not yet been reported.

Chick primary muscle cultures (PMC) and G-8 cells, a fusing cell line spontaneously transformed from mouse myoblasts, were evaluated by the fibrin over-lay and ^{125}I-fibrin plate methods. Plasminogen was purified from human plasma and dog serum; fibrinogen from bovine serum. Clones of PA secreting cells were identified and sub-cultured from G-8 cells plated at low density. PMC, before 6 days in vitro, did not produce detectable PA without media additions. However, after 7 days in culture or with 10^{-5}M ACh (with eserine) PA was detected in media. A dose response curve then demonstrated that half-maximal response was approximately micromolar. We next evaluated dibutyryl cyclic AMP (dbcAMP), adenosine and PGE_2. Slight increase in PA activity was found with all. Using SDS gel analysis of ^{125}I-labelled plasminogen incubated with conditioned medium from G-8 cells producing PA, both heavy and light chains of labelled plasmin were detected. Study of the kinetics of release of PA from cloned G-8 cells is in progress. This evidence suggests that PA released from muscle cells, cleaves plasmin, thus providing a potential extracellular mechanism for turn over of surface molecules at the NMJ that may play roles in plasticity, denervation and disease. The role of nerve in regulating such activity, by direct inhibition perhaps in the synaptic cleft or indirectly by "trophic" regulation of synthesis, transit to the surface or release deserves further exploration.

131.20 DEVELOPMENT OF CHOLINERGIC SYMPATHETIC INNERVATION OF ECCRINE SWEAT GLANDS IN RAT FOOTPAD. S.C. Landis and D. Keefe*. Dept. of Neurobiology, Harvard Med. Sch., Boston, Mass. 02115.

Sympathetic principal neurons dissociated from ganglia of newborn rats become cholinergic if grown with certain nonneuronal cells or medium conditioned by them. This induction occurs in immature adrenergic neurons which have acquired the ability to synthesize, store and take up catecholamines (CA); as the neurons acquire cholinergic properties, they lose the ability to synthesize CA but are still able to take up and store exogenous CA. In vivo a minority of principal neurons are cholinergic and some of these innervate sweat glands of cat and rat footpads. We have examined the development of these cholinergic sympathetic fibers in vivo to determine whether they undergo a similar transition from adrenergic to cholinergic function.

In the adult rat, acetylcholinesterase(AChE)-positive fibers form a loose plexus in the SG. In thin sections, AChE-staining surrounds axons which run in bundles partially ensheathed by Schwann cells. Axonal varicosities are separated from the myoepithelial and secretory cells of the SG by a thick basal lamina and often also by fibrocyte processes. In adults, no fibers contain histochemically detectable endogenous CA; however, if 1-10mg/kg α-methylNE is injected ip, a plexus of fluorescent fibers is present and corresponds in position and density to the AChE-stained plexus. CA uptake is blocked by prior injection of DMI (30mg/kg) and is absent from parasympathetic cholinergic fibers in irides and salivary glands of ganglionectomized rats. Thus this CA uptake is specific and a special property of cholinergic sympathetic neurons.

SGs form shortly after birth in the hindfeet. AchE-stained fibers are found in the forming gland at 7 days and increase in number and staining intensity as the SG differentiates during the next two weeks. At 7, 10 and 14 days, endogenously fluorescent fibers are also found in all developing glands and their distribution matches that of the AChE-positive fibers. At 21 days, only an occasional and faintly fluorescent fiber is observed in the absence of exogenous CA. One interpretation of these observations is that a single population of terminals is present, that cholinergic sympathetic neurons in vivo as in vitro initially possess adrenergic properties and that some of these properties are lost as cholinergic ones appear. An alternative possibility is that early-arriving adrenergic terminals retract or degenerate, leaving behind a second population of cholinergic terminals which constitute the adult innervation. Ultrastructural studies are in progress to distinguish these possibilities. Supported by ROI NS 15549. E. Lamperti participated in early studies.

132.1 ONSET OF HORMONAL REGULATION OF ODC ACTIVITY IN THE PERINATAL RAT. S. J. Geyer*, C. M. Kuhn* and S. M. Schanberg. Laboratory of Neuropharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Rapid cell proliferation and tissue growth is closely associated with elevated ornithine decarboxylase activity (ODC). It has been shown that levels of ODC activity in developing rats are altered by a variety of hormones and neural substances and that different organs show varying responsiveness to drug treatment. Recent data from our laboratory suggest that the regulation of tissue ODC activity by hormones changes during development. We have shown in rat pups as young as 2 days of age that a SC injection of growth hormone stimulated liver and brain ODC activity after 4 hr. However, growth hormone had no effect on tissue ODC activity when given SC to 17-21 day old fetuses in utero, whereas, placental lactogen effectively stimulated fetal tissue ODC activity (Hurley, T. W., Endoc. Soc. 1979). In the present study the changing regulation of ODC activity during development was examined by testing the sensitivity of liver and heart ODC activity to hormones and drugs in perinatal rats. ODC activity in fetal liver was found to increase 10 fold (per organ) between 15 and 17 days of gestation while a 10 fold surge in fetal heart ODC activity occurred later, between 17 and 20 days of gestation. Despite the dramatic increase of basal tissue ODC activity occurring in fetal tissues between 15 and 20 days of gestation, fetal liver and heart tissue was found to be completely insensitive to a variety of agents which we have shown markedly enhanced ODC activity in 8 day old pups. Phenylephrine, dexamethasone, dbcAMP, vasopressin, angiotensin II, insulin and PGE_1 were all ineffective when administered SC or IP to fetuses (15-20 days) in utero 4 hr before tissue collection. Furthermore, liver and heart ODC of 0-24 hr rat pups which were delivered naturally or by C-section on the 21st day of gestation also were unresponsive to SC administration of phenylephrine, dbcAMP, vasopressin and PGE_1. However, by 2 days of age, modest ODC increases (200% of control) were elicited in heart and liver tissue by phenylephrine and in the liver by dexamethasone. Starting at 3 days of age the ODC activity response to these drugs was more marked (300-500% of control). These results suggest that although basal tissue ODC activity is changing during the rapid growth phase of gestation, hormonal and neurotransmitter regulation of ODC activity characteristic of an adult develops only after several days of postnatal life. Supported by grants MH13688 and MH06489 from the USPHS.

132.2 SEXUALLY DIMORPHIC NUCLEUS IN THE RAT SPINAL CORD: RESPONSE TO ADULT HORMONE MANIPULATION, ABSENCE IN ANDROGEN INSENSITIVE RATS. S. Marc Breedlove and Arthur P. Arnold, Dept. Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

There is a sexually dimorphic, androgen accumulating motor nucleus in the fifth and sixth lumbar segments of the rat spinal cord, consisting of motoneurons innervating two striated perineal muscles, the levator ani and the bulbocavernosus. This nucleus, the spinal nucleus of the bulbocavernosus (SNB), is diminished or absent in female rats (Breedlove & Arnold, in press). We now report 1) the number of neurons in the SNB is not altered by adult gonadectomy or treatment with testosterone propionate (TP), 2) the size of SNB neurons is increased in the presence of androgen, 3) genetically male Tfm mutant rats with reduced androgen receptors do not have an SNB. These results support the hypothesis that the dimorphic nature of the SNB depends not on the adult hormone state, but on the interaction of androgens with their receptors early in development.

Adult male and female Sprague-Dawley rats were either gonadectomized or sham operated. Sham operated animals were given s.c. injections of vehicle, while the gonadectomized rats were injected with either vehicle or TP, 200 µg/100 g body weight/day for 28 days. Using thionin-stained 50 µm transverse sections, a blind observer made corrected counts of the number of nuclei of densely staining cells in the region normally occupied by the SNB. At least 10 cells from each animal were outlined using a camera lucida, and areas of the neurons and their nuclei calculated.

Across all three treatment groups, male rats had more and larger neurons with larger nuclei in the SNB region than females (2-way ANOVA, $p < .001$). Adult hormone manipulations had no significant effect on the number of neurons counted. Hormone treatment did have a significant effect on neuronal size ($p < .05$), with exogenous or endogenous androgen associated with larger neurons, although the 4 week treatment with TP was insufficient to bring the size of these neurons in gonadectomized females to the level of vehicle injected castrated males. Adult hormone state seems to have little effect on the dimorphism of the SNB, since TP treatment does not affect the number of cells, and only partially compensates for the sex difference in neuronal size.

Seven adult King-Holtzman male rats with the testicular feminization mutation which results in few androgen receptors, and seven normal male littermates were sacrificed and examined as above. Tfm males do not have an SNB, unlike their normal male littermates. In the SNB area, normal males have more and larger cells with larger nuclei than the Tfm rats (t-tests, $p < .001$).

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132.3 GLUCOCORTICOIDS INHIBIT MYELINATION IN DEVELOPING RAT. V.L.Friedrich,Jr. and M.C.Bohn. Lab. of Neuromorphology, Dept. of Biobehavioral Sciences, Univ. of Connecticut, Storrs, CT 06268

Glucocorticoids are known to affect the generation of neurons in vivo and of glial cells in vitro. Our study was undertaken to determine the effects of glucocorticoid treatment on myelination in vivo, using optic nerve as a model.

Most oligodendrocytes in rat optic nerve are formed during the second and third weeks after birth(Skoff et al., J.comp.Neurol. 169:313). Therefore, rats were injected daily with hydrocortisone acetate(HCA)(10 µg/g body weight, limited to 200 µg/injection), from postnatal days 7 to 18. Animals were sacrificed at 21 days after birth to determine acute effect of the treatment and at 60 days to assess lasting effects. Untreated litters were used as controls to avoid possible effects of increasing endogenous steroids through the stress of repeated injections. Animals(3 per group) were prepared for electron microscopy and the optic nerves analyzed by standard stereological techniques.

At 21 days after birth, the total amount of myelin (per whole nerve cross section) was reduced in the treated animals by 40%. This reduction was due entirely to a reduction in the proportion of axons myelinated, from 75% in the normal animals to 46% in the treated ones. The total number of axons, average size of myelinated axons, and average size of unmyelinated axons were not affected. In spite of the large deficit in myelin, the average amount of myelin per myelinated axon was normal, suggesting that myelination of individual axons proceeded normally once initiated.

At 60 days after birth, the total amount of myelin was normal, indicating a striking recovery from the substantial deficit at 21 days. This recovery reflected an increase in the proportion of axons myelinated, to the normal value(100%). The total number of axons, amount of myelin per axon, and average axonal size were also normal at 60 days.

To assess possible effects of HCA on gliogenesis, we injected tritiated thymidine on day 19 and sacrificed animals at 60 days. The total number of oligodendrocytes was normal. However, twice as many oligodendrocytes were labeled in the treated group as in the controls. We conclude that more oligodendrocytes were formed in the treated animals than in the controls, after day 18.

Our results show that glucocorticoid treatment of postnatal rats inhibits myelination of optic nerve. Following termination of treatment, we observed late generation of oligodendrocytes and complete recovery of myelination. Our findings raise the more general possibility that glucocorticoid treatment at any age may inhibit myelination or remyelination.

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132.4 THE CELLULAR NATURE OF THE CRITICAL PERIOD FOR SEXUAL DIFFERENTIATION IN THE ZEBRA FINCH. Mark E. Gurney Div. of Biology, California Institute of Technology, Pasadena, CA 91125

Within telencephalic brain nuclei which participate in the efferent motor pathway for song in the zebra finch (Poephila guttata) it has proved possible to follow over time the ability of individual neurons to undergo morphological sexual differentiation as a consequence of exposure to 17β-estradiol (E_2). The number of neurons in the nucleus robustus archistriatalis (RA) which are able to differentiate under the influence of exogenous E_2 declines exponentially with increasing age, while the magnitude of their growth response does not change. Such data may suggest that the critical period transition of RA neurons from E_2-responsive to E_2-unresponsive is autonomous to these cells.

Exposure of female zebra finch chicks to E_2 at hatching masculinized the cytoarchitecture of RA. When administered to one-year-old, gonadally intact adult females on the other hand, E_2 had no effect on RA's cytoarchitecture. To investigate the temporal constraints on responsiveness to E_2, female finches were implanted subcutaneously with a Silastic pellet which contained 50 µg of E_2 (Gurney and Konishi, Science, in press) either at hatching, at 3, 6, 9, 15, 30, or at 40 days of age, and then sacrificed for histological examination when sexually mature at 90 days of age. The morphological assay of the ability of RA neurons to respond to E_2 consisted of measurement of their maximum somal diameter and average packing in Nissl-stained material. The distribution of somal diameter for RA neurons in either males or females was unimodal and nonoverlapping. In males the somal diameter of RA neurons averaged 17.9 µm and in normal females averaged 8.2 µm. In females exposed to E_2 at hatching the distribution of somal diameter remained unimodal, but was shifted to an average diameter of 15.9 µm. In females exposed to E_2 at 40 days of age, the pattern of response to E_2 was quite different: neurons which responded to E_2 with an increase in somal size comprised only a fraction of the total neuronal population in RA and were confined within the core of the nucleus. The distribution of somal diameter was thus bimodal with a peak of responsive neurons at 14.6 µm while unresponsive neurons remained 8.7 µm in diameter. The maximum somal diameter attained by E_2-responsive neurons in RA was equivalent in all of the different treatment groups, while the number of E_2-responding neurons within RA fell exponentially with increasing age. The half-life with which RA neurons lost E_2-responsiveness was 17.3 days.

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132.5 NEONATAL PROGESTERONE AND SEXUAL BEHAVIOR OF RATS. E. M. Hull. Department of Psychiatry & Behavioral Sciences, University of Texas Medical Branch, Galveston, Texas 77550.

My coworkers and I have demonstrated an antiandrogenic influence of perinatally administered progesterone, manifested as decreased sexual and aggressive behaviors, improved active avoidance and impaired maze performance of intact male rats. We have also demonstrated that pre- and neonatally administered progesterone increased monoamine oxidase activity in whole brain homogenates of rat fetuses and neonates, respectively.

The present experiment investigated the effects of neonatal progesterone treatment on both masculine and feminine sexual behavior of both genders. Lactating females received daily injections of 3.3 mg/kg progesterone or oil, and their offspring were gonadectomized in adulthood and tested for both masculine and feminine sexual behavior elicited by estrogen, progesterone and testosterone regimens.

Progesterone treated males exhibited significant impairment of masculine behavior elicited by both estrogen and testosterone regimens. Latency and frequency of mounts and intromissions of those males which did engage in sexual behavior were not significantly different for the two groups; the major difference was in number of animals which did mount and/or intromit. Males exhibited insufficient feminine behavior for meaningful statistical analysis, though there was a slight preponderance of progesterone treated animals which showed lordosis responses. Progesterone administered to estrogen primed males did not facilitate feminine behavior in either group. There were no statistically significant differences between progesterone treated and control females in either masculine or feminine behavior tests, though there were trends toward reduction in masculine behavior of progesterone treated females during the testosterone regimen. Estradiol benzoate sufficient to elicit considerable feminine behavior failed to elicit any masculine behavior of females. There were no differences in body weight at any time nor in testis or accessory organ weights of males.

The results of this experiment confirm our previous finding of reduced sexual competence of male rats treated neonatally with moderate doses of progesterone, and indicates that this effect is not the result of diminished adult hormone levels. This finding is consistent with the hypothesis that progesterone interacts with testosterone's effects on developing neural circuits, though alterations in sensory mechanisms or in peripheral metabolism of hormones have not been ruled out. Neonatal progesterone appears to have minimal or no effect on feminine behavior patterns of rats.

132.6 THE INFLUENCE OF NEONATAL GONADECTOMY AND ANDROGENS ON THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA. R.A. Gorski, C.D. Jacobson, J.E. Shryne* & V. Csernus*. Dept. Anat. & Brain Res. Inst., UCLA, Los Angeles, CA. 90024

The volume of the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA), which is larger in the male, is influenced by androgen during the first week of postnatal life. To evaluate the role played by gonadal steroids during this period, we compared for each sex the influence of different doses of testosterone propionate (TP) or of a gonadal graft in the neonatally gonadectomized (Gx) rat on the volume of the SDN-POA in the adult. On day 1 of postnatal life (the day of birth) male (♂) and female (♀) Sprague-Dawley pups were Gx, sham operated (SHAM) or served as untreated controls (CONT). Additionally, Gx-♂ and Gx-♀ were given sc testicular or ovarian grafts. On day 2, Gx pups which did not receive grafts were given a single injection of either vehicle (OIL), 100µg or 1mg TP. On day 45, rats which had gonads were Gx, all other rats were subjected to sham surgery. When adult, rats were sacrificed and perfused with saline and with 10% formalin. Brains were frozen sectioned at 60µ, stained with thionin, and coded so that all analyses were performed blind. The volume of the SDN-POA for each rat was determined as previously described. In all cases, there is a sex difference in SDN-POA volume for rats which received the same treatment. In the ♂: SDN-POA volume in the CONT and SHAM groups is significantly greater than that in the Gx+OIL group. Also, 100µg or 1mg TP or testicular grafts reverses the effects of Gx since SDN-POA volume in these rats is equal to that of the SHAM group. However, an ovarian graft did not affect the resulting volume of the nucleus of the Gx rat. In the ♀: the volume of the SDN-POA in the Gx+OIL group does not differ from that of the SHAM, or Gx+ gonadal grafted rats. There is a similar pattern of response to TP in the ♀ as seen in the ♂. Gx+TP significantly increases SDN-POA volume as compared to that of the Gx+OIL group. These data confirm the importance of the steroidal environment in the ♂ during the first week of postnatal life since a single injection of TP or testicular implant in the Gx-♂ replaces the endogenous hormone which is normally present during the critical period for maturation of the SDN-POA. In addition, a single injection of TP increases SDN-POA volume in the Gx-♀. However, a testicular graft was not able to alter SDN-POA volume in the Gx-♀. The differing response of the developing ♂ and ♀ SDN-POA to the presence of a testicular graft indicates a differential sensitivity to the steroidal environment since in both sexes a larger amount of androgen is able to affect nuclear volume. Also, the steroidal milieu produced by an ovarian graft can not replace the testes in the ♂, or enhance the volume of the SDN-POA in the ♀. (NIH Grant HD-01182 & the Ford, Grant & Kroc Foundations.)

132.7 REGIONAL DIFFERENCES IN INTRANEURONAL α-FETOPROTEIN, ALBUMIN, AND TRANSFERRIN LOCALIZATION WITHIN THE DEVELOPING MOUSE BRAIN. D. Toran-Allerand. Center for Reprod. Sci. and Dept. of Neurol., Columbia Univ., Coll. of P&S, New York, NY 10032.

Immunofluorescence studies have shown the intraneuronal localization and coexistence of immunoreactive α-fetoprotein (AFP), albumin, and transferrin within the same neurons of the late fetal and post-natal mouse brain. These observations have important implications for the process of sexual differentiation of the brain. The intraneuronal presence of estrogen-binding plasma proteins (AFP, albumin) must bring estradiol (E_2) into the cell, forcing reconsideration of the concept that extracellular AFP protects the female rodent brain from exposure to maternal estrogens and providing support for the hypothesis of a need for E_2 per se in neural differentiation. The present study documents the unusual topographic distribution of these proteins with respect to the E_2-concentrating regions of the hypothalamus, preoptic area and the amygdala of the developing mouse. Antigen localization was carried out by single and double label immunofluorescence (combined direct and indirect method). 3-4um serial coronal sections of the brains of E-18, newborn and P-8 mice of both sexes were exposed to antibodies to mouse AFP, albumin, transferrin, or IgG, followed by goat anti-rabbit- or rabbit anti-goat IgG conjugated to either fluorescein or rhodamine, or to antibodies to mouse albumin directly conjugated to fluorescein. All appropriate controls were without fluorescence. Localization and coexistence of all three plasma proteins was observed in diverse cell groups throughout the brains of both sexes. In the hypothalamus, preoptic area (POA) and the amygdala, however, certain nuclear regions were rendered anatomically distinctive by the complete or almost complete and bilaterally symmetrical absence of fluorescence. These regions include: the diagonal band of Broca, the medial POA, the suprachiasmatic, arcuate, ventromedial, ventral premamillary nuclei of the hypothalamus, and the medial and cortical amygdaloid nuclei. These are all regions in the developing rodent that have been shown to contain E_2-receptors by ^3H-E_2 autoradiography. The significance of this staining pattern is unknown. It is unlikely that it is an artefact, however, since it has been observed in both sexes of different ages and in the newborn rat. By its association with presumed target regions of estrogen during the critical period for sexual differentiation these regional differences in staining raise intriguing questions regarding the role of E_2-binding proteins as mediators of steroidal effects on the developing brain. (Supported in part by grants from NIH (HD-08364); NSF (BNS 79-24775); W.T. Grant Foundation; The March of Dimes Birth Defects Foundation; and NIMH Research Scientist Development Award (MH-00192).

132.8 NEUROGENESIS OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA. Carol D. Jacobson and Roger A. Gorski. Dept. Anatomy and Brain Research Inst., UCLA, Los Angeles, CA 90024.

The Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA), is larger in volume in the male and hormone dependent early in postnatal life. Presently, we compared for each sex the time course of neuroblast proliferation which forms SDN-POA or adjacent medial preoptic area (MPOA) neurons, and the temporal gradient of production of neurons in relation to their final position within the SDN-POA of Sprague Dawley rats. Sperm in the vaginal smear defined day 1 postfertilization (PF). On day 13PF each pregnant rat was implanted with a right atrial cannula through which 3H-thymidine (*thy; 5µCi/g B.Wt.) was given on day 14,15, 16,17 or 18PF. Pups were sacrificed and perfused with neutral formalin when 30 postnatal days old. After histological processing, 6µ sections were coated with NTB-2 emulsion and stored in the dark at 4°C for 30 days, developed for autoradiography and stained with cresyl violet. All further analyses were performed blind. Three sections of the SDN-POA (anterior, middle and posterior) were analysed. Neurons were rated as either labeled or not. On each section neurons within the MPOA, but lateral to the SDN-POA were also analysed. Data were converted into the percent (%) neurons which were labeled. In the MPOA and the SDN-POA the % labeled neurons decreases as the day of injection of *thy approaches the end of gestation, but the time period in which neuroblast divisions occurred is markedly different for the SDN-POA as compared to that of the MPOA. DNA synthesis occurs as late as day 18PF for neurons which form the SDN-POA but ceases on day 16PF for those destined for the MPOA. There is a sex difference in neuronal production on both day 14 and 17PF for neurons destined for the SDN-POA. After injection on day 14PF the % labeled neurons is larger in the female than in the male, but after injection on day 17PF this is reversed. Analysis of SDN-POA neurons in relation to their final position within the nucleus revealed that the posterior section has a higher % labeled neurons than that seen in the anterior section after *thy injection on day 16,17 or 18PF, and after injection on day 14PF there is a higher incidence of division of neuroblasts which form the anterior SDN-POA. These data illustrate that the specific neurons which comprise the SDN-POA in both the male and female are being produced as late as day 18PF, whereas neurons located in the MPOA but not in the SDN-POA have all been born by day 16PF. Neuroblast division which produces the neurons of the SDN-POA may begin earlier and terminate sooner in the female than in the male. The difference in neuronal birth may partially account for the sexual dimorphism seen in the volume and neuronal number of the SDN-POA of the adult rat. (Supported by NIH grant HD-01182 and the Ford, Kroc and Grant Foundations.)

132.9 AGE-DEPENDENCY OF CORTICOSTEROID-INDUCED CHANGES IN BRAIN GROWTH

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The wet and dry brain weight of adult adrenalectomized rats is greater than that of sham-operated animals, and this difference holds for both hind- and forebrain (Devenport, Behav. Neur. Biol. 27:218, 1979). We have since found that this effect is mediated by the adrenal cortex. In the adrenalectomized rat, exogeneously administered corticosterone decreases both brain weight and body weight; deoxycorticosterone reverses corticosterone's effect on the body, but not its effect on the brain. We have found a combined dose of these hormones which, when administered daily to adrenalectomized rats, almost exactly duplicates the brain and body characteristics of intact animals.

Using this dose, we investigated the sensitivity of rat brain growth to the presence or absence of the hormones during two other stages of brain development: That occurring across ages 25-45 days, and that occurring across ages 45-65. This was accomplished in a design that also permitted assessment of the extent to which the effects of hormones or their absence are reversible.

Sprague-Dawley rats born and reared in our laboratory were adrenalectomized when 25 days old and maintained with isotonic saline. Some received daily s.c. hormone replacement (corticosterone, 8 mg/kg; deoxycorticosterone, 1 mg/kg in oil) or vehicle until 45 days old, after which conditions for some remained the same or, for others, hormonal conditions were reversed for another 20 days. Other animals were sacrificed at 45 days, and the remainder at 65 days of age. Perfused brains were removed and coded for "blind" weight determinations.

Brains of animals which received vehicle throughout the 40 day period were 12% heavier than those of rats receiving hormones during the same period. Of this differential growth, about 70% was accomplished in the second 20 day phase. Withdrawal of hormones during the first 20 days increased brain weight by only about 3.6% when sampled at the end of that phase. We conclude that for a time immediately following weaning, and perhaps before, the rat brain is relatively resistant to the atrophic action of corticosteroids, this resistance being lost with the approach of adulthood. Apart from this, evidence of reversibility of brain growth changes was also found.

133.1 INTRACEREBRAL INJECTIONS OF NERVE GROWTH FACTOR ALTER GLIAL RESPONSE TO BRAIN INJURY. D. G. Stein, A. C. Firl* and C. Craig*. Brain Research Lab., Clark Univ., and U. Mass. Medical Center. Worcester, MA 01610.

In a previous experiment, we noted that single injections of NGF facilitate behavioral recovery from caudate nucleus (CN) lesions. An analysis of the brain sections (6 months post lesion) revealed that the NGF treated rats had fewer glial cells in the area of the lesion than the control group. Because of the possibility that glial cells might play a role in mediating behavioral recovery after brain damage, we have examined the time-course and extent of glial reaction to brain injury after NGF treatment.

Bilateral, radiofrequency lesions were placed in the anterior CN of mature, albino rats. Immediately after, the rats received unilateral intracaudate injection of 2.5S NGF and, in the contralateral CN, an injection of saline solution. Following surgery and injections, groups of 4-5 rats were allowed to survive for 0, 10, 20, 30 and 60 days. Their brains were subsequently prepared for frozen sectioning, cut at 30 microns and then stained with the Cajal gold-sublimate method for reactive astrocytes. Astrocytes were counted in the lesion area and in the corpus collosum overlying the lesion area. Also, measurements of astrocyte size were taken by counting number of processes. Our preliminary analysis of size was not significant for any of the postoperative survival times, despite the fact that the NGF treated CN tended to have larger astrocytes in rats that survived 30-60 days after treatment. The measurements of astrocyte numbers did reveal effects--no differences between the saline and NGF-treated side of the brain up to 20 days post-operatively, but in those rats that survived 30 and 60 days, the CN treated with NGF had significantly more reactive astrocytes than the saline-treated side in the 2 areas we examined. Finally, as a control measure, 10 rats divided into groups with the same survival times as above, were given NGF on one side of the brain and saline on the other, but no lesions were inflicted. For all groups, analysis revealed no significant differences between the two sides of the brain in number and size of astrocytes.

It appears, therefore, that a single injection of NGF stimulates the reaction of astrocytes to brain injury but does not enhance glial proliferation in the absence of trauma. This response, however, was apparent only after 30 days following experimental treatment.

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133.2 ONTOGENESIS OF NERVE GROWTH FACTOR (NGF) AND EPIDERMAL GROWTH FACTOR (EGF) IN MALE MOUSE SUBMAXILLARY GLAND (SMG). P. Walker*, M.E. Weichsel, Jr., D. Eveleth*, and D.A. Fisher* Fetal-Maternal Res. Lab., Harbor-UCLA Medical Center, Torrance, CA 90509

NGF and EGF are found in highest concentrations in adult male mouse SMG. Little is known, however, of the postnatal ontogenetic pattern of accumulation of the two proteins. Using sensitive and specific radioimmunoassay (RIA) techniques, we measured SMG NGF and EGF concentration in SMG of 2,5,8,11,17,18,21,28,32,40 and 60 day old male Swiss-Webster mice. RIA sensitivities were 13 pg/tube and 21 pg/tube for NGF and EGF respectively. SMG extracts exhibited parallel displacement in both RIA systems and there was no significant displacement of radiolabeled NGF by unlabeled EGF or radiolabeled EGF by unlabeled NGF. SMG NGF and EGF content decreased from ages 2 to 8 days, the half-lives of disappearance being approximately 8 days and 5 days respectively. Mean EGF concentrations exceeded NGF concentrations from 2 through 8 days of age. SMG NGF and EGF concentrations increased exponentially after 11 days of age with 3 phases of increase being apparent for both proteins. Mean SMG NGF concentration increased 543 fold between 11 and 21 days of age from 0.19 $\pm$ 0.02 ng/mg protein to 0.1 $\pm$ 0.05 µg/mg protein, the greatest increase being observed between 18 and 21 days of age where NGF concentrations increased 314 fold. Mean EGF concentration also increased markedly from 0.24 $\pm$ 0.01 ng/mg protein at 11 days to 0.09 $\pm$ 0.005 µg/mg protein at 21 days, a 368 fold increase. Again, the greatest increase (218 fold) was observed between 18 and 21 days of age. The second phase of increase was noted between 21 and 32 days where NGF and EGF concentrations increased 26 and 28 fold respectively. In the third phase, (32-60 days of age), increases in mean SMG NGF (7.6 fold) and EGF (8.1 fold) concentrations were noted. Mean NGF and EGF concentrations were similar at all ages after 21 days. Conclusions: The postnatal period is characterized by three phases of increase in SMG NGF and EGF concentrations. Initially (11-21 days) NGF increases to a 50% greater extent than EGF. Since we have noted that thyroxine (T4) administered to adult female mice elevates SMG NGF to levels of 50% greater than EGF (unpublished), it is possible that this initial pronounced increase is thyroid hormone mediated. Whereas the factor(s) responsible for the second phase of increase between 21 and 32 days of age (to levels comparable to those in the adult female) cannot be explained, the third phase of increase between 32 and 60 days is most likely androgen dependent. Thus, the control mechanisms responsible for the observed developmental pattern of SMG and EGF appear to be similar, although the predominant factor(s) during early postnatal life may be different from those operative in mature animals.

133.3 A NEUROTROPHIC EFFECT UPON THE PHOSPHORYLATION OF CYTOSOLIC PROTEIN IN SKELETAL MUSCLE. S.P. Squinto*, J.A. McLane, H.C.* Yeoh and I.R. Held. Loyola Univ. Med. Ctr. and VA Hospital, Hines, IL 60141.

Many physiological responses induced by extracellular stimuli mediated by such diverse substances as hormones, neurotransmitters and perhaps neurotrophic factors may commonly act through phosphorylation of endogenous protein substrates by cyclic AMP dependent protein kinases. In an earlier study (Trans. Am. Soc. Neurochem. 11:118, 1980), we found that when the transmission of nerve impulses and unidentified neurotrophic factors to skeletal muscle are disrupted by denervation, the in vitro phosphorylation of a 40,000 dalton polypeptide from cytosolic fractions of the denervated muscle is increased. In this investigation, we have extended this finding by evaluating the endogenous phosphorylating activity of cytosolic fractions (105,000g supernatants) from rat soleus muscles which were denervated for 1,3,12,24,36,48,54, 60,66 and 72 hr by cutting the left sciatic nerve either at a low level of 2 mm or less, at a mid level of 17-20 mm or at a high level of 32-35 mm before its insertion into the muscle. When the nerve was cut distally at either the low or mid levels, a proximal cut was also made at the high level. Control samples were obtained from sham-operated, contralateral muscles. ^{32}Cytosolic phosphorylating activity was determined as fmoles ^{32}P incorporated into TCA-precipitable material/mg protein after incubation of 30-50 µg supernatant protein for 5 min in a phosphorylating media (pH 7.5) containing 2mM $MgCl_2$ and [γ-^{32}P] ATP (3000 Ci/mmole). We found that the cytosolic phosphorylating activity was similarly increased 75-100% whether the nerve was cut at the low, mid or high levels and remained significantly stimulated for as long as 12 hr. There was a marked difference, however, in the length of time after cutting the nerve at these three levels before the period of increased cytosolic phosphorylating activity was seen. With the shortest possible nerve stump of 2 mm or less the cytosolic phosphorylating activity was significantly increased at the 12 and 24 hr denervation periods. Marked increases were not seen, however, until 48 and 66 hr with the mid and high nerve stumps, respectively. The results of this investigation demonstrate that the cytosolic phosphorylating activity may be dependent upon a temporal alteration in the supply of axonally transported neurotrophic factors and not solely related to the contractile activity of the muscle.

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133.4 NEURONAL CONTROL OF METABOLIC TURNOVER OF JUNCTIONAL ACETYLCHOLINE RECEPTORS. R.H. Loring, T.A. Levitt* &M.M. Salpeter Lab. of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

After denervation the junctional acetylcholine receptor turnover rate increases while the average receptor site density does not change (Loring and Salpeter, PNAS, April 1980). We report here (using gamma counting after labelling with ^{125}I-α-bungarotoxin) that this increased receptor turnover rate is time-dependent and that by fifteen days the half-life of the denervated junctional receptors reaches thirty hours. We found however, that the acetylcholine receptors which are present at the junction prior to denervation increase their turnover rate only slightly over a fifteen day post-denervation period. Calculations show that these data are compatible with the hypothesis that there is a composite population of receptors at the denervated junction, and that as the original junctional acetylcholine receptors are degraded they are replaced by receptors with a half-life of about one day. EM autoradiographic analyses indicate that over this period there is no shift in the relative distribution of acetylcholine receptors between the tops and bottoms of the junctional folds. Thus, after denervation the junctional acetylcholine receptors are slowly replaced with metabolically unstable receptors, while their anatomical stability, as reflected by high density clustering on the specialized region at the tops of the junctional folds, remains.

133.5 EXTRAJUNCTIONAL MEMBRANE PROPERTIES OF DENERVATED SKELETAL MUSCLES REINNERVATED BY INTACT AND IMPULSE-BLOCKED NERVES. A.Cangiano, L.Lutzemberger* and P.C.Magherini*.Istituto di Fisiologia, Università di Pisa,56100,Italy.

Impulse activity, nerve borne "neurotrophic" factors, the neurotransmitter acetylcholine have all been proposed as mechanisms of regulation of the extrajunctional membrane properties of skeletal muscles. Part or all of these factors might be responsible for the return to normal of the extrajunctional membrane properties of skeletal muscles upon reinnervation. If impulse conduction is blocked in the rigenerating nerves, reinnervation cannot restore muscle activity and the possible normalizing action of nerve-borne chemical factors can be studied in isolation. For these purpose we have crushed in rats the nerves to the soleus and the EDL muscles of one side at a few mm of distance from the muscles without exposing their surface. The muscles of the contralateral side were denervated and reinnervation prevented by removal of a sciatic nerve segment. Eight days later, that is when reinnervation is beginning, a chronic blockade of nerve impulses of the sciatic nerve was established on the crushed side by implanting a tetrodotoxin impregnated silastic cuff. In a control group of rats with crushed nerves, cuffs were not implanted. After 9-11 days of chronic conduction block (i.e., 17-19 days since the initial crush) EDL and soleus muscles of the reinnervated and the denervated sides were examined in vitro for the resting membrane potential (RMP) and the extrajunctional resistance of the action potential to tetrodotoxin (TTX). In control muscles reinnervated by intact nerves,the TTX resistance characteristic of the denervated muscles had already practically disappeared whereas in the majority of the muscles reinnervated with blocked nerves it had retained high values comparable to those of the contralateral denervated muscles. Similarly, the RMP of the reinnervated paralized muscles had the same low values of the denervated muscles. Furthermore, no significant correlation was observed between the amount of TTX resistance measured in each reinnervated paralyzed fiber and the frequency of its miniature end-plate potentials (which is related to the age of the synaptic contact). Thus, muscle activity appears to be the principal factor that induces the return to normal of the extrajunctional membrane properties of denervated muscles upon reinnervation. Occasional failure of the long-lasting conduction block appears therefore to be the most likely explanation for the moderately lower TTX resistance observed in a minor number of reinnervated muscles in respect to their denervated counterparts, although the contribution of nerve-borne chemical factors cannot be entirely excluded. (supported by grants from the Muscular Dystrophy Association of America and the Consiglio Nazionale delle Ricerche of Italy).

133.6 THE ROLE OF ACH TRANSMISSION IN THE NEURAL REGULATION OF MUSCLE RESTING MEMBRANE POTENTIAL. E.F. Stanley, D.B. Drachman,* A. Pestronk,* D.L. Price*. Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

An intact motor nerve is necessary for the maintenance of many muscle properties. Elimination of the nerve's influence by surgical denervation results in changes in the morphological, physiological and biochemical properties of muscle fibers. However, the mechanisms by which the nerve regulates muscle properties are not clearly understood.

In this study we have examined the role of cholinergic transmission in the regulation of one muscle property, the resting membrane potential (RMP). A fall in the RMP is one of the first changes to occur in muscle following denervation. We have compared in vivo the effect of denervation with that of complete blockade of ACh transmission.

The soleus muscle of the rat was denervated by cutting the soleus nerve close to its point of entry into the muscle. Neuromuscular blockade was achieved by injecting 8 µg of purified α-bungarotoxin (α-BuTx) in Ringer solution directly into the muscle. In order to maintain an effective post-synaptic blockade, α-BuTx was infused into the muscle at a rate of 0.4 ug/hr by means of an implanted osmotic pump. RMPs were recorded from the soleus muscle in vivo after 6 to 48 hours of treatment.

The RMP began to fall 18 hours following denervation, as reported previously with in vivo recording (Stanley and Drachman, Exp. Neurol., in press). This fall reached a plateau of -65 mV by 42 hours. With α-BuTx treatment the onset, time course and extent of the fall in RMP were identical to the changes observed following denervation.

In order to exclude the possibility that α-BuTx also damages the nerve terminals, the muscles were examined by light and electron microscopy. Following 48 hours of α-BuTx treatment there were no changes in the structure of nerve terminals whereas denervation resulted in marked degeneration.

The effect of α-BuTx treatment on the RMP was identical to that of denervation. The only known action of α-BuTx at the neuromuscular junction is the blockade of ACh receptors. Cholinergic blockade is therefore equivalent to denervation and it is unnecessary to hypothesize that denervation involves the interruption of other neuroregulatory factors. Hence it is likely that the motor nerve normally regulates the RMP through ACh neurotransmission.

133.7 ACCELERATED DEGRADATION OF JUNCTIONAL α-BUNGAROTOXIN-ACETYLCHOLINE RECEPTOR COMPLEXES IN DENERVATED RAT DIAPHRAGM. R.S. Brett, S.G. Younkin, M. Konieczkowski* and R.M. Slugg*. Dept. of Pharmacol., Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

We labelled innervated and 5-day denervated hemidiaphragms in vivo with ^{125}I-α-bungarotoxin (BTX) and measured toxin binding 5 days later. Denervated hemidiaphragms contained only 17% of the BTX found in innervated hemidiaphragms. Because of the rapid degradation of extrajunctional toxin-receptor complexes essentially all of the toxin remaining 5 days after labelling is bound to junctional AChR. The decrease in junctional toxin binding seen could occur if denervation (1) decreases the number of junctional AChR, (2) interferes with the in vivo labelling of AChR or (3) accelerates the degradation of junctional toxin-receptor complexes. To evaluate junctional AChR in denervated muscle we microdissected AChE-stained endplates (mean length = 80 µM) from single fibers labelled with BTX. Innervated diaphragm labelled in vitro under saturating conditions bound 1.51 x 10^{-17} M BTX per endplate and 5-day denervated muscle bound 1.85 x 10^{-17} M per endplate, so denervation for 5 days did not reduce the number of junctional toxin binding sites. One day after labelling in vivo innervated muscle bound 0.44 x 10^{-17} M BTX per endplate whereas 5-day denervated muscle bound 0.23 x 10^{-17} M per endplate, so denervation appears to interfere with the in vivo labelling of junctional AChR. One day after in vivo labelling, 5-day denervated muscle contained 52% of the junctional toxin-receptor complexes found in innervated muscle. By 5 days after labelling, denervated muscle contained only 17% of the junctional toxin-receptor complexes found in innervated muscle. This suggests that junctional toxin-receptor complexes are degraded more rapidly in denervated muscle. To confirm this, innervated and 5-day denervated rat hemidiaphragms were labelled in vivo and toxin binding was examined 1,3,5, and 8 days after labelling. After correction for toxin binding to extrajunctional AChR we found that the loss of junctional toxin-receptor complexes in denervated muscle (t 1/2 = 2.0 days) was significantly faster than that in innervated muscle (t 1/2 = 10.7 days). In a separate experiment we labelled AChR in vivo immediately after denervation. This procedure was advantageous because it avoided both the denervation-induced interference with toxin binding and the need to correct for toxin binding to extrajunctional AChR. In this experiment the degradation of junctional toxin-receptor complexes in denervated left hemidiaphragms was apparently normal for three days and then increased significantly (t 1/2 = 3.7 days, 3-13 days after denervation). Taken together these results support the concept that continuous innervation is needed to maintain the stability of junctional AChR.

133.8 COMPARATIVE CHANGES IN THE MOLECULAR FORMS OF ACETYLCHOLINESTERASE DURING POSTNATAL DEVELOPMENT IN SOLEUS AND EXTENSOR DIGITORUM LONGUS MUSCLES FROM RAT. Douglas E. Groswald* and Wolf-D. Dettbarn. Dept. of Pharmacology, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232.

Previous studies have shown that acetylcholinesterase (AChE, EC 3.1.1.7) from various mammalian tissues exists in a number of molecular forms. These molecules, which may be characterized by their approximate sedimentation coefficients in sucrose gradients as 4s, 10s and 16s are present in skeletal muscle and the 16s form appeared to be associated with the muscle endplate.

It is generally accepted that physiological and biochemical characteristics of fast twitch and slow twitch muscles are different. However, nothing is known about differences in regard to AChE in these two muscle types.

The present study demonstrates the existence of a different molecular form of AChE between a slow tonic (Soleus-SOL) and a fast phasic (Extensor digitorum longus-EDL) muscle during postnatal development in the rat. Total AChE activity rapidly increases in both muscle types during the first two weeks postnatally and then decreases to adult levels by six weeks postnatally. A correspondingly rapid increase is seen in the larger molecular form (16s) of AChE in both muscle types, but in addition to the intermediate molecular form (11s) in the EDL and SOL muscles, a heavy intermediate (13s) appears in the soleus by two weeks postnatally and increases to its adult level by 10 weeks postnatally. As the molecular forms (11s and 4s) decrease in the soleus the 13s molecular form increases. The smaller molecular form (4s) which remains elevated in the EDL progressively decreases in the soleus. Decreases are evident in all the molecular forms, as total AChE activity decreases during development, but in a qualitatively different manner. These findings suggest that the specific cholinesterases in fast and slow muscle types may be under different regulatory mechanisms.

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133.9 COLCHICINE CONTAINING SILASTIC NERVE CUFFS ACT UPON THE NERVE TRUNK AND NOT THE TONGUE TO ELIMINATE TASTE RESPONSES AND TASTE BUDS. H. Sloan*, L. B. Jones* and B. Oakley (SPON: R. E. Davis) Div. Biol. Sci., Neuroscience Lab. Bldg., Univ. of Mich., Ann Arbor, MI 48109.

The effects of blocked axonal transport upon fungiform taste buds of the Mongolian gerbil were evaluated by placing silastic cuffs, containing 0-1% colchicine, around the combined lingual-chorda tympani nerve. Electrophysiological recordings were made from the chorda tympani proper in response to taste solutions applied to the tongue. Eight to fifteen days after cuff implantation ipsilateral taste responses were reduced or eliminated and fungiform taste buds were partially or wholly degenerated. Normal taste responses and taste buds were observed contralaterally to the cuffed nerve and with cuffs lacking colchicine. To determine whether the colchicine was acting at the level of the nerve trunk or the tongue, 1% (w/v) colchicine cuffs containing 0.79 μCi-7.86 μCi ^{3}H-colchicine (specific activity 10.1 Ci/mmole) were used. Liquid scintillation counting of aliquots of solubilized left and right halves of the tongue revealed closely similar colchicine levels. We conclude that colchicine acted at the level of the nerve trunk and not the tongue to eliminate ipsilateral chorda tympani taste responses and fungiform taste buds. Supported in part by NIH Grant NS-07072.

133.10 SPINAL CORD TRANSECTION INTERFERES WITH NORMAL DEVELOPMENT OF SYMPATHETIC PREGANGLIONIC NEURONS. Leonard L. Ross, Arnold J. Smolen and Joan Cherry*. (SPON: L. Pubols). Department of Anatomy, The Medical College of Pennsylvania, Phila., PA 19129.

The formation of a normal complement of synapses in a peripheral autonomic ganglion is dependent on the presence of an intact afferent input to the preganglionic neurons innervating the ganglion. Following neonatal spinal cord transection there is a marked reduction (>60%) in the number of ganglionic synapses formed, as demonstrated by biochemical studies in the rat (Hamill, Bloom and Black, 1977) or morphometric studies in the chick (Ross and Cosio, 1979). And, in the chick, this reduction in peripheral synapses is not accompanied by any loss of cells in the spinal cord preganglionic nucleus.

However, preganglionic neurons probably represent only 25% of the cell population of the nucleus (Caserta, Johnson and Ross, 1977). Therefore, the aim of the present study was to examine the effect of spinal cord transection on the sympathetic preganglionic neurons specifically innervating the adrenal medulla of the rat. The spinal cords of three-day old rats were transected between T5 and T7 and the animals were maintained until 16 or 24 days of age. At these times, the left adrenal was injected with horseradish peroxidase (HRP, 30% in saline). Following a 48-hour survival, the animals were sacrificed, perfusion-fixed and the spinal cords were cut serially at 50 microns. HRP reaction product was demonstrated in every fifth section between T9 and T11 using tetramethyl benzidine. The number of labeled cells were counted and compared with control animals of the same ages. These three segments were chosen because they have been shown to provide the major preganglionic input to the adrenal medulla (Ross, Glazer and Sparling, 1979).

In the spinal cord-transected animals, the number of retrogradely labeled neurons was reduced by 75% when compared with controls of the same age. Since transection does not appear to affect the number of neurons in the preganglionic nucleus, the most likely explanation for these results is an impairment of the cells' ability either to transport or to take-up and transport HRP. Thus, the reduction in the number of labeled cells could indicate that most preganglionic neurons fail to form an adequate number of synapses in the target organ or could be indicative of a general metabolic effect as a result of the transection.

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133.11 AXON TRANSPORT INHIBITION IN THE DEVELOPING VISUAL SYSTEM. M.A.Matthews, W.J.Cornell*, D.B.Clarkson*, L.West*, T.Alchediak* and D. Siegenthaler-Matthews*. Dept. of Anat., LSU Med. Ctr., New Orleans, La. 70119.

Establishment and maintenance of neuronal differentiation requires synchronized formation of appropriate synaptic arrays by which a physiological interdependence among functionally-related neuronal groups is created. The importance of proper synaptic input has been emphasized by studies in which surgical destruction or functional deprivation in primary afferent pathways results in retardation of growth patterns in neurons of secondary receiving centers. It has been proposed that "trophic" factors, carried by axon transport mechanisms, may mediate this long-term metabolic influence. Therefore, the developing visual system of the rat has been employed as a model to examine the effects of intraocular administration of axon transport inhibitors upon the differentiation of neurons in the dorsal lateral geniculate nucleus and superior colliculus.

Colchicine or anisomycin, (a protein synthesis inhibitor), was injected into the posterior chamber of rats ranging in age from neonate through 20 days postnatal and in doses ranging from 10^{-2}M – 10^{-5}M. EM analysis of the retina revealed swelling or dissolution of the lamellar plates of photoreceptors, a decrease in thickness of the plexiform layers, the appearance of cytoplasmic vacuoles, dilation of the endoplasmic reticulum and minimal ribosomal degranulation in ganglion cells, although quantitative analysis of these cells demonstrated no abnormal reduction in population density. Axon transport rates were determined by liquid scintillation spectroscopy following intraocular injection of ^{3}H Proline or Fucose 24 hours after colchicine injection. Fast and slow components of transport were inhibited in relation to dosage and the maturity of the animals. The optic nerve leading from the injected eye was reduced in diameter but EM studies revealed no evidence of axon degeneration.

Golgi analysis of relay neurons within the dorsal lateral geniculate nucleus revealed alterations in branching patterns as shown by Sholl methodology as well as a diminution in complexity of the post-synaptic component of the glomerular synaptic terminal of retinofugal fibers. A correlated EM study showed vesicular enlargement together with a reduction in size of these terminals but no evidence of degeneration. These findings support the efficacy of a deprivation model designed to address the role of axon transport in the maturation of neurons in functionally related centers.

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133.12 TROPHIC INTERACTIONS OF CRAYFISH GIANT AXONS.
Robert M. Grossfeld, George D. Bittner, Martis L. Ballinger*, and Terry A. Viancour. Department of Zoology, University of Texas, Austin, TX 78712 and Department of Biology, North Carolina State University, Raleigh, NC 27650.

The severed stumps of crayfish medial giant axons (MGAs) usually remain morphologically intact and functionally competent for 4-6 months. This survival is associated with hypertrophy and hyperplasia of glial sheath cells. Compared to intact axons, the sheaths of severed MGAs incorporate more labeled amino acid acids into proteins and the axoplasm contains more labeled protein (Meyer and Bittner, Br. Res. 143:145-211,212-232,1978; Ballinger and Bittner, Cell Tis. Res. in press,1980). We now report that both the ultrastructural appearance and the putative transfer of labeled proteins is not uniform along the entire length of a severed MGA. For example, labeled proteins often accumulate in the sheath or axoplasm near adaxonal glial cells. Furthermore, Lucifer yellow CH appears in all glial cells of the adaxonal layer within a few minutes after intracellular injection into an MGA. All these data suggest that glial cells and MGAs mutually exchange many substances, perhaps by exocytosis-pinocytosis of plasmelemma vesicles. However, we find that a decrease in extracellular [Ca^{++}] does not block the putative transfer, as reported in squid giant axons (Lasek et al, J. Cell Biol. 74:501-523,1977).

The severed stumps of crayfish lateral giant axons (LGAs) often survive for over 12 months with little or no change in axoplasmic or sheath morphology. However, LGA segments degenerate within one week if separated from both their own cell body and from LGAs in adjacent segments. After lesioning, the gap junctions largely disappear between adjacent LGAs, the coupling resistance between adjacent LGAs dramatically increases, but many 20-40 nm vesicles remain in the region of apposition between adjacent LGAs (Bittner et al. JEZ 189:13-36,1974; Anderson and Bittner, Br. Res. 184:224-228, 1980; Bittner and Ballinger, Cell Tis. Res, in press,1980). We now report that an entire set of adjacent LGAs can survive for many months if only one LGA remains connected to its cell body. Furthermore, horseradish peroxidase stain injected into one LGA appears in the vesicles and cytoplasm of adjacent LGAs within 30 minutes. All these data suggest that adjacent LGAs mutually exchange many substances, perhaps by exocytosis-pinocytosis of cytoplasmic vesicles which release or obtain their contents from the extracellular space. In fact, such a process may be a ubiquitous mechanism for trophic exchanges between various cell types in many organisms (Bittner, p. 507-532, Trophic interactions of crustacean neurons in Identified Neurons and Behavior, G. Hoyle, Ed, 1977). (Supported by an NIH RCDA 00070 and NINCHDS research grant to GDB).

134.1 ULTRASTRUCTURE OF REGENERATING CENTRAL NERVOUS CONNECTIONS AND IMPLANTED GANGLIA IN THE SNAIL MELAMPUS. Stacia Moffett. Dept. of Zoology, Washington State University, Pullman, WA 99164.

In the pulmonate snail Melampus behavioral and physiological studies have shown that neuronal circuitry necessary for restoring normal reflex responses is formed following transection of the cerebral commissure or removal of one cerebral ganglion (Moffett, Neurosci. Abs. 5, 254, 1979). Regenerating cerebral commissures have been examined eight days, two weeks, one month and four months postoperative and compared with cerebral commissures of control animals. In early stages of regeneration the commissure is composed of a relatively small number of large and medium sized axons (.1 micron up to 2 microns) very loosely associated with one another. In more advanced stages there is a progressive tendency for axons to be grouped together in bundles and smaller diameter axons (less than .1 micron) become increasingly common in the commissure profile. The cerebral ganglia of snails in early stages of commissure regeneration contain patches of degenerating tissue in close proximity to normal tissue. The rough endoplasmic reticulum is particularly well developed in many cells.

After the removal of one cerebral ganglion the nerves, connectives and commissure that were associated with it grow together and form an enlargement, the ganglion bud (Price, Cell Tiss. Res. 180, 529-36, 1977). The ultrastructure of the neuropile within this bud is indistinguishable from neuropile of control ganglia, except that axon tracts are lacking. The sheath covering the bud contains dense droplets characteristic of normal ganglion sheaths and neurosecretory release sites are present. In the margin of the ganglion buds a few neuron cell bodies have tentatively been identified, but because the distinction between glial cells and neurons is based primarily on the morphology of the nuclei, the presence of neurons in the ganglion buds is inadequately substantiated to date.

Cerebral ganglia implanted in host snails form connections with the host snail's nervous system and with peripheral structures. The connections have not been evaluated functionally but the ultrastructure of the cell bodies and neuropile of ganglia cultured two months indicates that the implants are viable. The gross appearance of ganglia cultured in vitro for two years is also normal.

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134.2 AXOTOMY INDUCES OUTGROWTH FROM CELL BODIES OF GIANT INTERNEURONS. E. Roederer* and M. J. Cohen. Dept. of Biol., Yale Univ., New Haven, CT 06511.

An insect central neuron characteristically possesses a rounded cell body from which a single initial process emerges to form both the dendritic tree and the axon. We report here that axotomy induces a proliferation of neurite-like outgrowths from cell bodies of identified central interneurons in the cricket Acheta domesticus. Approximately 40 days after transecting their axons in the abdominal nerve cord, the cell bodies of 7 identified giant interneurons in the terminal abdominal ganglion begin to form at least one additional large neurite that emerges opposite the initial process. These cytoplasmic extensions are irregular in shape and may reach lengths of 100µm. Homologous cell bodies of contralateral intact interneurons in the same ganglion appear normal. Intracellular recording indicates that the soma of the normal medial giant interneuron (MGI) does not support regenerative action potentials. The electrical properties of the soma membrane in the axotomized MGI do not seem substantially altered at 40 days after transection.

Sprouts emerge from the proximal axonal stumps of these interneurons as early as 2 days after transection and continue to grow for only 2-3 weeks. These sprouts never cross the site of transection into the distal stump of the sectioned connective. They follow an irregular path and each sprout ends in a terminal swelling that contributes to a neuroma formed at the proximal stump. Only well after the axonal sprouting stops does the outgrowth of neurites from the cell body of the same neuron begin.

Interrupting the giant axons by crushing a connective leads to axonal regeneration from the proximal stump across the lesion into the distal stump of the neural connective. This regeneration may continue through several abdominal ganglia. Cell bodies of regenerating interneurons whose axons have been crushed do not show the neurite-like outgrowth that is seen following axonal transection.

Cutting or crushing the axons do not modify the primary dendritic morphology of these giant interneurons as seen in whole mounts intracellularly stained with Lucifer Yellow or horseradish peroxidase. Intracellular recording from the medial and lateral giant interneurons indicates that synaptic input from cercal receptors is not obviously modified up to 72 days after axotomy.

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134.3 ANATOMICAL REGENERATION AND FUNCTIONAL RECONNECTION OF AN IDENTIFIED NEURON IN THE CNS OF A VERTEBRATE. Matt T. Lee. Biology Dept., UCSD, La Jolla, CA 92093.

The Mauthner (M) neurons are paired cells located in the medulla of fish and amphibians. Each cell gives rise to an axon that crosses the midline in the medulla and runs caudally in the ventral spinal cord. The large size, characteristic location, and morphology of the M cells make them readily identifiable. Consequently, they provide a potential system for the study of axonal regeneration and the re-establishment of functional synaptic contacts by an individual vertebrate central neuron.

In tadpoles of the African clawed frog, Xenopus laevis, the two M axons are clearly visible in the isolated brain and spinal cord, when examined with interference contrast optics. Each axon can be impaled with a microelectrode for intracellular stimulation, recording, and dye injection. In normal tadpoles at Nieuwkoop & Faber stages 49-54, current injected into the M axon produces a spike in that axon, followed by a short-latency compound action potential recorded from ipsilateral ventral roots. Intracellular recording from individual motor axons in these roots reveals a postsynaptic potential (psp) and spike following M excitation. The brief latency of the psp indicates a monosynaptic connection between M axon and motor neuron.

The spinal cords of tadpoles at stages 46-49 were transected completely at the level of the 5th myotome. At various times thereafter, the CNS was isolated and the fluorescent dye, Lucifer Yellow, was injected into each M axon. By 2 days following transection, fine processes can be seen emerging from the end of the proximal segment of the axon, which has usually retracted 1-3 segments from the lesion site. The first sprouts to cross the lesion can be seen within one week in some animals. Tadpoles examined at later times (3½-6 weeks) may have axonal sprouts that extend for 10 or more segments caudal to the lesion; this dye spread is comparable to that seen in M axons of normal animals. However, regenerating axons are often morphologically abnormal; they may branch at the lesion, and branches may run rostrally or caudally on either side of the cord.

By 3½ weeks after cord transection, firing of the M axon rostral to the lesion often elicits spikes in ipsilateral ventral roots caudal to the lesion. These spikes have a latency close to that observed in normal animals, and have been recorded as much as 10 segments caudal to the lesion. These results indicate that regenerating M axons are capable of re-forming functional synaptic contacts with their normal postsynaptic target cells. Occasionally, a regenerating axon will cross over to the opposite side of the cord at the lesion and synapse with motor neurons on that side. Thus, there is no apparent left-right specificity concerning trunk motor neurons as targets for these regenerating axons.

134.4 INCREASED ACTIVITY OF ORNITHINE DECARBOXYLASE FOLLOWING OPTIC NERVE CRUSH IN THE GOLDFISH. Shinichi Kohsaka* and Bernard W. Agranoff. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

We have previously demonstrated an increase in tubulin mRNA and tubulin labeling, as well as the activities of a number of enzymes of RNA precursor metabolism in the goldfish retina after its optic nerve had been crushed. Since it is generally believed that polyamines play an important role in RNA metabolism, we investigated effects of unilateral nerve crush on the activity of ornithine decarboxylase (ODC), a rate-limiting enzyme of polyamine synthesis.

ODC levels were measured in 6-7 cm goldfish at various times following optic nerve crush. Unlike the completely unilateral nature of biochemical alterations previously seen, ODC was increased in both retinas, although the activity was significantly higher on the side of the crush. Measurable increases in retinal ODC were seen 3 days following crush, peaked by day 5, and had returned to control values by day 7. ODC was also increased in brain and kidney. Various other treatments such as sham operation and behavioral stress secondary to electrical shock produced relatively minor increases in ODC compared with that seen following nerve crush. Unilateral eye enucleation resulted in increased ODC in the remaining eye and in the brain similar to that seen following nerve crush. A related study (Schwartz, Kohsaka and Agranoff, abstracts in this meeting) reveals that the addition of mouse ß-NGF to retinal explants leads to increased ODC activity in vitro.

The various results suggest that both local and humoral factors may participate in the retinal response to axotomy.

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134.5 RETINAL GANGLION CELL RESPONSE TO AXOTOMY AND NERVE GROWTH FACTOR ANTISERUM TREATMENT IN THE REGENERATING VISUAL SYSTEM OF THE GOLDFISH: AN IN VIVO AND IN VITRO ANALYSIS. J. E. Turner, R. K. Delaney* and J. E. Johnson. Dept. of Anatomy, Bowman Gray School of Med., Wake Forest Univ., Winston-Salem, NC 27103.

In vitro nerve growth factor (NGF) antiserum (anti-NGF) treatment was found to severely inhibit retinal ganglion cell neurite outgrowth in goldfish explant culture. Goldfish retinas, conditioned by a 14-day prior optic nerve crush, demonstrated a significant dose response inhibition of neurite outgrowth (i.e., up to a 50% reduction) if incubated with various concentrations of the antiserum (i.e., concentrations from full strength to 1:100 dilutions) before explantation for tissue culture. NGF (100 ng/ml) added to the culture medium containing antiserum (1:1.5 dilution) eliminated the inhibition of neurite outgrowth during the first four days of explant culture. Antiserum treatment at the higher concentrations (i.e., full strength and 1:1.5 dilution) caused a cessation of nerve growth from explants between culture days 3 and 4. However, preimmune serum controls at this time still exhibited vigorous neurite outgrowth.

In vivo treatment with anti-NGF administered intraocularly at 7 DPA) was found to reduce significantly the size and complexity of the retinal ganglion cell nucleoli when analyzed morphometrically at 14 DPA. No other cell parameters measured were found to be affected by the single antiserum treatment.

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134.6 A PREPARATION OF DISSOCIATED ADULT GOLDFISH RETINAL CELLS CAPABLE OF NEURITE OUTGROWTH IN CULTURE. James E. Johnson, James E. Turner and Rebecca K. Delaney*. Dept. of Anatomy, Bowman Gray Sch. of Med. of Wake Forest Univ., Winston-Salem, NC 27103.

The goldfish visual system represents a model of successful vertebrate central nervous system (CNS) regeneration. Retinal ganglion cells are capable of establishing functional connections with the optic tectum following optic nerve axotomy (Attardi and Sperry, Exp. Neurol., 7, 1963). Recent studies suggest that nerve growth factor (NGF) may effect the retinal ganglion cell response to axotomy in both the newt (Turner, Brain Res., 171, 1979) and in the goldfish (Turner, Brain Res., in press). We are reporting the development of cultures prepared from dissociated goldfish retinas as a model for further studies of neurotrophic phenomena.

Retinas from common goldfish (Carassius auratus) which had received either (1) an optic nerve crush 7, 14 or 21 days prior to placement in culture or (2) no prior lesion, were dissociated in 0.05% trypsin (Sigma). Following trypsinization, two million cells were seeded on poly-L-lysine (type VI B, Sigma) coated 35mm culture dishes in a Leibovitz (L-15, GIBCO) nutrient medium supplemented with 10% fetal calf serum (FCS, GIBCO), 20mM HEPES (pH 7.2, Sigma) and gentamycin sulfate (0.1 mg/ml, Sigma).

Highly refractile cells with neurite outgrowth were counted and examined by phase contrast, light and transmission electron microscopy. Preliminary results indicate that a population of cells dissociated from the goldfish retina were capable of attachment and extensive neurite outgrowth. Outgrowth from several large refractile cells were often found to form fascicles. Ultrastructural examinations of these cells and their processes also revealed the presence of microtubular complexes and organelle characteristics which are indicative of neurons. After three weeks in culture, retinas treated with an optic nerve crush 14 days prior to dissociation exhibited a 14-fold increase in the number of phase bright cells with neurite outgrowth over preparations which received no prior lesion. These results indicate that the administration of a prior optic nerve crush facilitates a significantly greater neurite outgrowth in dissociated cultures.

A dissociated cell population established from an adult vertebrate CNS tissue has demonstrated the capability for neurite outgrowth in culture. This in vitro preparation, represents a model for the analysis of possible neurotrophic phenonmena in successful CNS regeneration.

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134.7 INCREASED RATE OF REGENERATION IN GOLDFISH OPTIC AXONS RESULTING FROM INTRAOCULAR INJECTION OF CALCIUM IONOPHORE A-21387. Bernice Grafstein and Hamutal Meiri*. Department of Physiology, Cornell University Medical College, New York, NY 10021

At three and five days after the goldfish optic nerve had been crushed in the orbit, 3×10^{-10} moles of A-23187 in 3 µl of 10% ethanol-Ringer was injected into the eye. The rate of outgrowth of the regenerating axons was increased by about 30% compared to control animals which had been injected with 10% ethanol-Ringer. This was evident from the following observations:

a) The mean time for recovery of visual function (reappearance of the startle reaction to sudden illumination following 20 min of dark adaptation) was decreased from 14.2 ± 1.0 (SEM) days to 10.3 ± 0.6 days ($p < 0.001$).

b) The rate of advance of regenerating axons stained by cobalt filling, measured between 5 and 14 days after the lesion, was increased from 0.058 mm/day to 0.072 mm/day.

The proportion of retinal ganglion cells containing nucleoli, measured at 22 days after the lesion, was increased by the ionophore treatment from 73.9 ± 1.0 % to 89.8 ± 3.1 % ($p < 0.002$). The mean number of nucleoli per cell increased from 0.84 ± 0.03 to 1.15 ± 0.05 ($p < 0.001$). These findings indicate an enhanced level of ribosomal RNA synthesis accompanying the faster rate of regeneration.

The effect of the ionophore appeared to be dose-dependent, since doses smaller than 3×10^{-10} moles were less effective, as tested by the recovery of visual function. Doses of 10^{-9} moles and higher caused corneal opacity, intraocular bleeding, and bulging of the eye.

We conclude that modulation of the calcium level in the retinal ganglion cell may be an important factor in regulation of axonal outgrowth.

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134.8 ALTERED INTRACELLULAR TRANSPORT OF NEWLY SYNTHESIZED PROTEINS IN PERIKARYA OF REGENERATING GOLDFISH RETINAL GANGLION CELLS. Mark H. Whitnall and Bernice Grafstein. Dept. of Physiology, Cornell University Medical College, New York, NY 10021

Intracellular transport of newly synthesized proteins through the perikaryal organelles of regenerating goldfish retinal ganglion cells was studied using electron microscopic autoradiography. Retinas were removed 14 days after optic tract cut or sham operation, incubated in medium containing [^{3}H]proline for 5 min, and then incubated in chase medium containing unlabeled proline for up to 55 min, fixed and processed according to standard techniques. Organelle labeling was analyzed in terms of "crude relative specific activity", i.e., % grains ÷ % effective area (Williams, M.A., in Glauert, A.M.(ed.), Practical Methods in Electron Microscopy, vol. 6, pt. 2. Amsterdam: North Holland, 1977. Thus labeling time courses refer to relative concentrations, not absolute amounts of label.

The time courses for rough endoplasmic reticulum labeling were almost identical in regenerating and control cells, showing sharp decreases during the first 15 min of chase. Subsequently, a major portion of the label appeared in two main cell compartments: the Golgi apparatus, and a general category containing a combination of cytosol, free polyribosomes and small vesicles below the resolution of the autoradiographic technique. A greater proportion appeared in the Golgi apparatus in regenerating cells, suggesting a rerouting of newly synthesized proteins during regeneration. Also observed in regenerating cells, but not in controls, was a rise in plasma membrane labeling between 35 and 55 min of chase, indicating increased perikaryal plasma membrane turnover during regeneration. Since glycosylation in the Golgi apparatus is an important step in the preparation of plasma membrane proteins, the increase in plasma membrane turnover may be related to the observed increase in Golgi processing.

Between 35 and 55 min of chase, a significant increase in nuclear labeling occurred in regenerating cells but not in controls, showing that influx of newly synthesized protein into the nucleus increases during regeneration, possibly due to increased formation of ribonucleoproteins and/or other nuclear proteins involved in the regulation of the regenerative response.

Supported by an award from the Leopold Schepp Foundation to M.H.W., NIH Training Grant GM-01737 to the Dept. of Physiology, and USPHS grant NS-09015 from NINCDS to B.G.

134.9 RAPIDLY TRANSPORTED PROTEINS IN THE REGENERATING OPTIC NERVE OF GOLDFISH. L.I. Benowitz, V.E. Shashoua and M.G. Yoon. Depts. of Psychiatry and Biological Chemistry, Harvard Medical School, McLean Hospital, Belmont, MA 02178; Dept. of Psychology, Dalhousie University, Halifax, Nova Scotia (M.G.Y.).

In the goldfish, a severed optic nerve will regenerate within a few weeks of being cut, re-establishing the original pattern of connections between the eye and the brain and restoring visually-guided behaviors. To understand the molecular events which underlie this phenomenon, we have developed a double-labeling method to identify specific proteins whose metabolism is differentially altered during the regenerative process. Particular attention was paid to proteins which move down the optic nerve in the rapid phase of axoplasmic transport; this phase contains material which becomes added to the growing axon terminals and which is likely to play a role in such phenomena as intercellular recognition and synaptogenesis. Intraocularly injected [^{3}H]- and [^{14}C]-proline were used as precursors for contrasting the labeling spectra of proteins in a regenerating optic nerve and in the unoperated, contralateral side. Preliminary experiments to validate the method demonstrated that the eye gets labeled as a separate channel (i.e., no isotope cross-over occurs), and that no asymmetric uptake of the two labels occurs. The labeling time used, 5 h, was selected to exclude from our analysis most of the slower moving common structural elements of the axonal matrix. Among the rapidly transported proteins, our results showed the labeling pattern to shift radically during regeneration, with relative changes in the labeling of various proteins varying over a 3-fold range. Eight days after crushing the optic nerve, the greatest relative increases were found for species migrating on SDS-polyacrylamide gels with apparent molecular weights of 135,000 and 50,000 daltons; later in the regenerative process these peaks declined, and by day 29 the greatest relative change in labeling was seen for material with an apparent M.W. of $\sim$ 105,000 daltons. In general, the more common protein species of the optic nerve, i.e., those which stain prominently or which incorporate most of the label, were not among the ones whose metabolism was most altered. By day 62, the labeling spectrum on the regenerating side reverted to that of an unoperated eye. Our results demonstrate that regeneration involves a selective, time-dependent shift in the synthesis and transport of particular molecular species, rather than a nonspecific overall increase in the normal complement of transported proteins. [Supported by NIH 5-RO1 NS 14674-02 and a fellowship from the Alfred P. Sloan Foundation.]

134.10 QUANTITATIVE TWO-DIMENSIONAL GEL ANALYSIS OF SPECIFIC PROTEIN INDUCTION DURING OPTIC NERVE REGENERATION. M. Deaton*, J. A. Freeman, M. Willard*, and J. H. P. Skene*(SPON: M. I. Johnson). Depts. of Anatomy, Ophthalmology, and Physics, Vanderbilt Univ., Nashville, TN 37232; and Depts. of Anatomy-Neurobiology and Biological Chemistry, Washington Univ., St. Louis, MO 63110.

We have constructed a computer-controlled densitometric scanner to analyze and compare two-dimensional electrophoretic autoradiograms of radioactively labeled proteins. Regions of interest on the autoradiograms are scanned (512 x 512 points) and the data are stored in a mini-computer (PDP-12). A system of programs (similar to that described by Lutin, Kyle and Freeman, Electrophoresis '78, N. Catsimpoolas,ed., Elsevier, 1978) was developed which permitted the data to be smoothed transposed and plotted as contour maps or three-dimensional perspective displays. The computer also resolved individual polypeptide components from overlapping peaks and computed their concentrations.

We applied this analytical procedure to the identification and quantification of growth-associated proteins (GAPs) in the retinal ganglion cells of toads (Bufo marinus). During optic nerve regeneration in toads, the rapid axonal transport of three membrane-associated polypeptides (mol. wts. 24K, 43K, 50K), termed GAPs, is dramatically and specifically increased in retinal ganglion cells (Skene and Willard, submitted). Quantitative two-dimensional analysis of these GAPs labeled 9 days after the optic nerve was crushed showed that their relative labeling increased as follows: GAP-50, 14 fold; GAP-43, 5 fold; GAP-24, 35 fold. This method is also being used to study specific changes in gene expression during axon growth and synaptogenesis in the goldfish visual system, and it should be applicable to many other problems involving the analysis of changes in polypeptide compositions.

134.11 **ANOMALOUS GROWTH OF FROG OPTIC NERVE AXONS INTO THEIR OWN PIGMENT EPITHELIAL LAYER AFTER NERVE INJURY.** **R. Bohn*and P. Reier, Dept. of Anat., Univ. of Maryland Sch. of Med., Baltimore, 21201.**

As regenerating axons emerge from the proximal stump of injured peripheral nerves, they frequently exhibit variable patterns of outgrowth which appear to depend on the type and severity of the lesion and surrounding connective tissue response. Studies in this laboratory have also noted the formation of complex neuromas and aberrant bundles of fibers during initial stages of regeneration of the transected optic nerve in Xenopus tadpoles and adults. Whether such highly variable elongation of axons is due to altered patterns of outgrowth in the proximal (retinal) segment or to the cellular organization of the lesion zone has not been extensively studied. We describe here preliminary observations of an unusual growth pattern of axons in the retinal stump of adult Xenopus following resection of a 1 mm segment of the intraorbital portion of the optic nerve. During the first post-operative week the proximal stump was characterized by unmyelinated axons, reactive glial elements and myelin debris resulting from an initial retrograde response to injury. At 2-4 weeks the original nerve stump became circumscribed by a distinct peripheral zone of axons oriented longitudinally and fasciculated by radial glial processes. Near the eye, however, the outer ring of fibers changed from a longitudinal to a circumferential orientation and radial glial processes were no longer apparent. Circumferentially-oriented fibers were most apparent within the intrascleral portion of the nerve while fibers in the central region of the stump were still aligned parallel to the nerve's longitudinal axis. At the optic nerve head, bundles of axons emerged from the circumferentially-oriented fibers and entered the pigment epithelial layer of the retina. These fibers extended in some cases as far as the ciliary margin and generally grew between the epithelial cells internal to Bruch's membrane and the basal lamina. Penetration of more internal retinal layers by these axons did not occur. This novel pattern of axonal orientation has been seen following section of the nerve within the orbit and at the chiasm but not after ablation of the contralateral tectum. Moreover, this phenomenon has not been observed in either nerves from unoperated or sham-operated animals. Based on these initial findings, the observed growth pattern may represent a recurrent growth of axons from the lesion site along the subpial margin of the nerve. These results thus suggest that in addition to influencing the random orientation of fibers within the lesion zone, local environmental conditions at the cut end may compromise the emergence of fibers and induce retrograde growth. (Supported by NIH Grant NS-13836 and the Paralyzed Veterans of America)

134.12 REGENERATION OF OPTIC AXONS IN THE CHICK RETINA. S. Goldberg* and B. Frank* (SPON: J. de la Torre). Dept. of Anatomy, Univ. of Miami Sch. Med., Miami, 33101.

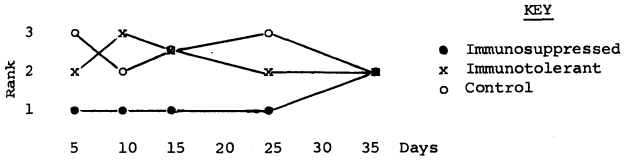
Lesions were produced in the retinas of newly-hatched chicks. Optic axon sprouting began within 2-3 days. Three types of growth patterns were observed. Optic axons that were deflected deep (sclerad) to the ganglion cell fiber layer (GCFL) grew randomly with no tendency to stick to one another. Some optic axons grew along the wound edge. Other axons made hairpin turns, remained within the GCFL, and grew in reverse direction, toward the retinal periphery. On the central side of the lesion, centrifugal axons behaved similarly, in mirror-image fashion. As compared with lesions in 5 day-old chick embryos (Goldberg, '77), axons in newly-hatched chicks showed a lesser degree of elongation, greater randomness of growth, and less tendency to adhere to other optic axons. Axons did not appear to bypass the lesion, as they did in embryos.

Anterograde and retrograde degeneration of optic axons proceeded more rapidly in newly-hatched chicks than in those 14 days post-hatching. More rapid degeneration of optic axons in neonates, as compared with older animals, has also been observed in the mouse retina (Goldberg and Frank, '80).

134.13 BASAL LAMINA AT THE SITE OF CNS INJURY IN TREATED ANIMALS. T. F. Kowalski, E. R. Feringa, H. L. Vahlsing*. Depts. Path. & Neurol. VA Med. Ctr. & Univ.Mich., Ann Arbor, MI 48105 & Dept. Neurosci., UCSD & VA Med. Ctr. San Diego, CA 92161.

After injury, functionally significant regeneration of long tract axons in mammalian spinal cord is not attained. Basal lamina (BL) caps the transected end of the rat spinal cord at the CNS-scar interface 20 days postsurgery. BL may impede the progress of growing axons. Since this BL cap is formed before regenerating axons would be expected to cross the transection site, we felt it might impede regeneration. To test this, we studied BL after two treatments which facilitate axon regeneration.

Fifteen adult (6 wk. old) rats immunosuppressed with 75 mg/Kg I.P. cyclophosphamide 48 hours after spinal cord transection were compared with 15 rats made immunologically unresponsive to CNS antigens by daily injections of 75 mg of emulsified isogeneic spinal cord on the first day of life and weekly until spinal cord transection at age 6 wks. These two groups and a 15 animal control group were studied at 5, 10, 15, 25 and 35 days post-transection with an epithelial BL specific immunohistochemical stain to determine the amount of BL present at the lesion site. At each time interval, animals from the 3 groups were ranked in a numerical hierarchy for the amount of BL observed at the CNS-scar junction. The group with the least BL was graded 1; the group with an intermediate amount, 2; and the group with the greatest amount, 3. These rankings were tested for significance by Students paired t test. The graph below shows the rankings of the groups at time intervals studied.



No significant difference exists between the immunotolerant animals and the controls. However, those animals treated with cyclophosphamide have a significant ($p < 0.05$) decrease in BL at the CNS-scar interface as compared to controls or the immunotolerant group.

Treatment of spinal cord transected animals with cyclophosphamide significantly slows the development of the BL cap over the transected end of the spinal cord. This delay may allow the earliest few axons to regenerate across the transection site. Later, a completed BL cap may block the remaining axons which attempt to regenerate.

134.14 REGENERATION OF VOMERONASAL NERVES INTO THE MAIN OLFACTORY BULB. P.C. Barber*. (SPON: J. Nagy) Laboratory of Neurobiology, National Institute for Medical Research, Mill Hill, London NW7 1AA

It has been previously shown (Barber, P.C. and Raisman, G., Brain Res., 147: 297, 1978) that after section of the vomeronasal nerves, the neurosensory cells of the vomeronasal epithelium undergo retrograde degeneration and die. They are subsequently replaced by newly-formed neurosensory cells, which arise from a stem cell present in the epithelium. In the present study, it is demonstrated that vomeronasal nerve section results in orthograde degeneration of neurosensory cell axons and their terminals in the glomeruli of the accessory olfactory bulb. The glomerular organisation collapses and the external plexiform layer of the accessory bulb becomes covered by glial lamellae. Re-innervation of the accessory bulb by vomeronasal axons does not occur, at survivals as long as 150 days. However, autoradiography of axonal transport after application of [^{3}H]-proline to the regenerated vomeronasal epithelium reveals that vomeronasal axons arising from newly formed neurosensory cells in the regenerated epithelium are able to innervate the main olfactory bulb, in regions where it is damaged or deafferented, and form glomeruli which resemble those of the accessory bulb. Electron microscope autoradiography shows that the regenerated vomeronasal axons within these glomeruli form morphologically normal terminals, with synapses contacting dendrites of the nearby main bulb neurons.

134.15 EM AUTORADIOGRAPHIC STUDY OF REGENERATING LOCUS COERULEUS FIBERS INTO IRIS TISSUE IMPLANTS IN THE RAT MESENCEPHALON. Amy Rothman Schonfeld, Cedric S. Raine and Robert Katzman, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Regenerative sprouting of adult central monoaminergic (MA) fibers after mechanically or chemically induced injury has been well documented light microscopically using histofluorescence and histochemistry. One model which has proven particularly suitable for demonstrating axonal regeneration involves implantation of peripheral tissue, especially the iris, into the brain or spinal cord (Svendgaard et. al., Adv. anat. embryol. Cell Biol. 57:7, 1975). A previous ultrastructural report from this laboratory revealed the presence of newly formed nonmyelinated axons and varicosities containing either dense or clear core vesicles within iris implants in the rat mesencephalon (Katzman et. al., Brain Res. 138:423, 1977) and light microscopic (LM) autoradiography established the locus coeruleus as the source of at least some of the fibers (Schonfeld and Katzman, Soc. Neurosci. 5:682, 1979). The aims of the present study were: (1) to determine what proportion of regenerating fibers within an implant are of locus coeruleus origin and (2) to establish the location and terminal arrangements of these fibers by electron microscopic (EM) autoradiography.

For this, heterologous iris implants were inserted in the caudal mesencephalon in the region of the dorsal tegmental bundle (DTB) in female rats previously subjected to ipsilateral superior cervical sympathectomies, a procedure known to sever the DTB (Katzman et. al., 1977). Approximately 2 weeks later, animals were injected with ^{3}H-leucine into the ipsilateral locus coeruleus and sacrificed 2 days later. After fixation with paraformaldehyde-glutaraldehyde, the areas of the implant and injection site were dissected out, post-fixed in 1% osmium tetroxide and processed for EM. One micron Epon sections were cut and prepared for LM autoradiography according to the method of Cowan et. al. (Brain Res. 37:21, 1972). Thin sections from selected blocks were prepared for EM autoradiography according to the method of Salpeter and Bachmann (J. Cell Biol. 22:469, 1964).

At the LM level, numerous labelled fibers were observed within iris implants and traversing between brain and implant, as previously reported (Schonfeld and Katzman, Brain Res., in press). Labelled fibers showed an affinity for smooth muscle and walls of blood vessels. Ultrastructurally, a number of fibers, predominantly thinly myelinated by Schwann cells or unmyelinated, were found to be labelled. The presence of labelled regenerated axons within the previously denervated implants further confirms that central adult MA fibers retain the capacity to regenerate. A morphologic analysis of this phenomenon will be presented.

134.16 CELL PROLIFERATION IN AREAS OF INTRASPINAL SCHWANN CELLS. S. A. Gilmore, J. K. Heard and T. J. Sims. Depts. of Anatomy and Pathology, Univ. Arkansas Med. Sciences, Little Rock, AR 72205.

Intraspinal peripheral nervous tissue (IPNT), consisting of Schwann cells, myelin, and connective tissue components, develops in immature rats following exposure of the spinal cord to x-rays (Gilmore and Duncan, '68; Beal and Hall, '74; Blakemore and Patterson, '75; Heard and Gilmore, '80). Areas of IPNT consistently occur by 2 weeks post-irradiation (P-I), when many of the intraspinal Schwann cells are labeled by ^{3}H-thymidine (Gilmore, '71). IPNT occurs first in the dorsolateral portion of the dorsal funiculus, and by one month usually extends throughout this structure and into the dorsal gray matter. At two or six months P-I, IPNT still occupies the dorsal portion of the spinal cord. Factors limiting the development of IPNT could reside within this tissue itself and/or in the normal constituents of the spinal cord. The present study was undertaken to examine the proliferative potential of cells in areas of IPNT.

Three-day-old Charles River CD^R rats were x-irradiated. A single exposure (4000R) was administered to a 5-mm length of lumbosacral spinal cord. Fifteen to 45 days P-I, groups of irradiated and sham-irradiated rats were injected with ^{3}H-thymidine and perfuse-fixed 1 hour later. Spinal cords were removed, and interrupted serial sections were prepared for light microscopic and autoradiographic evaluation. Spinal cords from other rats were used for ultrastructural studies.

The light microscopic appearance and the distribution of IPNT was the same as that reported previously from this laboratory. Labeled cells were noted throughout the IPNT at 15 and 20 days P-I, when IPNT was usually restricted to the dorsal and/or dorsolateral portions of the dorsal funiculi. By 25 and 30 days P-I, IPNT extended deeper into the dorsal funiclui and into the dorsal gray matter. The labeled cells occurred throughout the IPNT, but there was a tendency toward concentration of the labeled cells in the deeper portions of the IPNT, i.e., closer to the interface between central nervous system structures and IPNT. Labeled cells were present at 45 days P-I. These cells tended to be concentrated toward the depths of the IPNT, and sizeable areas of IPNT contained no labeled cells.

These data indicate that cell proliferation is in progress in areas of IPNT as late as 45 days P-I, a time when active Schwann cell myelination can be demonstrated ultrastructurally. Animals killed at longer post-irradiation intervals are under study, and the cellular composition of the areas of IPNT is being examined ultrastructurally.

(Supported by NIH Grant NS 04761.)

134.17 GROWTH OF OLFACTORY SENSORY AXONS INTO TRANSPLANTED BRAIN SLABS. P.P.C. Graziadei and G.A. Monti Graziadei* Department of Biological Science, Florida State University, Tallahassee Fla. 32306

After bulbectomy, olfactory sensory neurons are known to regrow into several portions of the spared forebrain, where they form glomeruli-like structures (Graziadei, Levine and Monti Graziadei, 1978, 1979). To test the ability of the olfactory sensory neurons to establish contacts with several neuronal populations of the C.N.S., besides the ones which can be reached after bulbectomy, we have resorted to the technique of transplantation.

In host mice 1 to 10 days old the olfactory bulb has been partially or totally removed. Brain slabs of medulla oblongata, cerebellum and occipital cortex, removed from donor mice embryos at 15 days of gestation, were positioned in the cavity resulting from bulbectomy. Care was taken to position the transplants immediately adjacent to the lamina cribrosa. After proper dressing of the surgical wound the animals were left to survive up to 6 months. The host mice were then perfused, the head embedded in paraffin and serial sections obtained. Alternate slides, with 4 sections per slide, were stained with iron hematoxylin and Bodian silver methods. Intermediate slides, judged of interest, were stained with the unlabeled antibody method (Sternberger et al. 1970) for the demonstration of the olfactory marker protein. The presence of this specific protein has allowed the recognition of the sensory axons and their terminals in the invaded, transplanted tissue.

It has been observed that the olfactory sensory axons can invade all the transplanted C.N.S. portions. When partial bulbectomy is performed the sensory axons can form glomeruli in the spared portions of the bulb, in the forebrain and in the transplanted tissue. A demonstation will be provided of the modalities of penetration of the sensory axons in the transplants and of the connections of these axons with the local neurons, as these contacts can be demonstrated at light microscope level.

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134.18 MATURATION OF FETAL SPINAL CORD IMPLANTS IN THE LATERAL CEREBRAL VENTRICLES OF ADULT RATS. P. J. Reier, M. J. Perlow and L. Guth (SPON: A. A. Zalewski). Dept. Anatomy, Univ. Maryland, Sch. of Med., Baltimore, MD 21201 and Unit on Geriatric Psych., Lab. Clincial Psychopharmacology, N.I.M.H., St. Elizabeths Hospital, Washington, D.C. 20032.

Implantation of fetal neural tissue to various regions of the adult rat brain has been shown to represent an important approach in the analysis of cellular mechanisms associated with development and regeneration. Although most regions of the CNS survive and differentiate as embryonic grafts, some variability has been noted in the extent of normal development achieved by various CNS structures. Notably, grafted segments of fetal spinal cords have been reported to develop an intrinsic cytoarchitecture which significantly departs from the normal cellular organization of the mature cord. Since the transected spinal cord in immature rodents has also been shown to have a limited regenerative capacity, the following histological and ultrastructural study was undertaken to examine further the degree to which injury compromises development of the spinal cord in the neural tissue transplantation model. Segments of cervical and lumbar spinal cord, 1-4mm long, were obtained from 15-day-old Sprague-Dawley rat fetuses, and several pieces of tissue were stereotaxically injected into the lateral cerebral ventricles of 600-800gm males. The recipients were sacrificed at intervals from 5 days to 7 months after implantation, and tissues were examined by light and electron microscopy. In all cases, the implanted cord segments survived and were extensively revascularized; no tissue rejection was evidenced. By 5 days the fetal cords exhibited numerous immature neurons, glioblasts and a well-developed neuropil in which early stages of synaptogenesis were evident. By 1½ months the implants had increased in size and closely approximated the parenchyma of the hosts' brains. Silver-stained sections revealed numerous axons traversing the interface between host tissue and implant. At this time, the grafts consisted of many identifiable neurons, including large motoneurons, glia, myelinated fibers and a highly differentiated neuropil with numerous synapses. Several distinct zones of white and gray matter were formed in the implants, although specific development of dorsal and ventral horns did not occur. Similar patterns of maturation were noted in grafts which were situated in regions of injured cerebral cortex near the initial site of tissue injection; however, axons extending between host parenchyma and implant were less apparent in these areas. Studies are currently in progress to define the nature of host-implant interactions and environmental factors which may influence neuritic outgrowth from grafted segments of fetal spinal cords. (Supported by NIH grants NS-13836,-12847 and The Paralyzed Veterans of America).

134.19 UNILATERAL LESIONS IN THE SUBSTANTIA NIGRA INDUCE PROJECTIONS FROM THE CONTRALATERAL SUBSTANTIA NIGRA TO THE IPSILATERAL THALAMUS. M. Pritzel* and J.P. Huston. Institute of Psychology III, University of Düsseldorf, Düsseldorf, FRG.

The efferent organization of the substantia nigra (SN) to the striatum, thalamus and superior colliculus is limited to ipsilateral connections. We recently found that hemidetelencephalization, which leads to transient ipsiversive turning in rats, also induces the development of crossed projections to thalamic nuclei which normally receive only ipsilateral projections. The present study sought to determine whether the development of crossed projections would follow other interventions known to produce behavioral asymmetries.

Rats were given a unilateral injection of 4 µl (4 mg/ml) 6-OHDA into the SN. The resulting ipsiversive turning had ceased by 6-8 days post-lesion. The animals were then administered an injection of HRP (Boehringer, grade I, 30-50%, 0.02-0.04 µl) into the ventrolateral/ventromedial thalamus, which is one of the projection sites of the SN. In addition to the ipsilateral nigro-thalamic projections that were spared by the lesion, we found mirror image contralateral projections from the SN to the ipsilateral thalamus. Similar results were obtained after unilateral lesions of the SN with kainic acid (2 mg/ml, 0.5 µl) alone, or in combination with 6-OHDA (which leads in both cases to contraversive turning).

We prefer to interpret these results in terms of morphological reorganization underlying behavioral reorganization following 'sensori-motor' asymmetries induced by unilateral insults to the brain.

134.20 HISTOFLUORESCENT OBSERVATIONS OF MONOAMINE AXONAL ELONGATION IN TRANSECTED RAT SPINAL CORD. Nylén, E., D.D. Rigamonti, W. Prominski* and L. Sigmund*. Department of Anatomy, Georgetown University School of Medicine, Washington, D.C. 20007.

Axonal growth of CNS monoamine (MA) fibers intrinsic to the brain stem has been documented following mechanical and chemical axotomies. The bulbospinal MA system also progressively regenerates following toxic lesions and "sprouting" is observed subsequent to mechanical induced injuries when the cord, meninges and the vascular supply has been interrupted. An improved spinal cord surgical transection technique which maintains the meningeal blood supply is currently being used in reconstruction experiments. Results from an anatomical study in the rat suggest that there is sprouting of primary afferents into an autologous peripheral nerve graft and also growth of intrinsic CNS neurons near the implant (Richardson et al., Nature, 284:264, 1980.) Our experiments were designed to study the reaction of fluorescent fibers, presumably descending MA bulbospinal axons, in the subpially transected adult rat spinal cord. In all, 16 animals were operated and observed at 21, 42 and 142 days. One group of animals received a sciatic nerve implant between the midthoracic cord stumps while a second group were subpially transected and left with no reconstruction. All animals showed MA fibers converging at the cranial side of the lesion, some of which projected into the transection area. Some fibers were also observed throughout the extent of the transection site. Immediate implantation of sciatic nerve segments in the transected area mainly influenced the pattern of MA growth which was then more uniform, and to a lesser extent the relative abundance of fibers in the distal zones of the transected area. We suggest that the maintenance of the meningeal vasculature is critical for axonal elongation and reconstruction experiments. (Supported by NIH Grant RR5360)

134.21 CEREBELLAR REGENERATION AFTER IMMUNOTHERAPY FOR GRAFT VERSUS HOST DISEASE. W.S.T. Griffin and J.R. Head*. Dept. of Cell Biology, The Univ. Tx. Health Science Center, Dallas, Texas 75235.

A tool with which cerebellar histogenesis could be turned off and then turned back on in vivo holds promise for the study of 1) events occurring during uninterrupted histogenesis and 2) the potential for regrowth and plasticity of developing CNS structures. Graft versus host disease (GVHD) induced alterations in rat cerebellar cell proliferation and function and the reversal of these alterations by therapeutic intervention affords such a tool. For this study, Fischer (FI) rat litters of not more than 12 pups were initially divided into two groups. One group contained one-third of the pups and received no treatment at birth. The other group contained two-thirds of the pups, and each of these pups was grafted by an intravenous injection of 20 x 10^6 lymph node cells from an allogeneic donor, viz., a DA adult rat. This latter group was further subdivided so that one-half of these pups received 3 injections, on postnatal days 11, 12 and 13, of syngeneic serum containing high titers of antibodies to their allogeneic graft, i.e., FI anti-DA serum. One-half of the control group also received three daily injections which had no apparent effect. Analysis of diseased animals on day 11 revealed that cerebellar DNA synthesis and total DNA content were significantly less than unmyelinated controls and further analysis indicated that synthesis and content of RNA were similarly affected. Histological analysis revealed that the mantle area which contains the newly formed cells of the external granular layer was smaller at day 11. These same kinds of analyses were performed on 14-day-old animals with GVHD, deprived of immunotherapy (E); animals "saved" from GVHD by treatment with alloantiserum (I); and littermate controls, both treated and untreated with alloantiserum (C). Analysis of cerebellum from E animals at 14 days revealed that DNA synthetic capacity had worsened between days 11 and 14 and DNA content was markedly less than the value from either I or C. DNA synthesis and content in I was significantly greater than E but remained less than C by a small but significant amount. RNA synthesis and content in 14-day-old E animals was significantly less than that in C at day 14, but synthesis of RNA from I (after only 3 days of alloantiserum treatment) was significantly greater than E and was similar to C. However, RNA content in 14-day-old I was still less than 14 days old C.

We conclude that the CNS has the ability to acquire new cells in an accelerated fashion when stress is imposed and then lifted and that this acquisition is accompanied by increased cellular functional capacity.

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135.1 CROSSED HINDLIMB REFLEXES DURING FICTIVE LOCOMOTION IN ACUTE SPINAL CATS. S. Rossignol and C. Julien*. Centre de Recherche en Sciences Neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

In decerebrate cats walking on a treadmill, a high intensity stimulation of the superficial peroneal nerve (SPN) on one side can evoke on the other side a crossed extension response during stance or a crossed flexion during swing (Rossignol, S. and Gauthier, L., Soc. Neurosci. Abstr., 4, 304, 1978). In the present work, similar stimuli were given during fictive locomotion in spinalized (th13) and paralyzed cats pretreated with Nialamide (50 mg/kg) and L-DOPA (50-70 mg/kg). Trains of 10 to 20 pulses at 100 Hz were delivered to the SPN at an intensity sufficient to induce contralateral responses recorded in the extensor Gastrocnemius or flexor Semitendinosus nerves by means of cuff electrodes. The innervation of the limbs was left intact except for the recorded nerves which were ligated distally to the recording site. Both limbs were placed in a standing position. The stimulation produced an ipsilateral flexion response in all phases of the step cycle. However, in the contralateral limb, crossed extension responses occurred when stimuli were given just before or during the first half of the locomotor extensor burst. Crossed flexion responses were seen with stimuli delivered towards the end of the extensor burst and during the first half of the flexor burst. Stimuli in the second half of the flexor burst often gave no response in either nerve, whereas stimuli in the second half of the extensor burst gave either an extension or a flexion. In periods when crossed flexion or crossed extension responses were usually elicited, mixed responses were seen on occasion. They consisted of a short inhibition in the ongoing locomotor burst and a short response in the antagonist after which the ongoing activity resumed and the burst was completed. Stimuli given during the crossed flexion burst shortened the cycle whereas stimuli during crossed extension lengthened it. The overall distribution of crossed responses and the effects of stimuli on the duration of the step cycle during fictive locomotion are strikingly similar to those observed in the cat walking on a treadmill except for the mixed responses which we have never seen in the latter. This suggests that crossed reflex pathways to extensor and flexor motoneurons are largely selected centrally during rhythmic activity and that afferent information originating from the walking limbs provides a corrective reinforcement of that selection to insure that the responses are appropriate for the phase of the movement.

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135.2 NEURAL FEEDBACK RESPONSES TO HAND PERTURBATION. Robert N. Stiles. Dept. of Physiology and Biophysics, University of Tennessee Center for the Health Sciences, Memphis, TN 38163.

Damped oscillations of the extended hand in response to a pulse perturbation occur at the frequency of postural hand tremor, when these are detected under similar conditions of added mass, displacement amplitude, and with rest between runs. Also, each kind of oscillation is accompanied by amplitude modulation of wrist extensor EMGs (forearm pronated) at the frequency of the oscillation, modulation that can be interpreted as resulting from neural feedback. Certainly the timing (or phase) of this EMG activity and the hand motion may be important relative to the amplitude of these hand oscillations. Hagbarth and Young (Brain 102: 509-526, 1979) noted that the repetitive EMG bursts tended to occur later in the oscillation cycle as the damped oscillations decreased in amplitude. The present report also considers the timing (and phase) between muscle activity and the damped hand oscillations that occur in response to a pulse perturbation.

Using a small accelerometer (phase and amplitude calibrated) and bipolar EMG electrodes, damped oscillations of the extended hand and surface EMGs from two wrist extensors were detected from each of ten normal human subjects. Damped oscillations of each subject's hand were produced by repetitively applying a downward pulse of force (a tap) at about 1/sec for 16 sec. The timing between the demodulated EMG and the negative of hand acceleration was obtained by cross-covariance analysis, and by the method of averaged transients.

The time (designated Δt) between when the peak upward position of the oscillating hand occurred and the time later that the maximum extensor activity occurred was consistently between 30-45 msec for all ten subjects. Visual analysis of the averaged transients indicated that Δt was relatively constant for each cycle of the damped oscillation. Further, the first average EMG response was a pulse of increased activity at 30-45 msec after the initial pulse of downward acceleration. With 100 g added mass, the maximum extensor activity at the frequency of the motion occurred at about the time of maximum downward velocity of the hand. With 600 g added mass, the oscillation frequency was reduced, Δt was little changed, and the extensor EMG maximum occurred prior to the maximum downward hand velocity. In all, the results suggest that: 1) Only one reflex pathway mediated the oscillatory EMG modulation. 2) The feedback delay varied little with oscillation amplitude. 3) The receptors that mediated this EMG response produced maximum afferent activity at the time when the muscle was maximally shortened. (Supported in part by USHPS Grant NS 14730.)

135.3 TIME-DEPENDENT EFFECTS OF SURAL NERVE STIMULATION ON EXTENSOR MOTOR POOL ORGANIZATION. H. P. Clamann and A. J. Robinson*. Dept. of Physiology, Med. College of Virginia, Richmond, VA 23298

It has been suggested that cutaneous nerve stimulation may have differential effects on select sub-populations of a motoneuron pool, and produce changes in the size-dependent recruitment order. The present study was designed to seek and quantify any changes in the recruitment order of monosynaptically activated medial gastrocnemius (MG) motoneurons produced by a conditioning train of stimuli to sural nerve. In cats anesthetized with chloralose, the sural nerve and MG muscle nerve were cut and placed on electrodes for stimulation or recording. Monosynaptic reflexes were evoked in MG by stimulation of L7 and S1 dorsal roots and reflex magnitudes were measured from the volleys recorded at the muscle nerve. The response of a single motoneuron was recorded with a microelectrode driven into its axon in a ventral root. A 10 msec train of pulses at 200 pps to sural nerve produced a brief inhibtion of a monosynaptic reflex initiated 1-2 msec after the end of the train; this inhibition was graded with conditioning stimulus intensity. In contrast, a 100 msec conditioning train to sural nerve facilitated a subsequent MG monosynaptic reflex.

When the sural nerve conditioning train was timed so as to facilitate MG, some changes in the recruitment thresholds of individual motoneurons were produced but these did not appear to be related to the conduction velocities of the cells. Sural nerve facilitation of MG did not alter the over-all size dependent recruitment order established in the monosynaptic reflex, but did reduce the correlation between recruitment order and conduction velocity.

When the sural nerve conditioning train was timed so as to inhibit MG, that inhibition appeared to occur selectively in motoneurons of low conduction velocity, causing their initially low recruitment thresholds to rise sharply. The same conditioning volley appeared to facilitate units of high conduction velocity, causing their initially high recruitment threshold to fall. This is supported by the following observation: when MG was tetanized to increase its monosynaptic reflex responses, 10 msec trains of sural nerve conditioning stimuli increased these responses further. As the effect of PTP wore off, the same sural nerve conditioning shocks produced inhibition of the smaller MG responses.

The nature of the effect of sural nerve stimulation (excitation or inhibition) depends in part on the level of activity of the MG motoneuron pool. This could contribute to the response of extensor muscles seen in 'phase dependent reflex reversal' of spinal cats during locomotion.

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135.4 TEMPORAL RELATIONS BETWEEN MOTOR CORTICAL AND ELECTROMYOGRAPHIC RESPONSES TO IMPOSED FORELIMB DISPLACEMENTS IN THE CAT. A.G.E. North* and W.G. Tatton, Playfair Neuroscience Unit, University of Toronto, Toronto, Ontario, Canada, M5T 2S8.

Imposed displacements of upper limb joints in the monkey evokes activity in motor cortical neurons (MCN's) that is appropriately timed to contribute to the "M2" response in the EMG activity recorded from the stretched muscles. Despite the appropriate timing, recent transection studies (Ghez, C., Shinoda, Y., Exp. Br. Res., 32, 55-69, 1978) do not support the proposal that the MCN output contributes to a second reflex EMG response peak in cat forelimb muscles considered to be analogous to the monkey "M2".

Simultaneous area 4 microelectrode and triceps EMG recording was carried out in nine chronically prepared cats. A "receiving" zone in area 4 containing MCN's responding to imposed displacements of the elbow was mapped for each animal. MCN afferent latencies ranged from 10 to 28 msec with two distinct modes at 14 and 18 msec. The latencies were constant within ± 2 msec despite variation of the initial displacement velocity by as much as ten fold (60 to 600 degrees/second) and variation of the initial included joint angle from 130° to 70°. Intracortical microstimulation at loci within the "receiving" zone was shown to evoke activity in triceps brachii. The EMG showed three response peaks identical to those of Ghez and Shinoda at latencies ranging from 10 to 14, 34 to 52 and 60 to 85 msec respectively. The latencies of the second peak varied by ± 10 msec depending on the velocity of the displacement and/or the initial joint angle.

Following chronic recording for 2 to 3 weeks, five of the cats were anaesthetized with chloralose and stimulation ($< 50\mu$amps) of the cervical cord at the C2 level was carried out to determine the antidromic conduction time to MCN's in the "receiving" zone and the efferent conduction time to triceps. Antidromic conduction times ranged from 1.0 to 7.0 msec (mode 1.0 - 2.0 msec) and efferent times to triceps of 4.0 to 7.0 msec. Calculation of the summed afferent-efferent times establish that MCN activity in the "receiving' zone does not contribute to the generation of the 2nd EMG response peak in support of the transection studies and in contrast to MCN activity related to the M2 response for monkey distal upper limb musculature.

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135.5 STRETCH REFLEX AMPLITUDE VARIATION IN PRIMATES.
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Monkeys maintained elbow angle at 90° (±1.5°) against constant extension force. Elbow angle and biceps EMG (from chronic intramuscular stainless steel wire electrodes) were monitored by computer. If correct angle was maintained for a randomly selected 1- to 2-sec period, and if the average absolute value of biceps EMG (sampled at 10 kHz) for the final 0.5 sec of the period fell within a preset range, a stimulus consisting of a 20-msec pulse of additional extension force occurred at the end of the period. It transiently extended the elbow (3-4°), eliciting a stretch reflex. The computer determined the amplitudes of the M1 and M2 components of the reflex. M1 amplitude was defined as the average absolute value of the EMG occurring 12.5-21.5 msec after stimulus onset, minus pre-stimulus EMG amplitude. M2 amplitude was defined as the average absolute value of the EMG occurring 31.5-56.5 msec after stimulus onset, minus pre-stimulus EMG amplitude. The computer also followed stimulus-induced elbow angle change. Under the Control condition, liquid reward was given 70 msec after stimulus onset. Under the M1+ or M1- condition, reward was given only if M1 amplitude was greater than (M1+), or less than (M1-), a preset value.

In initial work, data were obtained from four animals over continuous recording periods of up to 3 months. The computer tabulated number of trials, average pre-stimulus EMG amplitude, average M1 and M2 amplitudes, and average course of stimulus-induced elbow angle change, at hourly and daily intervals. Animals usually completed 3,000-10,000 trials per day. For each animal, EMG electrodes, pre-stimulus EMG amplitude, constant extension force, stimulus amplitude, and stimulus-induced change in elbow angle, remained unchanged over the recording period.

Marked acute (hourly) and chronic (daily and weekly) changes occurred in average M1 amplitude independent of change in average pre-stimulus EMG amplitude. These preliminary studies suggested: (1) When animals working in constant light complete equal numbers of trials around the clock, a clear circadian variation in M1 amplitude often occurs. M1 may be up to 40% greater at midnight than at noon. (2) Under the impetus of the M1+ or M1- condition, animals appear to be able to alter M1 amplitude progressively over a period of several days. (3) Without change in reward conditions, acute and chronic changes in M1 and M2 amplitudes can occur with considerable independence from each other.

In awake behaving primates, M1 amplitude at a given muscle length does not appear to bear a fixed relationship to alpha motorneuron tone. Whether the present findings are attributable to change in gamma motorneuron tone, other intra-segmental changes, and/or humoral effects on muscle spindle sensitivity, remains to be determined.

135.6 RELATION BETWEEN STIFFNESS AND FORCE IN THE ACTIVE CAT SOLEUS MUSCLE. T. M. Hamm* and L.D. Partridge. (SPON: B. R. Botterman). Dept. Physiol. Biophys., Univ. Tenn. Center Health Sci., Memphis, TN 38163.

In the active muscle, the relations between force and stiffness give insight into both current theories of contraction and the constraints imposed by muscle mechanics on the control of movement. The present report describes the relation between force and stiffness of the active soleus muscle (stimulated via sectioned sciatic nerve at 25 Hz) under isometric and anisometric (dynamic) conditions. Stiffness was measured as the ratio of force to length changes produced by small amplitude (< 0.2mm) vibration (200 Hz) imposed by an electromagnetic puller on the isometric muscle, and during 2-3 mm triangular and random stretches (< 8 Hz). By use of low- and high-pass filters, it was possible to separate low frequency changes in force and length from the high frequency changes used for the stiffness measurement.

"Shortrange stiffness" as described by Rack and Westbury (J. Physiol. Lond. 240: 331-350, 1974) was observed during triangular stretches. However, stiffness, as presently defined, was found to increase with force throughout the full duration of such stretches. In general, stiffness was found to be proportional to force under both isometric and dynamic conditions. At or near L_0, the slope of stiffness to force was similar for isometric and dynamic tests. At shorter lengths, (ascending limb of length-tension curve) this slope was greater for dynamic tests.

The dynamic relation between stiffness and force was examined by calculation of the cross-spectral density function of these two variables from records of random movements. At higher frequencies, the amplitude ratio of stiffness to force decreased, and stiffness began to lag force in phase.

In summary, the present results indicate that the mechanical properties of active muscle vary continuously with changes in force and length.

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135.7 A MODEL OF MEDIAL GASTROCNEMIUS MOTOR UNIT ACTIVATION PATTERNS IN THE STRETCH REFLEX OF THE DECEREBRATE CAT. P.J. Cordo and W.Z. Rymer. Neurological Sciences Inst., Portland, OR 97209 and Northwestern Univ. Medical School, Chicago, IL 60611.

Single motor unit recruitment and rate modulation data obtained from the medial gastrocnemius (MG) of the decerebrate cat during stretch reflexes was used to reconstruct a whole-muscle force-EMG relationship. This predicted relationship was compared to the measured force-EMG relationship to test whether the motor unit sample was representative of the population. Using the motor unit activation parameters quantified in these experiments (1), the model incorporated the following features: 1) a muscle of 150 motor units with 75 S and 75 FR twitch types (2), 2) a 5 mm, 5 mm/sec ramp stretch reflex commencing from an initial force of 100 g and rising linearly with time to a peak of 1600 g, 3) a single unit recruitment function (force threshold distribution) $N=150-150e^{-.0019xF}$, 4) an initial firing rate proportional to the units' force thresholds, 5) a smooth increase in firing rate of each recruited unit of 2.0 pps/100 g force increase up to a saturated rate, and 6) a step increase in firing rate at stretch onset of 4.6 pps for units which were active before stretch began.

Comparison of actual and predicted force-EMG plots revealed a recording bias for larger motor units had occurred. By adjusting the recruitment function to include an initial step in recruitment (c.f. the dynamic component of the IA afferent response to muscle stretch) and a slightly steeper decay ($e^{-.003}$), a good correlation between predicted and measured force-EMG relationships was obtained. The initial abrupt rise in multiunit EMG which was observed during stretch reflexes supports this alteration of the recruitment function. The adjusted model was then combined with isometric twitch tension data (2) to reconstruct the force trajectory of the experimentally observed stretch reflexes. The force trajectory predicted by the model generated substantially more force than actually observed suggesting that, at least initially, the net force production capability of isometric muscle exceeds that of lengthening muscle. More interesting, the predicted force trajectory had an early, marked discontinuity in it which may correspond to the early loss of muscle stiffness ("yield") which has been demonstrated in the electrically stimulated cat soleus (3). If the correspondence is real, this finding strongly suggests that the mechanical properties of the reflexly activated portion of MG (slow twitch units) are qualitatively similar to those of the soleus and that, under reflexive conditions, these stiffness discontinuities are largely compensated for by rapid and substantial motor unit recruitment.

(1) Cordo, P.J. & Rymer, W.Z. Soc. Neurosc. Abstr. 5:366 (1979).
(2) Burke, R.E. et al. J. Physiol. 234:723-743 (1973).
(3) Nichols, T.R. & Houk, J.C. J. Neurophysiol. 39:119-142 (1976).

135.8 Stimulation of and recording from axons within their myelin sheaths: A stable and nondamaging technique for studying single motor units. D.L. Zealear* (SPON: M. Hast). Dept. of Otolaryng., Northwestern Univ. Med. Sch., Chicago, Ill. 60611.

The contractile properties and motoneuron spiking patterns of single laryngeal motor units were studied by stimulating and recording intracellularly from their motoneuron cell bodies. Since these experiments were conducted in the brainstems of lightly anesthetized, spontaneously breathing cats, considerable difficulty was encountered in holding cell bodies with micropipettes. An alternative approach was therefore taken, that of penetrating motoneuron axons in the recurrent laryngeal nerve; this was found to be a very stable technique. In most axon penetrations, sudden d.c. voltage shifts were not recorded, indicating that axon axolemmas were intact. In such cases, activation and recording of action potentials occurred from the myelin sheaths of axons. As described previously (Tasaki, I., Jap. J. Physiol. 3:73:1952), myelin sheath penetrations were functionally similar to actual axon penetrations. Threshold activation occurred with small anodal current pulses (a few namps in magnitude), and positive action potentials were recorded, but were graded in size with the depth of sheath penetration. Activation from a myelin sheath, therefore, had the functional advantage of an intracellular approach: stimulus current sufficient to activate a motoneuron being recorded from was too small to activate other motoneurons. Myelin sheath penetrations were, nevertheless, physically outside motoneurons and circumvented damage or conduction block, which could occur when using an intracellular approach. Because of the stability and noninjurious nature of the myelin sheath approach, motor units have been studied for over an hour on a routine basis, and for as long as three or four hours in many instances. The factors which contributed to the success of the approach will be described.

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135.9 FREQUENCY ANALYSIS OF SHIVER IN HUMANS. R. S. Pozos, P. A. Iaizzo* and S. A. Burgstahler*. Dept. of Physiology, Univ. of Minn.-Duluth Sch. of Med., Duluth, MN 55812.

Shivering may be considered an involuntary oscillation similar to a reflex clonus but with possibly some central regulatory component (Perkins, J. F., Am. J. Physiol., 145: 264-271, 1946). However, the role of segmental as well as supraspinal reflexes on the generation of shiver and its maintenance and synchronization is still not well understood (Stuart, D. et al., Am. J. Physiol. Med., 45(2):61-102, 1966). The present study was undertaken to quantitate the frequency of shiver in humans using spectral techniques.

Human male and female subjects were seated in an environmental chamber set at 0°C. Surface electrodes had been placed on the masseter (M), pectoralis major (PM), trapezius (T) and soleus (S) muscles. An accelerometer was placed on the dominant knee of the seated subject, who held his leg in a plantar flexed position with the heel 2-4 cm off the floor. Core temperature was monitored using a rectal thermocouple and surface temperatures were recorded from thorax and abdomen regions. Signals were recorded on an FM tape recorder and later analyzed on a PDP-12 computer. Spectral as well as cross spectral techniques were employed to detect frequency and phase relationships between the various signals. Once an overall shiver was evident, subjects stayed in the chamber an average of 25 minutes. In most cases, core temperature rarely fell lower than 1°C. Peripheral temperatures, however, varied greatly depending on the amount of subcutaneous fat.

The analysis of the EMGs showed a number of major peaks. All the muscles oscillated in a range between 6-13 Hz. Accelerometer recordings of the ankle ranged between 5-8 Hz. In the case of the masseter, the EMG frequencies changed from one high frequency of 10 Hz to a higher one of 13 Hz. Throughout all records, there was a persistent major frequency of 4-6 Hz. Initially there was no significant coherence between the various EMGs and/or the accelerometer recording. However, as the shiver increased in intensity, significant coherence between the various EMGs (M, S, PM, T) as well as motion occurred at a frequency range of 5-6 Hz. At this time spectral analyses of the EMG usually showed at least two major peaks, one in the low range of 4-6 Hz and one at approximately 10-14 Hz.

This data indicates that during the initial stages of shiver the musculature is oscillating at a number of frequencies independent of each other. Eventually, as the shiver increases in intensity, there seems to be more synchronization between the various EMG signals.

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135.10 COORDINATION OF ARM MOVEMENTS AND ASSOCIATED POSTURAL ADJUSTMENTS IN STANDING SUBJECTS. L. M. Nashner and P. J. Cordo. Neurological Sciences Inst., Portland, OR 97209.

It has been shown that, prior to a voluntary arm movement, activation of the trunk and leg muscles occurs in such a way as to compensate for the destabilizing effect of the intended arm movement (1). We have examined the characteristics of these associated postural responses (APR's) during arm movements with a view toward determining some organizational features of the interaction between voluntary and APR activities.

Standing subjects held a handle in the right hand and either pulled (using elbow flexion) as quickly as possible following an auditory signal or were instructed either to resist or not to resist the perturbation. In one group of trials the subjects were allowed to lean their chests against a rigid cross-beam support ("supported trials") and in another group they stood unsupported ("free-standing trials"). Surface EMG electrodes recorded the activity of biceps brachii and involved muscles of the ipsilateral calf and thigh while mechanical transducers monitored anteroposterior sway angle and handle force.

During free-standing trials, APR's in leg muscles preceeded tone-triggered biceps activity by 10-40 ms. When the handle pulled unexpectedly upon the subject's arm, APR's occurred 50-60 ms following the onset of displacement (both instructions). These latter APR latencies were at least 40-50 ms faster than the fastest reaction times, possible when subjects intentionally activated either leg or arm muscles following a tone. When instructed not to resist, EMG activity in biceps was rarely discernable preceeding the APR, while an instruction to resist vigorously almost always resulted in biceps activation at myotatic (25-30 ms) latencies. During all supported trials, APR's were rarely discernable and biceps reaction times to tones were 10-150 ms shorter compared to unsupported trials. Biceps responses to unexpected pulls usually occurred at myotatic latencies (25-30 ms), regardless of the prior instruction. Finally, when subjects supported themselves with the handle during platform movements, APR's occurred in arm muscles at 50-60 ms latencies, while normal postural adjustments in leg muscles (90-110 ms latencies) were attenuated.

These findings suggest: 1) a hierarchically low level of organization of APR's because of their rapid action and versatility of organization, 2) the magnitude of the APR appears to be modulated by the "postural set" of the subject since it is markedly reduced or absent when postural demands are reduced, 3) an interdependence of APR's and voluntary movements because both voluntary reaction and stretch response times in biceps were delayed with an increase in the postural demands of the task. (1) El'ner, A.N. Biophysics 18, 966-971 (1973).

135.11 EFFECT OF CHLORPROMAZINE ON H-REFLEX RECOVERY CURVE. John Metz, Henry H. Holcomb*, and Herbert Y. Meltzer. Laboratory of Biological Psychiatry, Illinois State Psychiatric Institute and Dept. Psychiatry, University of Chicago, Chicago, Ill.

We have found that the recovery curve of the H-reflex, an electrically evoked monosynaptic spinal reflex, is abnormally high (fast) at intervals from 50 to 300 msec in about 25% of schizophrenic and affective disorder patients (Metz et al., Psychological Medicine, in press). We are now conducting a series of acute pharmacological studies in order to understand the neural transmitter mechanisms which may underlie this abnormality. Here we report that chlorpromazine (CPZ) significantly decreases the H-reflex recovery curve in a dose-related fashion in normal young adults.

We administered saline, 12.5 mg CPZ, or 25.0 mg CPZ intramuscularly to a total of 16 paid volunteers, 5 males and 11 females, aged 22 to 34 (median 27). Most subjects received all 3 doses on different days, with at least 3 days intervening between treatments. All subjects abstained from food, cigarettes, and caffein throughout the experimental day and for at least 9 hours preceding the first test. Subjects were tested on the H-reflex recovery curve according to standard procedures between 9 and 11 A.M. (baseline condition); they were then given an injection of CPZ or saline and were retested 2 hours later (drug condition). In each condition, we determined the ratio of an H-reflex to an equal stimulus reflex (at maximum H amplitude) at 10 interstimulus intervals between 50 and 300 msec. The average of these 10 values constituted our measure of recovery for that condition.

There were no statistically significant differences in the conditioning reflexes or direct muscle responses between baseline and drug conditions at any of the doses used. There were no recovery differences among baseline values obtained on saline, 12.5 mg or 25.0 mg CPZ days. There was also no difference between the baseline and saline condition. In contrast, both groups which received CPZ had recovery values significantly lower than baseline ($F=7.06$, $p<.005$; Scheffe comparison to saline, $p<.05$ for 12.5 mg group, $p<.01$ for 25.0 mg group). Eight of the 10 subjects who received both 12.5 mg and 25.0 mg CPZ had markedly lower recovery at the higher dose.

The dose-related reduction in H-reflex recovery following CPZ suggests that the excitability of the alpha-motoneurons may be controlled by catecholamines acting at either spinal or supraspinal levels. This finding is also consistent with an interpretation that abnormally high recovery curves in unmedicated psychiatric patients may be related to higher than normal catecholamine activity or sensitivity in these patients.

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135.12 EFFECTS OF BACLOFEN, GABA MIMETICS AND ADRENERGIC AGENTS ON THE FLEXOR REFLEX OF THE CHRONIC SPINAL RAT. J.G. Nutt, A.M. Marquez* and W.R. Woodward. Dept. of Neurology, Univ. of Oregon Health Sci. Ctr., Portland, OR 97201.

The antispasticity agent, baclofen, reduces flexor spasms (electrophysiologically analogous to the flexor reflex) in cord injured humans. As baclofen has some structural and physiological similarities to both GABA and phenylethylanine, we have compared the effects of baclofen with those of GABA-mimetics and adrenergic agents on the flexor reflex of chronic spinal rats.

The reflex was studied in rats three or more weeks after midthoracic cord transection when extensor spasms and hyperactive flexor reflexes were present. The rat was immobilized in a plexiglass restrainer, the flexor reflex elicited by constant current stimuli applied to the foot and the respnse measured by a force transducer attached to the leg. The parameters examined included maximum force, integrated force and duration of reflex response, as well as, threshold for eliciting the reflex. In chronic spinal animals, the maximum force of the flexor reflex is decreased and the duration increased as compared to that of intact rats.

Baclofen (3 and 6 mg/kg, i.p.) dose dependently reduced duration, and integrated force, increased threshold stimulus and slightly augmented maximum force. The "GABA facilitator" and antispasticty agent, diazepam (0.5 and 1.0 mg/kg), reduced maximum force, duration and integrated force of the reflex and increased threshold stimulus in a dose responsive manner. The GABA agonists, muscimol (up to 4 mg/kg) and THIP (5,10,and 20 mg/kg), produced catatonia and sedation respectively, without altering the parameters of the flexor reflex. The GABA transaminase inhibitor vinyl GABA (1000 mg/kg), doubled dorsal spinal cord GABA concentration but did not influence the reflex.

The α_2 adrenergic agonist, clonidine (0.06 to 0.5 mg/kg) increased the maximum force in a dose dependent manner and reduced the reflex duration but with no evident dose dependency. At low doses of clonidine the reflex force profile was similar to that produced by baclofen. In addition, preliminary studies indicate that the α_2 adrenergic antagonist, yohimbine (1mg/kg) antagonized the actions of baclofen and low doses of clonidine on the reflex.

In conclusion, the effects of baclofen on the flexor reflex of the chronic spinal rat did not resemble those of GABA-mimetics. There are, however, similarities between the actions of baclofen and those of the adrenergic agonist clonidine. The clinical implication is that GABA-ergic agents are unlikely to benefit patients with flexor spasms but that α_2 adrenergic agonists deserve further investigation.

This work has been supported by the Oregon Medical Research Foundation.

135.13 PHYSIOLOGICAL TESTS FOR THE STUDY OF SKELETOFUSIMOTOR (BETA) INNERVATION IN THE CAT. Léna Jami*, K.S.K. Murthy and J.E. Petit* (SPON: H.H. Kaufman). Laboratoire de Neurophysiologie, College de France, 75231 Paris-5, France.

Recent studies employing the technique of glycogen-depletion of muscle units have uncovered the existence of skeletofusimotor (beta) innervation by fast conducting alpha motoneurones in addition to the well established slow beta axons (Harker et al. J. Neurophysiology 40: 791-799, 1977). It is thus of interest to establish the physiological tests necessary for the adequate identification of beta fusimotor action exhibited by an alpha motor axon.

Experiments were performed on cats anesthetized with sodium pentobarbital. Up to ten muscle spindle afferents from peroneus tertius were isolated in dorsal roots L_7 or S_1. Single alpha motor axons isolated from ventral root filaments which increased the rate of discharge of any of the spindle afferents were subjected to a detailed investigation involving the following tests:

1. Test for the ability of the spindle afferent to respond to a sudden increase in stimulus rates, well above the frequency for maximal extrafusal tension.
2. Test for the occurrence of unfused "frequencygrams" of the spindle afferents (Bessou et al. J. Physiology 196: 37-45, 1968), when the motor axon was stimulated at rates above the tetanic fusion frequency for extrafusal tension.
3. Test for fusimotor action during a concurrently applied muscle stretch.
4. Test for difference in the fatiguability of junctional transmission between extrafusal and intrafusal end plates by repetitive high frequency tetanic stimulation.

A total of 116 motor units were studied. Thirty-six of these (31%) were identified as beta with the fusimotor actions of static versus dynamic in the ratio of 2:1. Static beta axons were found among the faster conducting alpha population (76-104 m/sec) compared to the dynamic beta axons which were exclusively among the slower conducting units (56-79 m/sec).

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135.14 DISCHARGE PROPERTIES OF MUSCLE SPINDLE AFFERENTS IN FATIGUED MUSCLE. D. L. Nelson* and R. S. Hutton, Dept. of Kinesiology, University of Washington, Seattle, WA 98195.

Fatigue to 60-50% maximum tetanic tension was induced in the isolated gastrocnemius muscle in five cats by sustained, 7xthreshold electrical stimulation (100Hz) of the cut L_7VR and S_1VR. Group Ia and II afferent responses to ramp stretches (5mm) and vibration (100Hz) applied to the Achilles tendon were monitored before and immediately after muscle tetany. Firing characteristics were similar when results from slower (summarized below) and faster (25mm/s) ramp stretches were contrasted.

Muscle Spindle Afferent Responses to Ramp Stretch (5mm/s) in Fatigued Muscle

	Resting Discharge				Static Response		
	↑	↓	NC	NA	↑	↓	NC
Ia	24	8	3	14	10	32	7
II	12	5	0	9	9	14	3
Sum(%)	36(48)	13(17)	3(4)	23(31)	19(25)	46(61)	10(14)

	Dynamic Sensitivity (Mean Frequency)			Dynamic Response (Peak)		
	↑	↓	NC	↑	↓	NC
Ia	30	12	7	19	22	8
II	15	6	5	14	11	1
Sum(%)	45(60)	18(24)	12(16)	33(44)	33(44)	9(12)

	Vibration Response				Response Latency		
	↑	↓	NC	NA	↑	↓	NC
Ia	42	3	3	0	3	45	1
II	19	3	2	2	1	22	3
Sum(%)	61(82)	6(8)	5(7)	2(3)	4(5)	67(90)	4(5)

↑ (increase), ↓ (decrease), NC (no change), NA (not active)

During muscle fatigue, both afferent types predominantly decreased in response latency to displacement and increased in resting discharge, mean frequency during stretch and frequency of firing to vibration. Static responses were generally lower indicating a decrease in position sensitivity. Resting muscle force and passive peak muscle stiffness were consistently higher following contraction. Under the same experimental conditions, we have previously reported (Med. Sci. Sport, 11:76, 1979) that group Ib excitability to passive stretch or muscle twitch is depressed for several seconds after sustained muscle tetany. It is therefore proposed that the sum effect of these adjustments in proprioceptive afferent and intrinsic muscle responses would be to reflexly and mechanically increase muscle stiffness during subsequent perturbations.

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135.15 SPIKE TRIGGERED AVERAGING OF EMG AS AN INDICATOR OF SKELETOFUSIMOTOR ACTION ON MUSCLE SPINDLE RECEPTORS. W. Zev Rymer, F.R. Edwards* and C.L. Cleland (Spon: A.R. Gibson), Depts. of Physiology and Neuroscience Program, Northwestern University Medical School, Chicago, Illinois 60611.

In previous presentations, we have argued that changes in muscle spindle receptor discharge arising above extrafusal threshold are legitimately attributed, at least in part, to actions of skeletofusimotor neurons on intrafusal muscle fibers. However, these arguments were indirect in nature, and more direct evidence supporting skeletofusimotor action is to be preferred. If skeletofusimotor innervation arises simply as branches from ordinary (i.e., skeletomotor) axons, then it follows that changes in intrafusal state should be paralleled by discernible changes in skeletomotor state. Such changes in intrafusal state would be expected to induce modifications in spindle receptor discharge.

We examined the discharge of primary spindle receptor afferents from the soleus muscle of the decerebrate cat preparation. This discharge was recorded from small natural dorsal root fascicles; most of the dorsal roots were left intact. Muscle force and length were recorded by appropriate transducers, and emg by extensively bared intramuscular nichrome wires (.005" diameter). Afferent discharge was recorded during spontaneous or reflexively induced changes in muscle force, under both isometric conditions and during changes in muscle length.

Evidence for skeletofusimotor discharge was sought by compiling a spike triggered average of soleus emg, force and length, using the afferent fiber spike to trigger this average. In approximately 50% of the primary endings, this spike was associated with an increase in emg activity in the soleus muscle arising about 5.0 msec. before the afferent spike. There was also typically a correlated force increase, peaking shortly after the spike occurrence.

It is argued that the prior increase in emg is precisely what would be expected if the intrafusal fibers were induced to contract by a descending impulse which also activated extrafusal muscle fibers. The increase in intrafusal tension would heighten the probability of discharge of the spindle receptor, while the impulse to extrafusal fibers would give rise to correlated emg and force increases.

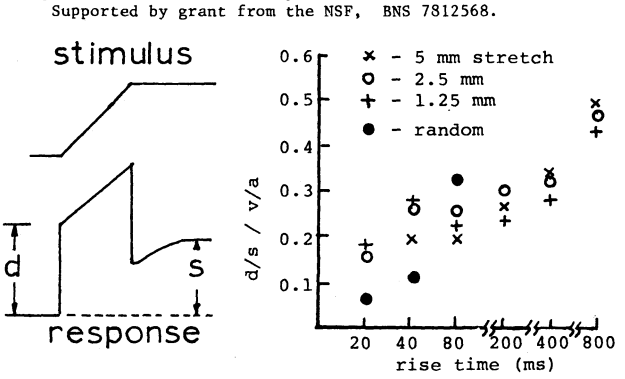
135.16 STRETCH DURATION DETERMINES THE RATIO OF VELOCITY TO PROPORTIONAL SENSITIVITY IN PRIMARY MUSCLE SPINDLE RECEPTORS. R.E. Poppele. Lab of Neurophysiology, University of Minnesota, Minneapolis MN 55455.

The behavior of primary endings of deefferented cat spindles to relatively large amplitude stretches was explored using randomly applied stretches and ramp-and-hold stretches. The response of the primary endings was expressed as a ratio of its dynamic to static responses to a ramp-and-hold stretch (see below). For a linear device, this fraction (d/s) should be proportional to the velocity-amplitude ratio (v/a) of the stimulus, so that the fraction (d/s / v/a) should be constant. For the spindle, that fraction is not constant, but depends on the amplitude to velocity ratio of the applied ramp or its rise time as plotted below for three stretch amplitudes.

The finding is confirmed with random stretches. Applied as a filtered Poisson process, the mean stretch velocity per unit amplitude could be varied by suitable choice of filter time constant and shot amplitude. A frequency domain analysis was done to determine the ratio d/s of the response. This was divided by the mean velocity to mean amplitude ratio of the stretch and is plotted as a function of the mean rise time of the random stretches below.

The results suggest that the primary endings, in the absence of fusimotor influence, provide predominantly velocity feedback in the presence of slow stretches and predominantly proportional feedback for rapid stretches.

Supported by grant from the NSF, BNS 7812568.



135.17 TENDON ORGAN RESPONSE TO RANDOM ACTIVATION OF SINGLE AND MULTIPLE MOTOR UNITS. E. K. Stauffer, G. P. Moore, and R. A. Auriemma*. Univ. Minn.-Duluth Sch. Med., Duluth, MN 55812 and Dept. Biomed. Engr., USC, Los Angeles, CA 90007.

Previous studies of motor unit-tendon organ (TO) interactions have tended to emphasize the stereotyped responses of these receptors to isolated twitches or short tetani. In this report we examine the effect of patterns of motor unit activity in which summation of individual twitch forces over time and between different motor units associated with a single receptor can take place.

Single slow twitch motor units from the cat soleus were functionally isolated from ventral rootlets at the L7-S1 level. Single TO afferents were identified and isolated from corresponding dorsal rootlets. Afferent responses were recorded during periodic (2 Hz) stimulation of each motor unit and PST histograms were constructed to show the average response of the TO spike train to periodic motor unit contractions. Average motor unit twitch force also was calculated. Following this the same TO was tested as motor units were stimulated with random Geiger counter driven pulse trains at mean rates of about 10 Hz for periods of 10-20 minutes duration, either singly or with multiple, concurrent, and independent stimulus trains.

When the firing pattern of one or more slow twitch motor units produces forces which exceed some minimum load, the TO undergoes a transition from a basically silent receptor with transient responses to a receptor more or less tonically active whose firing rate is modulated by the force pattern. Only when summation of random twitches from a single motor unit takes place is there distinguishable a time varying effect of each motor unit twitch on the TO firing probability, an effect characteristic of that motor unit and relatively unaffected by the existence or particular pattern of concurrent firing in other motor units.

Thus the average Ib afferent response has 1) a baseline discharge rate related to the mean total force coupled to the receptor capsule; 2) a transient burst associated with and characteristic of the rate of change of each motor unit's development; and 3) a slower, more prolonged response that closely follows the entire force profile of the twitch.

A striking nonlinear effect of twitch summation within a single motor unit was revealed by a new two dimensional PST histogram technique. Less dramatic nonlinear interactions were revealed when two or more motor units activated a common tendon organ. The random stimulus technique shows for the first time the dependence of tendon organ discharge on the entire twitch waveform (and hence type) of the motor units connected to its capsule and on the detailed firing pattern by which each is activated.

(Mn. Med. Found. and NIH grants #NS11298 and GM23732)

136.1 RECIPROCAL INHIBITION IN THE MEDULLARY RESPIRATORY NETWORK. Stephen R. Muza* and Donald T. Frazier. Department of Physiology and Biophysics, University of Kentucky, Lexington, KY 40536

A current notion with respect to the genesis of the respiratory rhythm involves the existence of two separate pools of brainstem neurons which are reciprocally inhibitory. If the hypothesis is true, maneuvers which increase inspiratory neural activity should cause expiratory neurons to receive more inhibition and vice versa. To test the hypothesis, experiments were conducted in dial-urethane anesthetized cats. Firing patterns of inspiratory (nucleus tractus solitarius-NTS) and expiratory neurons (nucleus retro-ambigualis-NRA) were recorded with extracellular glass microelectrodes. Inspiratory and expiratory neural activity was independently modulated by adding graded airway resistances either on inspiration or expiration. Since inspiratory and expiratory neural activity is 180° out of phase, the hypothesized inhibition would be arriving during the respective cell's silent period. However, when a cell is silent an extracellular electrode cannot distinguish whether the cell is silent because of lack of excitation or the presence of inhibition. Therefore glutamate, which raises the excitability of respiratory neurons, was used to unmask the existence of inhibitory inputs. L-glutamate was microiontophoretically applied to produce neural activity in the normal silent phase. Thus a reduction of the L-glutamate induced activity during resistive loading in the opposite respiratory phase would indicate the presence of an inhibitory input. The data was analyzed on a PDP 8/e computer which provided inspiratory (T_I) and expiratory (T_E) durations and plotted unit activity histograms. The presence or absence of an inhibitory input was statistically evaluated using the Sign Test. Application of L-glutamate substantially increased the firing rate of a single respiratory neuron throughout the entire breath but did not alter the animal's respiratory activity. As previously shown in our laboratory, inspiratory and expiratory resistive loading increased T_I and T_E respectively. Induced inspiratory unit activity during the normal silent period was decreased by expiratory resistive loading. Likewise, induced expiratory neuron unit activity was decreased by inspiratory loading. The occurrence of inhibition of the L-glutamate induced activity during resistive loading in the opposite phase was highly significant. The results suggest that NTS inspiratory and NRA expiratory neurons receive phasic inhibitory inputs during the opposing phase of respiration and are consistent with the reciprocal inhibition hypothesis.

136.2 RESPIRATORY AND MORPHOLOGIC CHANGES FOLLOWING KAINIC ACID (KA) LESIONS OF THE VENTROLATERAL NUCLEUS OF THE SOLITARY TRACT (v1-NTS). Albert J. Berger and Karen A. Cooney*. Dept. Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195.

KA may destroy CNS neurons having glutamate receptors while sparing axons in passage (Coyle et al. J. Comp. Neur. 180:301, 1978). Medullary respiratory neurons are markedly excited by glutamate (Denavit-Saubié et al. Brain Res. 155:55, 1978). Therefore we used KA to destroy neurons within the v1-NTS, which is known to contain a major concentration of respiratory neurons. These neurons may have a role in respiratory pattern generation.

Under anesthesia we exposed the dorsal medullary surface and using a microsyringe injected stereotaxically KA dissolved in mock cerebrospinal fluid (CSF). At 0, +1, and +2 mm rostral of obex 0.3 or 0.5 µg of KA was injected at each site, either unilaterally (2 cats) or bilaterally (5 cats). In 2 control cats mock CSF was injected. Two mos. later the medulla was removed. Cells of somal diameter ≥ 20 µm and lying in a quadrant of 300 µm radius placed ventrolateral to the tractus solitarius from 0-2 mm of the obex were counted. In two unilaterally lesioned cats the total number of cells was reduced from 305 and 353 cells on the unlesioned side to 74 and 81 cells on the lesioned side, respectively. The ipsilateral solitary tract and the dorsal motor vagal nucleus appeared normal, while the number of cells in the XII n. nuc. was also reduced.

Hyperoxic breathing patterns were determined before and after lesion placement during wakefulness and under pentobarbital anesthesia. Awake studies were done on 4-5 separate days prior to lesion and usually at 1, 2, 4, 6, and 8 weeks post lesion. Anesthesia studies were done 2 weeks prior to and 2 months post lesion. During wakefulness control and unilaterally lesioned animals did not have significant ($P > .01$) changes in any respiratory variable pre vs. post lesion. Some bilaterally lesioned animals did have significant ($P < .01$) changes: ventilation ($\dot{V}$) reduced (2 of 5); tidal volume (V_T) reduced (1 of 5); breathing rate (f) reduced (3 of 5); inspiratory time (T_I) increased (2 of 5); expiratory time (T_E) increased (2 of 5). All anesthetized unilateral and bilateral but not control lesioned animals exhibited marked reductions in $\dot{V}$ and f and marked increases in T_I and T_E, but no consistent change in V_T for any group. These results demonstrate that: 1) KA can destroy most of the v1-NTS. 2) During wakefulness rhythmic respiration is sustained and in some but not all animals is not significantly altered following bilateral KA lesion of the v1-NTS. 3) Anesthetized unilaterally and bilaterally lesioned animals have altered rhythmic respiratory patterns. We suggest that during wakefulness or anesthesia the v1-NTS is not solely responsible for respiratory rhythmogenesis. (Supported by USPHS Grant NS 14857 and RCDA NS 00378.)

136.3 ABSENCE OF FAST PERIODIC OSCILLATIONS IN PNEUMOTAXIC AREA NEURONAL DISCHARGE DURING SLEEP. Gary C. Sieck*, and Ronald M. Harper. Dept. of Anatomy and Brain Research Inst., UCLA Sch. Med., Los Angeles, CA 90024.

High frequency oscillations (HFO; 60-110 Hz) are present in phrenic activity and medullary respiratory unit discharge (Cohen, M. I., Acta Neurobiol. Exp. 33:198-218, 1973). Periodic stimulation of the nucleus parabrachialis medialis (NPBM; Pneumotaxic Area) can drive the HFO of both phrenic activity and medullary respiratory neuron discharge, suggesting that HFO of the respiratory system are effected by oscillatory input from the pneumotaxic area. Moreover, HFO appears to be susceptible to the state of arousal of the animal, as is the discharge of NPBM neurons (Sieck, G. C. and Harper, R. M., Exp. Neurol. 67:79-102, 1980.). The purpose of the present study was to examine the discharge of NPBM neurons during different sleep and waking states to determine whether the activity of this area displayed fast periodic oscillations that might drive the HFO of the respiratory system.

Adult cats were instrumented with electrodes for monitoring sleep and waking states. A bundle of 10 fine wire electrodes (62 µ insulated nichrome wire) was implanted in the NPBM through the guide tube of a miniature microdrive. During recording sessions, the animals were unrestrained except for a recording cable coupled to a connector atop the animal's head. The physiological signals were recorded both on analog tape and polygraph paper, and the two records were synchronized using a time code. Segments of the polygraph record were selected that represented samples of each sleep-waking state. The corresponding analog tape records were replayed, and the neuronal spike data were passed through a Schmitt trigger circuit. The times of occurrences of the neuronal events were stored in a computer file and periodicities in neuronal discharge were assessed by calculating autocorrelation functions (bin width=1 msec) on these data. The activity of a total of 60 NPBM neurons was recorded during each sleep-waking state. Fast periodic activity was not observed in the discharge of any NPBM neuron during any sleep-waking state, despite the presence of respiratory-related activity in many of these cells (N=27). In contrast, recordings of the activity of medullary respiratory neurons (N=10) in awake intact cats show the presence of HFO in the discharge of these neurons. Thus, these data indicate that fast periodic oscillations in activity present at several levels of the respiratory system are not present at the level of the pneumotaxic area. (Supported by Grant HL-22418-02 from the NIH, and by NS05349-03 PHS Research Fellowship Award to G.C.S.)

136.4 IS RESPIRATORY ACTIVITY EVERYWHERE? A. Netick* and J. Orem. Psychology Dept., Calif. State Univ., Hayward, CA 94542 and Dept. of Physiology, Texas Tech Univ. Sch. of Med., Lubbock, TX 79430.

After recording 800 mesencephalic neurons, none of which was ostensibly respiratory, we began to doubt the claim by Vibert and his colleagues (Neuroscience Letters,11:29,1979) that 38.5% of the cells in this region are respiratory modulated. Although there were differences in the preparations (our animals were intact, chronic and unanesthetized while theirs were semi-chronic and locally anesthetized with bilateral vagotomy and spinal section, these differences seemed less significant than the fact that both preparations avoided the use of general anesthetics. A more important difference between the studies was that they used computer techniques to discriminate respiratory cells while we simply looked at and listened to the neuronal activity. With this in mind, we tested the respiratory modulation index (RMI) developed by these authors to discriminate respiratory neurons (RMI≥.7). Computer simulations revealed that the RMI is an inadequate statistic that can and often does characterize nonrespiratory activity as respiratory.

We compared RMI with two standard statistical techniques: a parametric test, the analysis of variance (F), and a nonparametric test, Friedman's analysis of variance for ranked scords (χ^2). We used a Monte Carlo technique of drawing repetitively samples from simulated distributions of neuronal activity and allocating them to successive bins (n=22) representing the respiratory cycle. Allocation to bins was made randomly so that only a chance relationship existed between the simulated activity and the respiratory cycle. For F and χ^2, the theoretical rate of false positives was set at 5% using standard statistical tables. Over wide variations in sample size and in the shape of the distribution of spike activity, the actual rate of false positives conformed closely to the expected rates. In contrast, RMI, under the same conditions, wildly fluctuated with up to 100% false positives. Similarly when respiratory activity was simulated, χ^2 and F proved more sensitive to the relationship than RMI.

The RMI evaluated 54% of a sample of 41 midbrain cells as respiratory. In fact, only 12% and 7% were actually respiratory as determined by F and χ^2 respectively. These latter values are possible chance events, and although there may be some small percentage of respiratory cells in this region, the high incidence as reported by Vibert and coll. for this and other brain regions (Bertrand et al., Exp. Brain Res. 16:383, 1973) is based upon the faulty RMI and is highly questionable.

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136.5 DIFFERENTIATION OF CENTRAL AND PERIPHERAL ACTIONS IN SOMAN-PRODUCED RESPIRATORY ARREST. D.L. Rickett, M.L. Adams*, K.J. Gall*, S.F. Rybczynski*, and T.C. Randolph*. Physiology Branch, US Army Biomedical Laboratory, Aberdeen Proving Ground, MD 21010.

Soman (1,2,2-trimethylpropyl methyl-phosphonofluoridate), an organophosphorus anticholinesterase, resists treatment with atropine and/or oximes. This is thought to be due to the rapid rate at which the soman-acetylcholinesterase complex ages. The lethality of this agent is generally attributed to respiratory arrest. Recent demonstrations of the efficacy of the quaternary carbamate pyridostigmine (3-dimethylcarbamoyloxy-1-methyl-pyridinium bromide) in raising the subcutaneous LD50 of soman have raised questions concerning the relative importance of the central and peripheral effects of soman in producing respiratory arrest. The present study was designed to differentiate acute central and peripheral effects of soman in Dial anesthetized cats. Medullary respiratory-related unit activity, contralateral phrenic nerve activity, diaphragmatic electromyographic activity and contractions, blood pressure, and airflow were monitored. Soman was infused via the femoral vein at the rate of 1 ml/min (15 ml equivalent to an iv LD50). Soman infusion was stopped when spontaneous respiration ceased. At that time, neuromuscular blockade was tested by administration of supramaximal stimulation of the phrenic nerve. The results show that at the time of soman-produced respiratory arrest there was a loss of synchronous firing of respiratory-related units. Furthermore, contractions elicited by phrenic nerve stimulation demonstrated that the neuromuscular junction was not blocked. These contractions were subsequently blocked by Flaxedil. The present data support the notion that soman-produced respiratory arrest is mediated centrally and that, while soman's peripheral effects may be important, they are not the primary cause of respiratory arrest. The central actions of soman remain to be elucidated.

136.6 THE NEUROCHEMICAL MECHANISM BY WHICH AMINOPHYLLINE STIMULATES RESPIRATION. R. A. Mueller, D. B. A. Lundberg* and G. R. Breese. Departments of Anesthesiology, Pharmacology and Psychiatry, Univ. of N. Carolina, Chapel Hill, NC 27514, and Dept. of Anesthesiology Sahlgrenska Sjukhuset, Göteborg, Sweden.

Aminophylline has been employed for several years as a respiratory stimulant in infants displaying the neonatal apnea syndrome. The present study examined the neurochemical basis of the aminophylline-induced increase in respiratory activity using halothane-anesthetized rats. Male Sprague-Dawley rats with cannulae in the tail artery, the trachea, the esophagus, and the peritoneal cavity were given 0.7% halothane in O_2 and placed in a closed body plethysmograph for recording of tidal volume, respiratory frequency, blood pressure and heart rate. After 20 min of the above mixture, the response to 5% and 10% CO_2 in oxygen was measured for 2 five min periods and hypoxia (10% oxygen, 90% nitrogen) for 5 min. After aminophylline administration (i.p.) the hypercarbic and hypoxic tests were repeated 15 min later. Brain concentrations of norepinephrine, dopamine, and serotonin were measured at the conclusion of these respiratory tests with aminophylline. Aminophylline increased basal and hypercarbia or hypoxia induced respiratory stimulation. Division of the ninth cranial nerve together with removal of the carotid body did not alter the response, whereas vagotomy changed the response from one of increased frequency to an increase in tidal volume. Both awake and anesthetized rats evidence a decrease in $PaCO_2$, and in awake animals a decrease in brain stem dopamine content was observed.

Treatment of infant rats with 6-hydroxydopamine, which selectively reduced brain stem dopamine content to less than 30% of control, abolished the effects of aminophylline on both basal and CO_2, or hypoxia-induced respiration when tested as adults. Neonatal treatment with 5,7-dihydroxytryptamine, which reduced brain stem serotonin content to less than 30% of vehicle treated controls, potentiated the effects of aminophylline on basal and stimulated respiratory parameters. Since haloperidol, a dopamine antagonist, blocked the response to aminophylline it is possible that aminophylline may stimulate respiration by indirectly altering dopamine receptor activation in the brain. Alternatively, aminophylline could be stimulating respiration by blunting the effects of tonic inhibitory serotonin receptor activation.

137.1 ANTAGONISM OF THE REGIONAL ELECTRICAL EFFECTS OF GAMMA-HYDROXY-BUTYRIC ACID BY PETIT MAL ANTICONVULSANTS. L. J. Bearden and O. C. Snead. Neurosciences Program, Dept. of Pediatrics, and Dept. of Biomedical Engineering, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Gamma-hydroxybutyric acid (GHB) is known to be a normal constituent of human and animal brain. An increase in the concentration of GHB in the brains of animals has been found to produce an unresponsive state which is characterized by a fixed gaze, and an accompanying spike-wave pattern in the electroencephalogram. These behavioral and electroencephalographic effects are quite similar to those observed during petit mal seizures. The similarity between the GHB induced behavioral depression and petit mal seizures is increased by the fact that clinically effective petit mal anticonvulsants also antagonize the behavioral and electrical effects of GHB. Since GHB has been found to inhibit the firing of dopaminergic cells in the pars compacta of the substantia nigra (Roth et al., in "Frontiers in Catecholamine Research", p. 567, 1973), this inhibition (via GABA receptors, Roth and Nowycky, Biochem. Pharmacol. 26:2079, 1977) has been proposed as the means by which the behavioral and electrical effects of GHB are achieved. However, this inhibition was not specific for dopaminergic cells. In addition, more recent experiments have shown that the inhibitory effects of GHB on nigral and neocortical cells are not mediated via activation of bicuculline sensitive GABA receptors (Olpe and Koella, Eur. J. Pharmacol. 53:359, 1979). Therefore, the mechanism of action and the neural origin of the behavioral effects of GHB remain undefined. The present experiments were conducted to examine the subcortical electrical effects of GHB and the alterations of these effects by petit mal anticonvulsants. Power spectral analysis of the electrical response of cortex, hippocampus, caudate-putamen, and ventral thalamus to GHB indicates a variable sensitivity of these tissues to the effects of this compound.

137.2 CHANGES IN LOCOMOTOR ACTIVITY IN RATS "KINDLED" IN THE AMYGDALA AND NUCLEUS ACCUMBENS SEPTI. C.L. Ehlers*, G.F. Koob, S.J. Henriksen, and F.E. Bloom (SPON: W. Vale). A.V. Davis Ctr. for Behav. Neurobiol., Salk Inst., La Jolla, CA 92037.

"Kindling" is a process whereby a progressive increase in neural and behavioral responsivity develops during spaced and repeated epileptogenic stimulation of specific brain sites. In the present study we sought to determine if changes in interictal behavior, initially assessed by monitoring locomotor activity, accompanies the development of "kindling" following electrical stimulation of the amygdala (AMYG) or Nucleus Accumbens Septi (NAS). Wistar rats were stereotaxically implanted with bipolar electrodes aimed at either the AMYG or NAS. Additional screws were placed in the calvaria over the frontal and occipital cortex. The kindling stimulus (a 1 sec train of 1 msec biphasic square wave pulses, 60 Hz, 200 A) was applied once daily until 3 fully-kindled convulsions were observed. Although fully kindled responses in NAS and AMYG animals were similar, the order of presentation of behavioral signs during the development of kindling was different, as was the number of stimulations necessary to produce fully-kindled responses. Control animals were implanted and handled but not electrically stimulated. Locomotor activity of kindled and control rats was assessed in hanging wire cages equipped with 2 photocell beams. Activity was measured as total beam interruptions counted and recorded every 10 minutes over 4 hr test sessions. NAS and AMYG kindled animals exhibited a significant decrease in interictal locomotor activity compared to implanted controls. Significant decreases in locomotor activity were also found post-ictally in rats monitored for 2 hours immediately following a kindled seizure. NAS and AMYG kindled animals also exhibited a significant attenuation of the locomotor-stimulating effects of amphetamine (0.5 mg/kg) administered post-ictally. All these effects were more prominent in NAS kindled animals compared to those kindled in AMYG or controls. These results suggest that interictal changes in behavior accompany the development of kindling in the rat, and that a differential response is seen in NAS vs. AMYG kindled animals (supported by DA 01785 and the Klingenstein Foundation).

137.3 KINDLING PHENOMENON: PHARMACOLOGICAL AND ELECTROPHYSIOLOGICAL CORRELATES. R.L. Borison, R. Kowatch*, P.J. Maple* and B.I. Diamond. Illinois State Psychiatric Institute, Mount Sinai Hospital, and Rush Medical College, Chicago, IL 60612.

The kindling phenomenon was first described electrophysiologically as repeated subconvulsive stimulation inducing generalized seizure activity. Pharmacological kindling has now been described behaviorally for drugs which work via brain dopamine systems, such as L-DOPA. We now report experimental studies correlating pharmacological kindling and behavior, with electrophysiological events. Subjects were white male Sprague-Dawley rats, with recording electrodes stereotactically placed bilaterally into the caudate-putamen nucleus and the nucleus accumbens (NA). Animals were treated daily with either d-amphetamine (dA) (3.75 mg/kg) or phenylethylamine (PEA) (50 mg/kg) in doses per se subthreshold for producing behavior. Both of these drugs are capable of producing stereotyped behavior and psychosis in man. After drug injections, the EEG was recorded while the animal was freely moving. We found that repeated injection of PEA or dA resulted in a gradual increase in stereotyped behavior during the first three weeks and plateauing at five weeks. The EEG records showed that acute or subacute dA or PEA failed to affect electrical activity. However, by week three, there was a significant increase in fast, high amplitude activity lateralized to the right caudate in dA treated animals, and the right NA in PEA treated animals. At five weeks, PEA treated animals showed bilateral high amplitude activity in the NA, whereas dA treated animals showed similar activity, but localized to the caudate-putamen. The behavior induced by dA and PEA are identical, yet the EEG indicates differing sites of origin. Moreover, changes in behavior were correlated with changes in EEG activity, indicating that electrical kindling is coincidental with behavioral kindling.

(Supported by the State of Illinois Department of Mental Health and Developmental Disabilities grant #8009-11)

137.4 LATERALIZATION OF STATE-DEPENDENT LEARNING PRODUCED BY HIPPOCAMPAL KINDLED CONVULSIONS. K. A. Stokes* and D. C. McIntyre* (SPON: Dr. Metuzals). Physiological Psychology Labs, Carleton University, Ottawa, Ontario, Canada, K1S 5B6.

Convulsions kindled from the amygdala readily produce state-dependent learning. The hippocampus also kindles easily, and because of its implication in learning and memory, it was decided to study the state-dependent effects of hippocampal kindling. Electrodes were implanted in either the left or right hippocampus of 87 male Wistar rats and kindling was begun. One week after the fifth generalized convulsion, the rats were trained in an inhibitory avoidance task under one of two conditions: normal (N) or convulsed (C). Testing took place 24 hours later under either the same or changed state conditions. Thus, there were four groups for each hemisphere: N/N, C/C, N/C and C/N. Results showed that animals trained and tested in the normal state, for both hemispheres, could learn and retain the task adequately. For both hemispheres, animals trained in the normal state while tested in the convulsed state were severely impaired in their retention. Differences between the two hemispheres occurred in the C/N and C/C conditions. Left C/N animals showed markedly impaired retention, while right C/N animals were less impaired, although significantly different from normals. Left C/C animals were somewhat impaired in their retention, while right C/C animals were comparable to normals and controls in their retention. Seizure data could not adequately account for these differences beyond a tendency for the left hemisphere to give longer electrographic afterdischarge than the right hemisphere. Thus, a convulsion produces an encoding deficit as well as a recall deficit in the left hemisphere, but only a recall deficit in the right hemisphere, except if the state remains the same at training and testing. The results were interpreted as a hemispheric asymmetry, either in terms of relative impairment of function after a convulsion (functional lesion effect) or in terms of lateralization of information processing.

137.5 INCREASE IN SENSITIVITY TO CONVULSANTS FOLLOWING INTRAVENTRICULAR INJECTION OF GANGLIOSIDES. Karpiak, S.E. and Rapport, M.M., Div. of Neuroscience, N. Y. State Psychiatric Inst. and Depts. of Psychiatry and Biochemistry, Columbia Univ., New York, 10032.

Several studies (1-4) have implicated brain gangliosides in seizure activity. Work in our own laboratory showed that the intracerebral injection of antibody to GM1 ganglioside induces recurrent epileptiform activity. Both Westmoreland et al. and Yu et al. have reported increases in ganglioside content in animals following chemically induced seizures. Recently Kostic et al. reported ganglioside changes in the CNS following pentylenetetrazol (Metrazol) induced seizures in the rabbit. The following study was undertaken to determine if an intraventricular injection into the rat brain of brain gangliosides would decrease or otherwise alter seizure thresholds to Metrazol. Metrazol thresholds were determined (5) in rats (Sprague-Dawley, 250 grams) on Day 1. On Day 2 the rats were given (under ether anesthesia) an intraventricular injection of 10 mg of total brain ganglioside in saline (10 µl). Controls were injected with saline. On Day 3 Metrazol thresholds were redetermined. Animals injected with gangliosides showed a decrease in threshold ($p<0.01$) whereas thresholds of controls were unaffected. The results indicate that gangliosides injected into the brain can alter the properties of neurons in such a way as to increase seizure susceptibility. Since the mechanism of action of Metrazol is hypothesized to be that of a "synaptically acting convulsant" which activates excitatory synapses rather than blocking inhibitory synapses (6), exogenously administered gangliosides may be exerting their effect on excitatory synaptic pathways. It will be of interest to see if gangliosides affect seizure thresholds induced by convulsants which block inhibitory pathways.

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137.6 DENDROTOXIN: CONVULSANT AND POSSIBLE ANTICHOLINERGIC ACTION IN FROG SPINAL CORD. P.A. Smith, A.L. Padjen, M. Quik* & B. Collier. Dept. of Pharmacology, McGill Univ., Montreal, H3G 1Y6, Quebec.

Several fractions of the toxin (DTOX) isolated from the venom of Dendroaspis viridis (the green mamba) have been proposed as ligands for the study of nicotinic receptors in the nervous system (1). In the present study the sucrose gap technique was used to record potentials from the dorsal and ventral roots of the isolated hemisected frog spinal cord. Fractions of DTOX were tested as potential antagonists of synaptic and amino acid evoked responses. A fraction of DTOX resembling 4.11.3 of Banks et al. (2) induced spontaneous and stimulus coupled rhythmic convulsant discharges in both roots (cf.1). These convulsions, which appeared within 7 min of toxin exposure, were not the result of the antagonism of neutral amino acids since DTOX did not block dorsal and ventral root responses to GABA, glycine, β-alanine or to glutamate and aspartate (measured in the presence of tetrodotoxin or high calcium low magnesium Ringer). This same toxin fraction also completely antagonised the ventral root evoked dorsal root potential (cf.1) (VR-DRP). Unlike its convulsant action, this second action of DTOX occurred only after 30-50 min exposure to the toxin and could be partly reversed after 24 hours washing. The convulsant action of the toxin persisted even after 40 hours washing. This may indicate that these two actions of the toxin occur through independent mechanisms. Since DTOX was ineffective in antagonising responses to putative amino acid neurotransmitters it is possible that its selective blockade of VR-DRP resulted from an action at the cholinergic synapse of the polysynaptic VR-DRP pathway. We were unable, however, to protect the VR-DRP from the action of the toxin with the nicotinic antagonist dihydro-β-erythroidine. These experiments therefore raise some doubt as to the value of DTOX as a ligand for central nicotinic receptors especially since we were unable to reduce the binding of I^{125} DTOX to brain or spinal cord members with the cholinergic ligands, nicotine or d-tubocurarine (10^{-4}M). We also found that DTOX did not block nicotinic receptors in bullfrog sympathetic ganglia. If, however, DTOX does block VR-DRP by an action at the cholinergic synapse this may indicate a differential sensitivity of central vs. peripheral synapses to this substance.

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137.7 KAINATE NEUROTOXICITY IS SUPPRESSED BY SOME ANTICONVULSANTS BUT NOT OTHERS. Terry A. Fuller and John W. Olney. Washington University School of Medicine, St. Louis, MO 63110.

Systemically administered kainic acid (KA), a potent excitotoxic analog of glutamate, induces convulsions and acute neuronal necrosis in several regions of the adult rat brain, including hippocampus, amygdala, olfactory cortex and lateral septum. Previously we reported that pretreatment with diazepam (20 mg/kg) effectively inhibits seizures and markedly suppresses the brain damage so that only mild histopathology was detected in the CA3 and CA4 regions of the hippocampus and occasionally in the lateral septum or the amygdaloid complex. Others have reported that the neurotoxicity induced by direct intra-hippocampal injection of KA is suppressed, not only by diazepam, but by other anticonvulsants, including phenobarbital (PB), and diphenylhydantoin (DPH). Here we report that phenobarbital (PB), like diazepam, suppresses both the convulsions and the brain damaging effects of systemically administered KA but large doses of DPH or valproic acid (VA) do not suppress either phenomenon.

Adult male Sprague-Dawley rats were injected subcutaneously(sc) with KA (12 mg/kg). Pretreatment 15 min to 1 hr earlier was either with an anticonvulsant sc or an equal volume/weight of H_2O sc. Brain regions of interest were examined by light microscopy at survival times of either 4 or 24 hrs. In control animals receiving only KA + H_2O (n=27) the incidence of both convulsions and brain damage was 96%. In animals pretreated with 50 mg/kg PB (n=16) there were no convulsions and brain damage, restricted to very mild changes in the CA3-CA4 hippocampal region and occasionally lateral septum or amygdaloid complex was detected in 62.5%. DPH at 50 mg/kg (n=13) or 100 mg/kg (n=6) and VA at 27 mg/kg (n=6), 100 mg/kg (n=7) or 400 mg/kg (n=7) exerted no suppressant effect on either convulsions or brain damage; 100% of animals in all groups sustained both convulsions and brain damage. In a subgroup of rats (n=3) we administered DPH (50 mg/kg) chronically for 8 days prior to KA treatment and this chronic pretreatment regimen also was ineffective in suppressing either convulsions or brain damage.

Our finding that the potent convulsive property of KA is inhibited by diazepam and phenobarbital but not by diphenylhydantoin or valproic acid and that the agents which suppress convulsions also suppress KA-induced brain damage, suggest that KA-induced convulsions and brain damage are closely associated phenomena. These findings lend additional credence to the usefulness of KA as a tool to study epilepsy and the condition known as "epileptic brain damage" that has been reported in human epilepsy. Supported by NIH grants NS-09156, DA-00259, ES-07066 and RSD Award MH-38894 (J.W.O.). The valproic acid was a gift from Abbott Laboratories.

137.8 NEUROPATHOLOGIC AND BLOOD BRAIN BARRIER CHANGES IN KAINIC ACID INDUCED LIMBIC SEIZURES. D.K. Zucker, E.W. Lothman and G.F. Wooten; Depts. of Neurology & Psychiatry; Wash. Univ. Sch. Med., St. Louis, MO 63110.

Electrical and 2-deoxyglucose autoradiographic studies have shown that intravenous kainic acid (KA) is a reliable means of selectively provoking seizures in limbic centers. The severity of the seizure activity varies with the dose of KA, and can be graded with behavioral markers (Neurology:30, 386, 1980). Injections of greater than 7 mg/kg of KA cause neuropathologic changes in the same regions as the electrical seizures (Soc. Neurosci. Abstr. 4:712, 1978).

We have done a dose/time study of the neuropathologic changes associated with intravenously administered KA in adult rats. These changes were correlated with the animal's behavior and changes in the blood brain barrier permeability, studied with α-aminoisobutryic acid (αAIBA). Animals received 1, 4, 7 or 12 mg/kg of KA and were observed over intervals from 1/2 hour to 7 days. At the end of the given interval they received 10µCi of ^{14}C-α-AIBA (New England Nuclear, 51.6 mCi/mmol) intravenously. Ten minutes later the animals were perfused and decapitated. The brains were studied with ^{14}C-autoradiography, and Nissl and H. & E. Stains.

Behavioral response to KA was variable at a given dose level. Necrosis of neurons and neuropil was seen in entorhinal cortex, hippocampal formation, amygdaloid nuclei, and thalamic nuclei in the animals who had major motor seizures. Only scattered neuronal changes were seen in animals with seizures of lesser intensity. Increased blood brain barrier permeability to α-AIBA was seen acutely only in those animals who had major motor seizures. These changes were seen as early as 1 hour after injection. The distribution of the increased permeability seen acutely conformed to the pattern of necrosis recognized after longer survival in other animals with major motor seizures. Minor seizures did not lead to changes in blood brain barrier permeability.

These results show that, with a given dose of KA, behavior is a reliable prediction of ultimate neuropathologic findings. It is suggested that, in the KA model, the breakdown of blood brain barrier is an epiphenomenon to tissue necrosis.

137.9 FACILITATION OF PENICILLIN-INDUCED INTERICTAL SPIKES BY ATROPINE
Jeffrey H. Goodman*and R. M. Lebovitz. Dept. of Physiol., Univ. Texas Health Sci. Center at Dallas, Dallas, Texas 75235

Recent experiments have shown that atropine increases the interictal spike rate in the amygdala of kindled rats. This effect of atropine is believed to be mediated through a cholinergic mechanism (Fitz and McNamara, Brain Res.,178:117, 1979). It is unclear to what degree this result can be generalized to other forms of experimental epilepsy. The purpose of this study was to determine the effect of atropine on another seizure model,viz., the interictal spikes of epileptic foci created by the topical application of penicillin.

The left hippocampus of Long-Evans rats was exposed under nembutal anesthesia. Sodium penicillin was applied to the CA1 region of the dorsal hippocampus and this area was then covered with warmed artificial cerebrospinal fluid (CSF). Once a baseline interictal spike rate was established, the hippocampus was superfused with atropine sulfate dissolved in artificial CSF. The concentrations of atropine tested ranged from 10^{-5}M to 10^{-2}M. It was observed that atropine in doses ranging from 3×10^{-4}M to 10^{-2}M significantly increased the interictal spike rate. The latency of this facilitatory effect was dose dependent and the increased rate continued after the atropine was removed. Repeated washing with atropine-free artificial CSF did not cause a reversal of any facilitatory effect. Furthermore, subsequent superfusion with acetylcholine dissolved in CSF (10^{-2}M) did not reverse this effect.

When atropine was administered IV no facilitation of the interictal discharges was observed. In only one out of four animals in which atropine was injected IP (50mg/kg) did an acceleration of the interictal spike rate occur. This effect was not long-lasting.

It is well established that atropine has convulsive properties of its own (Daniels and Spehlmann, Electroenceph. Clin., Neurophysiol., 34:83,1973). Whether this apparent pacing of the penicillin focus is due to the epileptogenic action of atropine or through a direct cholinergic mechanism remains to be determined.

137.10 EFFECTS OF BEMEGRIDE ON THE SENSORY RESPONSES OF NEURONS IN THE RETICULAR FORMATION AND HIPPOCAMPUS.
C.L. Faingold, W.E. Hoffmann* and J.D. Stittsworth Jr. Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708.

Studies from this laboratory have shown that the sensory responses of brainstem reticular formation (RF) neurons are greatly enhanced following administration of subconvulsant doses of pentylenetetrazol or strychnine (Faingold, Neuropharmacology, 19:53-62, 1980. Soc. Neurosci. Abs. 4:142, 1978). This investigation was undertaken to examine further the generality of this action. The effects of bemegride (1.9 mg/kg/min i.v.) were evaluated on the neuronal responses of locally anesthetized, paralyzed and respired cats using poststimulus time histogram (PSTH) analysis. Many RF neurons which were not clearly responsive to visual, auditory or somatosensory stimuli before drug became responsive to one or more modalities of stimulus after subconvulsant doses of bemegride. Initially responsive RF neurons became more responsive after bemegride as shown by increases in amplitude and/or duration of the peaks in the PSTHs. This occurred in both mesencephalic and bulbar RF regions. Most hippocampal neurons examined thus far have shown relatively minor changes in response to sensory stimuli with bemegride, although a small number of hippocampal neurons showed some response enhancement at high doses. Some increase in stimulus-induced inhibition of firing of hippocampal neurons and modest decreases in PSTH peak latency were also observed. Simultaneously held RF neurons showed extensive response enhancement. These data suggest that hippocampal neurons are considerably less involved than RF neurons in the initiation of seizure generalization by sensory stimuli under these conditions. This finding and the observation of minimal convulsant-induced changes in lateral geniculate neuronal responses (Faingold and Stittsworth, Electroenceph. clin. Neurophysiol. 1980 in press) suggest a relative selectivity of the effect for the RF.

Bemegride-induced enhancement of the responses of RF neurons is quite similar to that seen with strychnine, pentylenetetrazol, and in preliminary studies with physostigmine. This neuronal mechanism subserves the enhancement of sensory evoked field potentials induced by these agents. These findings suggest that the 14 other convulsant drugs which also enhance sensory evoked field potentials (Faingold, Prog. Neuro-Psychopharmacology, 2:401-422, 1978) may also produce such neuronal response enhancement. These data further support the concept that the enhancement of RF neuronal responses to sensory stimuli is a general action of convulsant drugs which can contribute to the initiation of sensory-induced seizures.

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137.11 KINDLING AND CYCLIC NUCLEOTIDES: EFFECTS OF THEOPHYLLINE, DIBUTYRYL-cAMP, AND 8-BROMO-cGMP.
Donald P. Cain and Carol A. Routhier. Department of Psychology, University of Western Ontario, London, Ontario, Canada N6A 5C2.

The cyclic nucleotides have been implicated recently in cellular processes that could be involved in the neuroplastic changes thought to underlie the kindling effect. Thus, the effects of manipulations of cyclic nucleotide levels on the development of kindled seizures was assessed in male hooded rats. The animals carried bilateral amygdala electrodes and were electrically stimulated with low intensity square wave currents at 60 Hz following i.p. or intracerebral administration of one of the following drugs: theophylline, dibutyryl-cAMP, or 8-bromo-cGMP. Control groups received either the vehicle or artificial CSF. This procedure was repeated at 24 or 48 hour intervals until a generalized seizure developed.

The theophylline group developed seizures more rapidly and displayed longer afterdischarges relative to controls. The dibutyryl-cAMP and 8-bromo-cGMP groups also developed seizures significantly more rapidly than their respective control groups, but did not display longer afterdischarges. Various control procedures showed that the accelerated kindling was not due to a pharmacological kindling effect, and that the resulting kindling was permanent.

The results suggest that the cyclic nucleotides may play a role in the neuroplastic changes thought to underlie kindling.

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137.12 AUDITORY AND VESTIBULAR PATHOLOGY OF SEIZURE-PRONE CHICKS.
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The mutant sex-linked lethal recessive px (paroxysmal) gene, expressed in White Leghorn chicks (Gallus domesticus) causes seizures beginning on approximately day eight posthatching. Seizures are spontaneous and induceable by auditory but not by photic stimulation. Prior to seizure onset px chicks are indistinguishable from non-px siblings. With seizure onset is a decrease in food intake which causes deterioration and death by 4-8 weeks of age. In a preliminary histological study conducted on 22-day-old chicks, brains were perfused and treated according to an improved cupric-silver staining technique. Nuclei and fiber tracts of px auditory and vestibular systems were extensively degenerated; control brains showed essentially no degeneration. A second experiment was performed on 5, 10, 15, and 20-day-old chicks to determine whether and to what extent degeneration occurs prior to seizure onset. Nuclei of px cerebellum appeared to be the first affected (5 days of age). Extent of degeneration progressed steadily over time through 20 days of age, by which time all components of the 2 systems were maximally affected. In a third experiment, one group of px and control chicks was raised in a quiet to low-level noise environment; a second group was subjected to systematic noise stress from day of hatching. Noise did not affect onset or extent of degeneration, nor did it affect onset of seizures. A sequel study indicated that GABA levels are implicated in abnormal auditory function of px chicks.

137.13 REDUCED SUSCEPTIBILITY TO DRUG-INDUCED SEIZURES AFTER SOMATOSENSORY DEAFFERENTATION IN THE CAT. S.S. Bowersox* & M.B. Sterman. Brain Research Institute, University of California, Los Angeles & V.A. Medical Center, Sepulveda, CA 91343

We have evaluated the effects of somatosensory deafferentation on monomethylhydrazine (MMH)-induced seizures in adult cats. Enforced restraint was shown previously to decrease susceptibility to MMH seizures in cats (Bowersox, Siegel & Sterman, Exp. Neurol 61:154, 1978) and infrahuman primates (Sterman & Kovalesky, Exp. Neurol., 65:78, 1979). It was hypothesized that reduced somatosensory inflow contributed to these effects.

To test this hypothesis, eleven adult cats equipped with indwelling cortical and subcortical electrodes, received bilateral lesions of the dorsal columns at either high cervical (C_1-C_3) or low cervical (C_5-T_1) levels. After a three week postsurgical period, each animal was administered an intraperitoneal injection of monomethylhydrazine at the established convulsive dosage of 10 mg/kg. Behavior was monitored continuously, and seizure latency was measured from the time of drug injection to onset of tonic-clonic convulsions. Latency data were compared with those obtained from a similar group of non-lesioned animals. Results are given below:

	Range of Latencies (min)	Group Mean (min)
Intact (N=18)	41-80	59.5±4.05
Low Cervical (N=5)	60-93	81.4±6.29
High Cervical (N=6)	70-222	134.0±22.04

Data showed a progressive increase in seizure latencies with more encephalad transection of the dorsal columns. Multiple t-test comparisons of individual group data revealed significant differences between intact group latencies and those of both lesion groups (Intact vs. Low: $t=-2.10$; $p<0.05$, Intact vs. High: $t=-4.26$, $p<0.05$). There was no significant correlation, however, between extent of damage to the dorsal columns at each level and latency to seizure (Spearman Rank Correlation Coefficient; High: $r_s=-.77$, $p>0.25$, Low: $r_s=.58$, $p>0.25$).

Two possibilities might account for these findings. First, somatosensory deafferentation was associated with significant enhancement of specific electrocortical patterns known to correlate with the inhibition of motor functions (e.g., sensorimotor rhythms). Resistance to seizures observed here might relate to the facilitation of these patterns. Alternatively, protection against seizures might be provided by the reduction of cutaneous and proprioceptive input which normally constitutes a source of convulsion inducing excitatory feedback.

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137.14 ON THE LOCALIZATION OF STRUCTURES RESPONSIBLE FOR SEIZURE ANTAGONIZING EFFECT OF A MIDBRAIN TEGMENTAL LESION. R. A. Browning and F. J. Turner*. Southern Illinois University, School of Medicine, Carbondale, IL 62901.

We have previously reported that bilaterally-placed mechanical lesions of the midbrain tegmentum inhibit the hindlimb extensor (HLE) component of both maximal electroshock (MES)-and maximal pentylenetetrazol-induced seizures (Neurosci. Abst. 4, 139, 1978). These lesions were produced by lowering a stainless steel rod (1.2 mm diameter) 9 mm below the surface of the brain, 6 mm posterior to the bregma and 1 mm lateral to the midline. Histological evaluation of the lesion revealed damage to the superior colliculus and its commissure, periaquaductal gray (PAG), the dorsal and ventral noradrenergic (NA) pathways, superior cerebellar peduncles, lateral aspects of the tectospinal tract, and the reticular formation. In an attempt to determine which of the damaged structures is responsible for the seizure antagonizing effect of the lesion, we varied the location of the lesion within the midbrain in separate groups of rats, and examined the response to MES 2 weeks later. Histological evaluation of each lesion was also carried out. The present findings show that inhibition of the HLE component of the MES seizure is observed only if the lesion is bilateral, and only if the damage extends ventrally beyond the superior cerebellar peduncles (7-7.5 mm below surface of brain). Movement of the lesion 1 mm in the lateral direction, while retaining the same AP and ventral coordinates, spares the PAG and the NA fibers, but still produces seizure antagonism. Furthermore, movement of the lesion 2 mm in the anterior direction interrupts the ascending NA fibers, and the PAG, without producing seizure antagonism. On the basis of these findings we can suggest that the following structures are not involved in the seizure antagonizing effect of our original lesion: superior colliculus and its commissure, PAG, NA pathways, and the tectospinal tract. This leaves the superior cerebellar peduncles and the reticular formation as probable candidates. Studies are currently underway to determine whether either or both of these are involved.

137.15 AUTORADIOGRAPHIC IDENTIFICATION OF THE CORTICAL LAMINAE INVOLVED IN THE GENERATION OF A DISCRETE INTRACORTICAL EPILEPTIC FOCUS. A.B. Chatt and J.S. Ebersole. Dept. of Neurology, Yale Univ. School of Medicine, New Haven, Ct. 06510 and Neurology Service, VA Medical Center, West Haven, Ct. 06516.

Previously, we have described an in vivo model of epileptogenesis within the cat striate cortex induced by picoliter volumes of pressure-injected sodium penicillin (NaPen). These methods produced an epileptic focus that was: discrete, progressive, temporary and repeatable. Since response activity was being driven by natural visual stimuli, this model allowed neurons or neuron populations of interest to be characterized before, during, and after the development of an epileptic focus. Our objective, here, was A) to determine whether the methods used to induce this highly localized electrocorticographic epileptic focus could also be employed in determining whether a laminar sensitivity to NaPen exists within the striate cortex and, if so, B) to identify the cortical depths and layers (using 14-C) which are most sensitive to NaPen.

In the first series of experiments, ejections were made at each of several cortical depths (200,600,1100,1600,2000μ)within an animal and responses were recorded at the level of ejection. Using these methods, we have been able to isolate a band of intracortical tissue that is most sensitive to the epileptogenic effects of penicillin. This band is confined to cortical depths located roughly between 700-1600μ during relatively large volume ejections and to approximately 1000-1300μ when smaller more discrete volumes are employed. Penicillin ejections made either superficial to or deeper than these values were not effective in producing an epileptic focus. Since the site of maximal penicillin sensitivity always occurs where the response to visual driving is greatest, we feel that cortical layer IV may be the site of penicillin focus generation. It is in this layer that the sensory-specific subcortical afferents terminate most densely.

In our autoradiographic studies, microejections were made at these same cortical depths but were limited to 1 depth/animal. Following ejection and 3 minutes of recording time, the brain area of interest was excised immediately and prepared for autoradiographic analysis. The results, thus far obtained, support the preceeding data in demonstrating the largest concentration of [14-C] NaPen in cortical layers IV and V whenever epileptogenic abnormalities were seen. Discrete ejections made in other laminae (I-III,VI), which were unsuccessful in producing epileptogenesis, were largely confined to those layers with only an occasional sparse labeling of IV and V.

Future work will correlate the spatial extent of [14-C] NaPen with the level of epileptic abnormality induced.

137.16 MULTIPLE VS SINGLE HYPERTHERMIA-INDUCED CONVULSIONS IN THE DEVELOPING RAT. Corinne Manetto*, James A. McCaughran, Jr.*, and Nisson Schechter. (SPON: I. Fand). Departments of Psychology, Psychiatry and the Long Island Research Institute, SUNY at Stony Brook, Stony Brook, New York 11794.

We have proposed a model for human infantile febrile convulsions using hyperthermia-induced convulsions (HICs) in the rat pup. In this study, we compared the effects of a single HIC (SHIC) to multiple HICs (MHICs) to determine if additional changes in the parameters of the convulsion or later susceptibility to pentylenetetrazol (PTZ) will occur.

At 5, 10, 15, or 20 days of age rat pups were subjected to a SHIC, hyperthermia alone, or handled. In another group, pups were subjected to 7-10 HICs starting from 5 days of age and ending at 20 days of age. No more than 1 HIC was induced per day in the MHIC group of pups.

The latency to the HIC increased with age in both the SHIC and MHIC groups. However, at 15 and 20 days in the MHIC group the latency was significantly shorter than that of 15 and 20 day pups in the SHIC group (means = 8.8 min and 8.4 min vs 10.9 min and 11.5 min, respectively). The duration of the convulsion between the SHIC and MHic groups did not differ significantly at any age point. Within each group the duration of the convulsion in the 15 and 20 day pups was significantly longer than that of the 5 and 10 day pups ($ps < .01$). Furthermore, within each group the rectal temperature associated with the start of the HIC was generally higher in the older pups.

Rats subjected to a SHIC were more susceptible to the convulsant effects of PTZ as adults than rats that had been subjected to hyperthermia alone or handled (means = 9.5 min vs 11.7 and 12.1 min, respectively). Infants that had been subjected to MHICs were found to not only be more susceptible to PTZ as adults than rats from their control groups (means = 6.9 min vs 11.4 min, respectively) but were also more susceptible to PTZ than rats that had undergone a SHIC (means = 6.9 min vs 9.5 min, respectively).

Overall, the parameters of the MHIC are similar to those of the SHIC with the following exceptions. Although the rectal temperature did not differ between the MHIC and SHIC groups, the latency to the HIC in the 15 and 20 day MHIC pups was shorter than that of similar rats in the SHIC group. This is discussed in terms of a 'kindling-like' effect or a breakdown of the thermoregulatory ability of the rat. Furthermore, MHICs have a greater effect on the later susceptibility to PTZ than a SHIC. This result is discussed in terms of its similarity to human infantile febrile convulsions.

(Supported by the Long Island Research Institute).

137.17 HYPERTHERMIA-INDUCED CONVULSIONS IN THE RAT PUP: AN ANIMAL MODEL OF HUMAN INFANTILE FEBRILE CONVULSIONS. James A. McCaughran, Jr.* and Nisson Schechter. (SPON. R.E. Whalen). Departments of Psychology, Psychiatry and the Long Island Research Institute, SUNY at Stony Brook, New York 11794.

Infantile febrile convulsions are a serious pediatric neurological problem. Although the incidence of these convulsions is second only to head injuries in the United States, little experimental work has been done towards elucidating the underlying causes of this problem. To a large extent this can be attributed to the lack of an experimental animal model of the disorder. In the present report data is presented that supports the use of hyperthermia-induced convulsions (HICs) in the developing rat as a useful model of human infantile febrile convulsions.

At 5, 10, or 15 days of age rat pups were subjected to a single HIC, hyperthermia alone, or handled. The latency to the convulsion in the HIC group was significantly influenced by age with the youngest rats displaying the shortest latencies ($ps < .01$). The duration of the convulsion was also affected by age. Pups of 15 days of age had longer convulsions than either the 10 day or 5 day old rats ($ps < .01$). Although the older pups generally had higher rectal temperatures associated with the start of the convulsion, the effect of age on the rectal temperature was not significant.

Male and female rats that had been subjected to a HIC were more susceptible to the convulsant effects of pentylenetetrazol (PTZ) as adults ($ps < .01$). When the data were analyzed according to the age at which the HIC was elicited, only the 15 day old males differed from their appropriate control groups. These males were more susceptible to PTZ ($ps < .01$). The duration of the PTZ-induced convulsion was not different between any of the groups ($ps > .05$).

Induced hyperthermia using infrared radiation is a reliable and quantifiable technique for eliciting convulsions in infant rats. The parameters of the convulsion are age-related and are similar to human infantile febrile convulsions. The clinical data indicate that younger children are more susceptible to the convulsant effects of a febrile illness than the older children. We have found that younger rats are more susceptible to the convulsant effects of hyperthermia than older rats. Clinical reports also indicate that epilepsy is a major neurological sequela to severe febrile attacks. In the present study, a single hyperthermia-induced convulsion in infancy increased the susceptibility of adult rats to the convulsant effects of PTZ. These results indicate that a long-term change in the physiology of the central nervous system resulted from this early convulsion.

(Supported by the Long Island Research Institute).

137.18 SELF-INDUCED REDUCTION OF INTER-ICTAL HIGH VOLTAGE SHARP WAVES BY EPILEPTIC PATIENTS. W.J. Jackson, A.V. Nelson*, D.W. King* and J.L. Veal*. Depts. of Physiology, Biomedical Engineering and Neurology, Medical College of Georgia, Augusta, GA 30912

Several epileptic patients having prominent high-voltage sharp waves in their inter-ictal EEG's were given cues (4 hrs. per day for 7 days) upon the occurrence of the spikes. The spike detection circuitry was an expansion of techniques described by Whisler, et al. (Proc. Epilepsy International Symposium, Vancouver, 1978). During the training, the patients were instructed to accomplish two objectives: (1) To attempt recognition of mental events associated with the high-voltage sharp waves and (2) To attempt to reduce the frequency of the sharp waves by self-inducement of the mental state during which (in the patients' evaluation) the least number of sharp waves occurred.

Findings were concurrent with Stevens, Milstein, & Dobbs (EEG & Clin. Neurophysiol., 23: 57-66, 1967) in that none of the patients could recognize any mental event concurrent with the sharp waves. On this basis Stevens, et al. had hypothesized that operant conditioning would not be effective in reducing such sharp waves, but did not actually make the attempt. To the contrary, however, most of our patients demonstrated that they could reduce the numbers of sharp waves even without recognizing them. All successful patients reported that their strategy was to remain alert and to avoid drowsiness. Subsequent analysis of the patients' EEG's verified that there were more spikes present against a background EEG of 1-8 Hz than were present against a background of 8-16 Hz or 16-24 Hz.

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137.19 EATON-LAMBERT SYNDROME: DECREASED QUANTUM CONTENT OF NEUROMUSCULAR TRANSMITTER RELEASE PRODUCED BY SERUM FACTORS. Yong I. Kim,* Donald B. Sanders and T.R. Johns, (SPON: G.R. Hanna) Department of Neurology, University of Virginia School of Medicine and the University of Virginia Jerry Lewis Neuromuscular Center, Charlottesville, Virginia 22908.

The Eaton-Lambert syndrome (ELS) is a defect of neuromuscular transmission, frequently associated with carcinoma of the lung, characterized by a reduced number of acetylcholine (ACh) quanta released per nerve impulse. We wished to determine whether serum factors from a patient with this condition could produce in vitro a neuromuscular blockade resembling that found in that patient. Our findings show that ELS serum, when applied to rat skeletal muscle in vitro, produces a pronounced neuromuscular block as a result of decreased quantum content of the evoked transmitter release.

Using intracellular microelectrode recording techniques, direct quantal analysis was performed on rat flexor digitorum longus muscles bathed in a physiologic buffer solution containing a high magnesium concentration (10mM Mg^{++}). Control measurements were made of miniature end-plate potentials (MEPPs) and indirectly stimulated end-plate potentials (EPPs) from 10-15 end-plates. Similar measurements were repeated in the same muscle as it was perfused with the high-magnesium control solution containing either ELS or normal human serum.

Perfusing the muscle with normal serum produced no significant effect on the amplitudes of MEPPs and EPPs, and thus had no effect on the calculated ACh quantal content. ELS serum, however, applied under the same conditions, produced a marked reduction in the EPP amplitude, with no change in MEPP amplitude. Thus there was a reduction in the number of ACh quanta released from the motor nerve terminals. This prejunctional blockade of neuromuscular transmission was only partially reversed by prolonged washing with the control solution. Although normal and ELS sera caused a slight decrease in the frequency of MEPPs, neither had an effect on the resting membrane potential of the end-plates.

This study demonstrates that serum from a patient with ELS contains a factor that diminishes the release of ACh quanta from motor nerve terminals and can produce the physiologic abnormality characteristic of the syndrome. Future investigations are aimed at confirming this observation within a larger group of patients with the Eaton-Lambert syndrome.

(Supported by a center grant from the Muscular Dystrophy Association).

137.20 NEUROMUSCULAR BLOCKING PROPERTIES OF SUXAMETHONIUM AND DECAMETHONIUM IN NORMAL AND MYASTHENIC RAT MUSCLE. Bruce R. Johnson*, Yong I. Kim*, and Donald B. Sanders. Department of Neurology, University of Virginia School of Medicine and the University of Virginia Jerry Lewis Neuromuscular Center, Charlottesville, Virginia 22908.

The neuromuscular blocking properties of suxamethonium (Sux) and decamethonium (Deca) were compared in forelimb flexor digitorum longus muscle from normal rats, and from those with experimental autoimmune myasthenia gravis (EAMG). EAMG was induced by immunization with an initial subcutaneous injection of 50 µg Torpedo acetylcholine receptor protein in Freund's complete adjuvant and a booster injection of 25 µg given at least two weeks before experimentation. Conventional intracellular recording techniques were used to measure endplate depolarization and miniature endplate potential (MEPP) amplitude.

In normal muscle at 1, 5, 10, and 25 µM concentrations we found that Sux produces a greater depolarization than Deca. Depolarization with Sux was typically maintained until a saline wash. Depolarization produced by Deca tends to reverse after reaching its peak despite continued application of the drug. With both drugs MEPP amplitude reduction is maintained until a saline wash and begins and ends more quickly than does the depolarization. EAMG endplates respond in a similar qualitative manner to each drug. However, the induced depolarization is significantly less in EAMG than in normal muscle; it occurs and recovers at a slower rate than in normal muscle; and it appears to be proportional to the MEPP amplitude measured before drug application. When MEPP amplitude was reduced in normal muscle by treatment with 0.03 µg/ml d-tubocurarine, the depolarization induced by Sux and Deca was similar to that in EAMG muscle. In both EAMG and normal muscle neostigmine (1 µM) antagonized the MEPP amplitude reduction produced by Sux and Deca.

In human myasthenic muscle, the blocking effects of Sux and Deca are less than in normal muscle. Neostigmine potentiates the depolarizing effects of Deca in normal muscle, but reverses the neuromuscular block produced by Deca in myasthenic muscle. We have shown that curarized normal rat muscle and EAMG muscle also have diminished sensitivity to Sux and Deca. However, we found no differences in neostigmine and depolarizing drug interaction between normal and EAMG rat muscle. Thus, while EAMG may mimic myasthenia gravis in some respects, differences such as those observed in this study should be considered when extrapolating from the animal model to human myasthenia. (Supported by a Center grant from the Muscular Dystrophy Association.)

137.21 THE EFFECTS OF AZATHIOPRINE AND CYCLOPHOSPHAMIDE ADMINISTRATION ON THE PATHOGENESIS OF EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS. I. J. Griffith and N. L. Norcross*. Dept. of Microbiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853.

Myasthenia gravis (MG) is an autoimmune disease affecting humans. As clearly shown in the animal model for MG, antibodies capable of binding to the nicotinic acetylcholine receptor (AChR) are critical to the pathogenesis of the disease. Due to the disease's immune nature, many current therapies for MG involve the use of immunosuppressive agents. It is possible to use the animal model, designated as experimental autoimmune myasthenia gravis (EAMG), to evaluate the mechanisms of immunosuppression and effectiveness of these therapies in controlling disease pathogenesis. We report here the effect of two immunosuppressive agents, azathioprine (Imuran®) and cyclophosphamide (Endoxan®), on the pathogenesis of EAMG in the rat.

As previously described (Soc. Neurosci. Abstr. 4:397, 1978), rats inoculated with AChR purified from Torpedo characteristically develop two distinct periods of decreased activity corresponding to the recognized acute and chronic phases of EAMG. This activity assay was used as a sensitive and objective method with which to evaluate the effects of drug therapy on the development of the clinical weakness during disease pathogenesis. Activity was also correlated with levels of antibodies capable of binding to both Torpedo and rat AChR, AChR content of the limb muscles, and percentage of endogenous AChR complexed with antibody.

Azathioprine (AZA), an antimetabolite, has been used effectively in the treatment of many patients with MG. However, the drug had no effect on the pathogenesis of EAMG in rats when administered biweekly in intraperitoneal injections of 160 mg/kg beginning 9 days after inoculation with Torpedo AChR. This is in contrast with the finding that AZA can block the induction of EAMG when administered shortly after inoculation with AChR (J. Immunol. 117:225, 1976).

The intraperitoneal administration of cyclophosphamide (CY) at a dose of 100 mg/kg (60% LD_{50}) 14 and 28 days after inoculation with AChR had a pronounced effect on disease expression. This was reflected in activity, AChR content of limb muscles, and level of endogenous AChR complexed with antibody. CY administration did not affect the levels of antibodies capable of binding to Torpedo AChR in vitro.

The implications these studies have for understanding the pathogenesis of MG will be discussed.

(Supported by the Muscular Dystrophy Association of America.)

137.22 MOLECULAR FORMS OF ACETYLCHOLINESTERASE IN DYSTROPHIC MOUSE SKELETAL MUSCLE. K.A. Skau & W.S. Brimijoin, Mayo Foundation, Rochester, MN. 55901

It has been suggested that murine muscular dystrophy may be related to a "functional denervation" of muscle fibers leading to an abnormality of locomotion as well as to biochemical changes. Denervation of normal muscle results in a variety of biochemical changes including profound alterations in the distribution of the molecular forms of the enzyme acetylcholinesterase (AChE). We have examined the forms of AChE in both intact and surgically denervated dystrophic mouse muscle as compared to muscle from normal littermates to test the possibility that dystrophic muscle resembles denervated muscle. The hemidiaphragms and extensor digitorum longus (EDL) muscles from REJ/129 (dys/dys) mice and their normal (+/?) littermates were homogenized in tris buffered medium containing 1% Triton X-100 and 1M NaCl to solubilize AChE. Low speed supernatants from these homogenates were centrifuged on 5-20% linear sucrose density gradients as described by Hall (J. Neurobiol 4:343, 1973) to resolve the AChE molecular forms. In hemidiaphragms we found no difference in total AChE activity between dystrophic and normal littermates; however, the distribution of the molecular forms of AChE differed markedly. In the normal littermate muscles three peaks of AChE activity were evident sedimenting at 4S, 10S and 16S. In dystrophic muscle only the 4S and 16S forms showed clear-cut peaks; small but consistent peaks indicated that presence of minor amounts of 13.5S AChE, not observed in controls, and 10S enzyme. Although 16S activity was slightly lower in dystrophic muscle (118 ± 7.0 units) vs control (148 ± 16 units), the pattern did not resemble changes induced by surgical denervation of muscle. AChE activity in dystrophic EDL was significantly lower than control EDL (168 ± 22 vs 394 ± 29 units) and, as with hemidiaphragms, exhibited major peaks at 4S and 16S. The percentage of 16S enzyme in dystrophic EDL was slightly less than the percentage in control muscle; however, in one week surgically denervated muscles the reduction of 16S AChE was similar in both types of muscle (dys = 32% vs control = 34%). Since 16S AChE is generally regarded as an endplate marker and declines upon surgical denervation, the data suggest that dystrophic muscle is not exhibiting denervation-like effects. The unusual distribution pattern of AChE further indicates a biochemical abnormality of enzyme synthesis that may be related to neuronal or muscular aberrations or both. (Supported by NIH grants NS11855 and NS14304. WSB is a recipient of Research Career Development Award NS 00119 from NIH.)

137.23 STUDY OF A FATAL AUTOIMMUNE DISEASE. L.-P. Chao, K.-S.K. Kan and C. Angelini. Dept. Neurol. Sch. Med. UCLA, Los Angeles, CA 90024 and Clin. Neurol. Padova Univ., Padova, Italy.

When a fraction of choline acetyltransferase (ChAc) was injected weekly into guinea pigs with complete Freund's adjuvant, the animals showed progressive weakness, weight loss, reduction of respiratory rates, signs of cyanosis and then died about 14 days from the initial injection. These results suggest that the death is from respiratory failure. The incidence of the disease is 100% and reproducible. Some animals were sacrificed 3-6 days after the second injection. The brain, spinal cord, sciatic nerve, liver, intestines, lung, heart and various muscles, the diaphragm, soleus, biceps, gastrocnemius and iliopsoas, were removed for histological and histochemical studies. Guinea pigs injected with saline substituted for ChAc were used for controls. These animals appeared normal throughout the experimental period. They were sacrificed and tissues were removed for histological and histochemical examinations. Histological studies involved: a) Hematoxylin and eosin staining for all sections; b) Silver and myelin stains were performed on sections of nervous tissue. Histochemical staining of muscle sections included succinic dehydrogenase; adenosine triphosphatase at pH 9.4, 4.6 and 4.3; NADH-tetrazolium reductase; alkaline phosphatase and acid phosphatase. The histological and histochemical results showed various degrees of muscle atrophy and lymphocyte infiltration in certain areas of the muscles. However, the infiltration was found to be concentrated at the neuromuscular junctions and not randomly distributed. In addition, we recently localized ChAc in the presynaptic nerve terminals and axons of the neuromuscular junctions (Muscle & Nerve in press). Therefore, the pathogenesis of this autoimmune disease is most likely from denervation and may be considered a presynaptic neuropathy. Some lymphocyte infiltration was also observed in the lung, liver and intestines. No significant abnormality was found in the central nervous system of the injected animals. The control guinea pigs revealed no pathological changes.

137.24 NON-HEME INTRACELLULAR IRON IN MULTIPLE SCLEROSIS BRAINS. Michael W. Migdal* and William Craelius, Dept. of Biology, Lafayette College, Easton, Pa., 18042.

Elemental analyses of multiple sclerosis (M.S.) brains indicated that unaffected areas of M.S. white matter had elevated concentrations of calcium, iron, manganese and zinc compared with controls. M.S. plaques were particularly concentrated with these elements. Histochemical confirmation of non-heme iron contained within cells of M.S. brains is reported in this study.

Cerebral cortex samples of 5 victims positively diagnosed with M.S. and of 6 controls of similar ages, were fixed in 10% Formalin at autopsy and processed identically. White and grey matter samples were dissected out with glass knives, embedded in paraffin, and sectioned for light microscopy. Serial sections were stained with either luxol fast blue to show myelin or with Perl's acid ferrocyanide to demonstrate reactive ferric iron bound to hemosiderin and ferritin.

Sections from 6 control brains, processed simultaneously with the M.S. brains, showed no positive ferric iron (Prussian blue) reactions. In each of 5 M.S. brains examined, positive Prussian blue reactions were observed in certain myelinated areas. Numerous hemosiderin-loaded cells were observed in some areas of the cortex, which appeared to be large macrophages. Staining was heaviest along the cell perimeter. Myelin staining of adjacent sections indicated that the iron containing macrophages were within circumscribed areas of white matter of variable myelin density. Hemosiderin, apparently in association with myelin, was also observed following along axonal tracts. Perivascular lymphocytes were also found to contain hemosiderin.

Further confirmation of the iron deposits was obtained using Mallory's hematoxylin stain for non-heme iron. Serial sections of tissue which stained positively with Prussian blue were also positively stained with Mallory's. Preliminary ultrastructural observations on unstained sections of M.S. brains demonstrated electron dense particles along the perimeter of cells similar in appearance to the iron loaded macrophages seen histochemically.

The iron-loaded macrophages are similar in morphology to spleen macrophages described previously. The iron deposits observed in association with axons and lymphocytes are novel and unexplained.

137.25 GLUTAMATE TOXICITY IN HUNTINGTON'S DISEASE FIBROBLASTS. P.N. Gray and P.C. May*. Dept. of Biochem. and Molec. Biol., Univ. Oklahoma Coll. of Med., Oklahoma City, OK 73190.

Fibroblast cultures derived from persons with Huntington's Disease (HD) show loss of cell viability, with cell degeneration, when treated with L-glutamic acid (Glu). Control fibroblast cultures, after similar treatment, do not respond in the same manner. The cell degeneration is apparently a specific response to L-Glu and occurs at concentrations of Glu in the range of normal neural levels. Huntington's disease (HD) is a progressive neurodegenerative genetic disorder. Pathologic examination of the brain reveals degeneration in the neostriatum and cerebral cortex. This degeneration is accompanied by consistent decreases in the levels of glutamate decarboxylase and γ-aminobutyric acid. Intrastriatal injections of two excitatory amino acids, glutamic acid and its analog kainic acid (KA), cause degeneration of neostriatal neurons in rats and mice which mimic the neuronal degeneration observed in HD brains at autopsy. Preliminary experiments indicating an increased Glu uptake in HD fibroblasts, and the KA and Glu induced neuronal cell degeneration in rats led us to examine the effects of these compounds on HD skin fibroblasts in cell culture. The effects of Glu, at concentrations up to 50 mM, were determined on nine HD fibroblast cell cultures. Non-HD cultures, matched for sex, approximate age, and passage were tested in parallel. All HD cell cultures showed characteristics of cell degeneration, within 16 hrs following treatment with Glu in excess of 20 mM. HD cells grown in Glu concentrations equal to or less than 10 mM decreased in viability by only 20%. Glutamate at 30 mM and 50 mM was extremely toxic to HD cultures with significant loss of viability occurring 10 to 12 hrs after treatment. Cell death and degeneration were not observed in any of the matched control cell cultures grown in 30 mM Glu. HD cells did not show cell degeneration in the presence of 30 mM D-glutamic acid, pyroglutamate, γ-aminobutyric acid, aspartic acid, cysteic acid, proline, glutamine, asparagine, ornithine or sodium chloride. Kainic acid, 30 mM, was also nontoxic to all cells tested. The present studies with HD skin fibroblasts indicate that specific toxic effects can be induced in non-neural tissues and that the effects can be blocked by Gln. The data here provide evidence pertinent to both the primary membrane defect and the cause of cell degeneration in the HD brain; namely, an excessive effect of glutamate on neurons that may have defective membrane functions.

This research was funded by a grant from the DHEW, NINCDS #NS 14642.

137.26 DISTRIBUTION OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS (LCMV) ANTIGENS IN THE CEREBELLA OF NEONATAL RATS DURING THE ACUTE PHASE OF INFECTION. Robert L. Stoughton[1], Manuel del Cerro[1,2] and Andrew A. Monjan*[3]. 1) Center for Brain Research, Univ. of Rochester Med. Ctr., Rochester, NY 14642. 2) Center for Visual Science, Univ. of Rochester, Rochester, NY 14627. 3) Dept. of Epidemiology, School of Hygiene and Public Health, The Johns Hopkins Univ., Baltimore, MD 21205.

LCM virus infection of neonatal rats produces an acute, destructive immunoneuropathy of the cerebellum which has previously been studied with immunofluorescence (Monjan et al., 1973). We thought it worthwhile to re-examine the spread of the infection using the much more sensitive unlabeled antibody technique applied to 1 μ sections, and to correlate the results with optical microscopy of 1 μ sections and with electron microscopy. Rats were infected intracerebrally with LCMV at age 4-5 postnatal days (PND) and subsequently sacrificed by intracardial perfusion with a variety of aldehyde fixatives at several ages up to 21 PND. Slices of cerebellar vermis were post-fixed in OsO_4, stained with uranyl acetate, and embedded in Spurr's resin. Unlabeled antibody immunocytochemistry was performed on 1 μ sections (del Cerro et al., 1980), with adjacent sections being stained for optical microscopy. Finally, the blocks were trimmed and thin sections for electron microscopy were cut.

The antisera used were raised in rabbits against a 20% suspension of LCMV in mouse brain. To control for the staining of various brain antigens, 1 μ sections of non-infected cerebella were also prepared for immunocytochemistry; these did, indeed, reveal a diffuse staining pattern. However, when compared to the non-infected tissue, infected cerebella clearly showed a pattern of staining "superimposed" on the background diffuse staining. The most prominent staining appeared in the white matter. At 13 PND, a band of white matter nearest to the internal granular layer (IGL) stained, while by 17 PND much more of the white matter stained. Electron microscopy of these areas revealed an increased extracellular space filled with a flocculent material. A grainy distribution of labeling appeared over the IGL. The vasculature and hematogenous elements yielded variable results. Some endothelial cells were unstained, while others appeared to be faintly labeled. Occasional labeled monocytes were seen within the lumens of vessels. Slightly more frequently, foamy cells in the neural parenchyma showed label. A striking finding was the staining associated with cell-free perivascular areas, especially in the meninges.

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137A.1 MODELING OF RECURRENT CYCLIC INHIBITION IN NETWORKS OF NON-SPIKING NEURONS. W. Otto Friesen and Charles E. Mitchell*. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

Experiments to identify the physiological basis of repetitive animal movements are now beginning to provide some insights into the neuronal mechanisms generating the underlying rhythms. These experiments have been especially fruitful in the invertebrates where both specific neuronal circuits as well as specific mechanisms for rhythm generation have been identified. Swimming movements in the leech, for example, were shown to result from the phasic activity of interneurons whose interactions form a network of inhibitory rings. Modeling studies have shown that phasic activity in such inhibitory rings can replicate the rhythmic activity observed in leech neurons and that the origin of phasic activity results from the mechanism of recurrent cyclic inhibition (Friesen, Poon and Stent. J. exp. Biol. 75: 45-63, 1978). In insects as well, rhythmically active interneurons occur which function to generate rhythmic movements. Unlike in the leech, however, the rhythmic activity appears to result from interactions of non-spiking interneurons (Pearson and Fourtner. J. Neurophysiol. 38: 33-52, 1975). We report here on experiments using neuromimes, designed to examine rhythm generation by the mechanism recurrent cyclic inhibition in networks of such non-spiking neurons.

The modeling was carried out with a system comprised of two types of analog circuits. One circuit type, the spike analog (SA), is designed to simulate membrane potentials near the spike initiating zone. The potential of this SA circuit is controlled by its input from the second circuit type, the integrative analog (IA), which simulates the neuronal integrative region. The IA potential is in turn, controlled by the potential of the SA. Thus activity in neuronal networks can be simulated by suitable interconnections of SA and IA circuits. Three of the SA circuits were interconnected via inhibitory IA circuits to form an inhibitory ring. In addition, a common source of non-phasic excitation was provided to each of the SA circuits. Action potentials in the SA's were eliminated by setting thresholds to the maximum values. The results show that with sufficient excitation the SA circuits exhibit phase-locked voltage oscillations similar to those observed in such networks of spiking neuromimes (Friesen and Stent. Biol. Cybernetics. 28: 27-40, 1977). However the activity in non-spiking circuits differs from that observed previously in two important respects: the cycle period of the oscillations in non-spiking circuits is much reduced and the cycle period for non-spiking networks is nearly independent of the level of extrinsic excitatory drive. (Supported by the UVA-NIH Biomedical Sciences Support Program and by NIH grant NS 14965).

137A.2 ROLE OF CA^{2+} IN CYCLIC NUCLEOTIDE ALTERATION OF ACTIVITY IN APLYSIA NEURONS. P. F. Drake and S. N. Treistman, Dept. Biology, Bryn Mawr College, Bryn Mawr, PA. 19010.

Bursting pacemaker activity of cell R15 of Aplysia is altered by synaptic and hormonal stimulation, and this alteration may be mediated by cyclic nucleotides (Treistman and Levitan, Nature, 1976; PNAS, 1976). Cyclic nucleotide agents are capable of inducing bursting activity in normally silent neurons (Treistman and Drake, Brain Research, 1979). Steady state I-V plots under voltage clamp indicate that the induction of activity in the silent metacerebral giant cell (MCC) with 8-benzylthio cAMP is associated with an increased Na^+-dependent negative slope resistance (NSR) and an increased conductance to hyperpolarizing pulses. In cell R15 the 8-benzylthio cAMP derivative enhances only the conductance to hyperpolarizing pulses.

We have investigated the possible role of Ca^{2+} in the cyclic nucleotide-induced alterations. Steady state voltage clamp data is examined under two circumstances: 1) EGTA injection and subsequent nucleotide treatment, and 2) Ca^{2+}-free perfusion followed by cyclic nucleotide perfusion. These procedures should inhibit intracellular accumulation of Ca^{2+} and transmembrane Ca^{2+} fluxes, respectively. The I-V plot of cell R15 shows that the injection of EGTA moderately increases the conductance to hyperpolarizing pulses. Upon subsequent perfusion of 8-benzylthio cAMP the slope conductance to hyperpolarizing pulses is further increased. The injection of EGTA into the MCC linearized the I-V curve. Perfusion of the cAMP derivative returns the hyperpolarized portion of the I-V curve to control values and dramatically enhances an NSR region in the depolarized portion of the I-V plot.

Perfusion of cell R15 with Ca^{2+}-free ASW has two primary effects: 1) an increased conductance to hyperpolarizing pulses, and 2) an increased magnitude of the NSR. Subsequent perfusion of the cAMP derivative caused a further enhancement of the slope conductance to hyperpolarizing pulses suggesting that the action of cAMP does not require a transmembrane Ca^{2+} current. Perfusion of the MCC with Ca^{2+}-free ASW induces bursting activity and the I-V curve exhibits a marked enhancement of the NSR. This enhanced NSR is further increased when 8-benzylthio cAMP is added to the bathing solution. In addition to an enhanced NSR in Ca^{2+}-free ASW, an increase in the slope conductance to hyperpolarizing pulses is observed; this effect also increases when the cAMP derivative is introduced. (Supported by PHS Grant #NS15195-01.)

137A.3 PARAMETER CONSTRAINTS FOR OSCILLATORY NETWORKS. D.K. Hartline, W.M. Roberts‡ and C.L. Baker Békésy Lab, U. Hawaii, Honolulu, HI.

A study was made of repetitive bursting in neural network models to help assess the origin of such behavior in real nets. Constraints on "emergent" oscillations were examined in a series of models ranging from a physiologically detailed computer simulation to a simplified but analytically solvable model still retaining the important aspects of the physiology.

A simple N-cell linear network model was generated by solving:

(1) $f_i(t)=e_i(t) + \sum_j \int_{-\infty}^{t} p_{ij}(t-t')f_j(t')dt'$

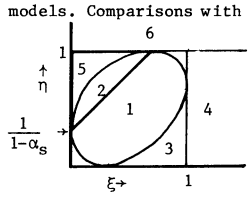
where f is the response (V_m or frequency); e=excitation; $p_{ij}(t)$= interaction time-course = $(\alpha_{ij}/\tau_{ij})H_o(t)\exp(-t/\tau_{ij})$ where H_o is the step function, α_{ij} the PSP area, and τ_{ij} its time-constant (Ratliff et al PNAS 62:733; Coleman & Renninger math Biosci 38:123). For the case $p_{ij}=p_x$ for $i\neq j$ and $p_{ii}=p_s$ for all i (simulating adaptation), oscillatory solutions to a step in e occur when:

(2) $[\xi-\eta_\ell+1/(1-\alpha_s)]^2-4\xi(1-\eta_\ell)/(1-\alpha_s)<0$

where $\xi=\tau_x/\tau_s$; $\eta_\ell=(\ell N-1)\alpha_x/(1-\alpha_s)$; $\ell=0$ or 1 (inside ellipse of figure below). For $\alpha_s<0$ ("normal" adaptation case) oscillations are damped in region 1; singular (growing indefinitely) in 2; overdamped solutions occur in 3 and 4; and non-oscillatory singular ones in 5 and 6. With step-function excitations, sustained oscillations do not occur except on the boundary between 1 and 2.

A similar picture holds in a physiologically more realistic 2-cell computer model which eliminates negative "frequencies" ($f\geq 0$). A "reverberatory" circuit ($\alpha_x>0$) and a reciprocal inhibition circuit ($\alpha_x<0$) were examined. In both cases stable sustained oscillations occur in region 2, and for $\alpha_x<0$, in 5 as well. It is of physiological interest that the range of parameters for stable oscillations is restricted, especially for $\alpha_x>0$. With a ceiling on f ($f\leq f_{max}$), the range of stable oscillation for $\alpha_x>0$ includes region 5, although firing frequency saturates during the bursts.

In all cases except the f_{max} constraint, oscillation frequency is independent of excitation, in contrast to most real networks. For 2 cells with unequal PSP areas, the diagram below is still useful taking $\alpha_x=\sqrt{\alpha_{12}\alpha_{21}}$. Parallel results have been obtained with still more physiologically faithful models. Comparisons with real nets will be presented. These results exemplify the quantitative constraints which must be met before an emergent hypothesis for rhythmic bursting in a network is tenable. Stabilizing influences of special cellular properties on oscillatory behavior will be discussed. Supported by NIH NS13138 and 15314.



137A.4 MODULATION AND GENERATION OF SPIKES IN THE PERIPHERAL BRANCHES OF A MOTOR AXON. P.J.Stephens and H.L.Atwood. Dept. of Zoology, Univ. of Toronto, Toronto, Ontario M5S 1A1, Canada.

In the limb of the crab Pachygrapsus the stretcher muscle is innervated by branches from 3 motor axons; the excitor (E), the specific inhibitor (SI) and the common inhibitor (CI). Preparations were made from autotomised limbs so that the 3 motor axons could be stimulated individually or in combination. At normal temperatures a single stimulus shock applied to the E axon evoked a single excitatory junctional potential (ejp) in the stretcher muscle fibers. However, above a critical temperature single E axon shocks produced 2 successive ejp's. Further temperature increases resulted in a progressive increase in the number of ejp's recorded from stretcher fibers following a single stimulus. Experiments using animals acclimated to different temperatures demonstrated that the threshold for repetitive firing is dependent upon the animal's thermal history.

When single axon shocks elicited repetitive ejp's in the stretcher muscle fibers, each ejp was associated with an E axon spike. Latency measurements and extracellular E axon recordings from 2 locations showed that spikes produced after the initial action potential are generated at the periphery.

If the SI axon was stimulated at the same time as the E axon all peripherally generated E spikes were abolished, with the result that only one (orthodromic) E spike and one ejp were recorded. Moreover, in preparations exhibiting prolonged ejp discharges following E axon shock, stimulation of the SI axon shortly after the E axon shock caused the abolition of only a portion of the secondary E response. These results suggest that activity in the SI axon can modulate or curtail the peripherally generated E axon spikes and the concomitant ejp's. Since our results were obtained from autotomised limbs we suggest that SI modulation of E axon activity must take place in the peripheral axon branches, possibly at the axo-axonal synaptic terminals. Further evidence for this was obtained from bath applications of GABA and picrotoxin at concentrations of 5×10^{-5}M. GABA is considered to be a crustacean inhibitory synaptic transmitter (Otsuka, et al, Proc. Natn. Acad. Sci. 56, 1966) and abolished the repetitive firing following E axon shocks. In contrast, picrotoxin is an antagonist to the action of GABA (Takeuchi and Takeuchi, J. Physiol. 205, 1969) and not only abolished all ijp activity following SI axon spikes, but also prevented SI modulation of peripherally generated E axon spikes.

This work was supported by grants from the National Research Council of Canada and the Muscular Dystrophy Association of Canada.

137A.5 EVIDENCE FOR ELECTROTONIC COUPLING BETWEEN CA3 PYRAMIDAL CELLS OF RAT HIPPOCAMPUS: DYE-COUPLING AND SIMULTANEOUS INTRACELLULAR RECORDINGS. Brian A. MacVicar and F. Edward Dudek, Dept. Zool., Univ. Toronto, Toronto, Ontario M5S 1A1.

Hippocampal pyramidal cells are well-known for their susceptibility to seizure. Electrotonic coupling between pyramidal cells could mediate the synchronization and spread of epileptiform discharges. We have used two independent strategies to test the hypothesis that some CA3 hippocampal pyramidal cells are electrotonically coupled.

Lucifer yellow (LY) was injected intracellularly to determine if CA3 pyramidal cells are dye-coupled. LY has been shown to cross gap junctions in numerous other systems. Intracellular LY injections (10-17 min, 8 nA hyperpolarizing pulses) were continuously monitored and immediately terminated if the impalement was lost. In 10 out of 20 injections, two or more cells were stained when only one was injected. LY is most likely crossing through gap junctions into other dye-coupled cells since (a) in 7 extracellular ejections of LY, there was no evidence of uptake, (b) the cells were not disrupted by iontophoretic injection, and (c) no extracellular LY was present.

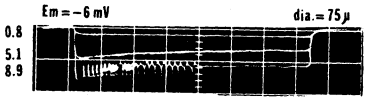
In a second series of experiments we have used simultaneous intracellular impalements of CA3 neurons to demonstrate electrotonic interactions directly. Recordings were obtained with two microelectrodes positioned as close as possible in the cell body layer. Coupling ratios were estimated using reciprocal current pulses. The simultaneous recordings revealed two situations. (A) There was perfect synchrony between recordings and coupling ratio was 1:1. In this case the two electrodes were evidently in the same cell. (B) Weak bidirectional coupling was observed between recordings and action potentials in one evoked fast prepotentials in the other. We interpreted these latter cases to be recordings from two electrotonically-coupled cells and not simultaneous impalements of soma and dendrite of the same cell since: (a) in several cases after the electrodes were in the same cell (case A) one electrode was advanced a few microns, another impalement was obtained and weak coupling was observed; and (b) in one successful case, Ni injection through one electrode and HRP through the other electrode showed two stained neurons and consequently the recordings were obtained from two cells.

Therefore, dye-coupling after LY injections and simultaneous recordings from hippocampal pyramidal cells have provided evidence for electrotonic coupling, which may be the basis for synchrony and spread of seizure. Furthermore, many fast prepotentials may be electrotonic coupling potentials rather than dendritic spikes.

Supported by grants from NSERC.

137A.6 INTRACELLULAR POTENTIALS FROM FROG SARTORIUS MUSCLE FIBERS, T. M. Mikiten. Department of Physiology, University of Texas Health Science Center, San Antonio, Texas 78284.

Frog sartorius muscle fibers were stimulated with intracellular glass microelectrodes filled with either KCl or K acetate, using constant current pulses 10-300msec long. A recording electrode 10-500μM away detected responses to the stimuli. Hyperpolarizing pulses elicited repetitive responses that were not altered by TTX or by removing Na^+ from the medium. The apparent conduction velocity of the responses was 10^{-3} to 10^{-2} meters/sec. Raising the extracellular K^+ concentration to 150mM or replacement of Cl^- by methylsulfate did not block the responses. The repetitive responses could be halted by relocating the stimulating electrode to a different location along the long axis of the fiber. The response returned when the electrode was replaced. For these reasons, it seems possible that the responses arise from intracellular sites, especially the internal membrane system of the fiber. Special thanks to Robert Woodward for his technical assistance.



Repetitive Firing in a Fiber

Responses to three stimuli at successively greater intensity; from the top down: 0.8, 5.1 and 8.9 x 10^{-6}A.
Ringer Solution: Na^+ free, 150 mM K-Methylsulfate
Vertical Scale: 20mV/div
Horizontal Scale: 50msec/div
Electrode Separation: 300μM

137A.7 ELECTROPHYSIOLOGICAL STUDIES ON RECURRENT INHIBITION IN THE HIPPOCAMPAL DENTATE GYRUS. R.S. Sloviter and J.D. Connor. Dept. of Pharmacology, Penn State Univ., Col. of Med., Hershey, PA 17033

Recurrent inhibition in the dentate gyrus of the urethane-anesthetized rat was studied using extracellular recording techniques. Fourteen repetitively firing (RF) cells in 14 rats exhibited high discharge frequency (450-700 Hz) for long durations (up to 200 msec) in response to activation of the perforant path, a physiological input to the dentate gyrus. RF cell bursts were dependent on stimulus frequency. At 1 Hz, RF cell discharges were not synchronized with the stimulus pulse. At or above 3 Hz the RF cell discharges followed the stimulus closely (8-10 msec). In other rats, granule cells (G-cells) were recorded that fired spontaneously in bursts of 2 or 3 spikes. During a series of triplet bursts from a G-cell, a RF unit (700 Hz) recorded simultaneously apparently converted the triplets to singlets. When the G-cell discharged in singlets, the RF cell did not discharge. These recordings presumably represent frequency dependent discharges of dentate basket cells which have been assumed to mediate recurrent inhibition in the dentate gyrus. Since these cells are rarely encountered, experiments were designed to determine the extent to which the G-cell evoked potential might serve as a model for studying the influence of inhibitory interneurons on G-cells. Stimulation of the perforant path evokes a field potential which reflects EPSPs in G-cell dendrites. Superimposed on this potential is a "population spike" which represents action potentials generated simultaneously in many G-cells. The amplitude of the spike is proportional to the number of G-cells firing (Lømo, 1971). At low frequencies (≤0.1 Hz), the population spike is relatively large. At 1 Hz, the spike amplitude is small, but at 2-4 Hz the spike amplitude increases again. The decrease in spike amplitude from 0.1 to 1.0 Hz is blocked by bicuculline (i.v. or ivt.). The twin pulse paradigm (Andersen et al., 1966) was also used to examine recurrent inhibition. At low frequencies (≤0.1 Hz), the second population spike of two evoked potentials (40 msec apart) is larger than the first, indicating potentiation of the test pulse by the conditioning pulse. At higher frequencies (>2 Hz), the first spike grows larger and the second is small or absent. This inhibition of the test spike represents frequency dependent recurrent inhibition of G-cells and is blocked by bicuculline or picrotoxin but not strychnine. As stimulus frequency is increased, or after administration of bicuculline, recurrent inhibition is abolished and epileptiform activity (large amplitude, repetitive spiking of granule cells) is observed. These observations are consistent with the view that loss of GABA-mediated recurrent inhibition results in uncontrolled epileptiform activity.

Supported by USPHS grants DA 02007 and NS 15663.

137A.8 REACTION-DIFFUSION MODELS FOR CALCIUM FLUXES IN NERVOUS TISSUE. A. F. Lawrence*, S. M. Bawin* and W. R. Adey (SPON: E. Schwartz) Research Service, VA Hospital, Loma Linda, CA 92357

Data from recent experiments involving the efflux of $^{45}Ca^{++}$ from the brains of live cats and from synaptosomes extracted from rat brains indicate that the steady-state kinetics of calcium exchange between cell membrane and extracellular pools may exhibit more complex phenomena than the usual simple (multi-) exponential decay of amounts of labeled atoms in various tissue pools. Evidence for periodic oscillations has been observed in the efflux of $^{45}Ca^{++}$ from both preparations. These oscillatory states may be induced by stimulation with weak electromagnetic fields generated externally to the preparation. A reaction-diffusion model based on a two-stage calcium binding mechanism (which has been observed in several proteins) may be used to fit the data. Explicit parabolic partial differential equations describe the reaction diffusion process, and hyperbolic-partial differential equations describe the propagation of the field. Coupling between the reaction-diffusion process and the induced field is shown to cause a shift in the steady-state solutions of the modeling equations between field-absent and field-present conditions. Oscillatory states in the numerical solutions are shown to parallel the experimental observations. Similar coupling effects between Ca^{++}, Na^+, K^+ and H^+ fluxes and electromagnetic fields may provide a mechanism for the cooperative action of neurons. (Funding: DOE DEAI101-79ET29078, FDA R01-FD00963-03, and Southern California Edison Company.)

138.1 GLUCOCORTICOIDS INDUCE EXPRESSION OF THE ADRENERGIC PHENOTYPE IN A RAT SYMPATHETIC GANGLION. I.B. Black, M.C. Bohn, E.M. Bloom and M. Goldstein Division of Developmental Neurology, Cornell Univ. Med. Coll.,New York, N.Y. 10021 and Dept. Psychiatry, New York Univ. Med. Ctr., New York, N.Y. 10016.

The effect of glucocorticoid treatment on expression of the adrenergic phenotype in rat superior cervical ganglion (SCG) was monitored immunocytochemically. The adrenergic phenotype was defined as the presence of immunoreactivity to PNMT (phenylethanolamine-N-methyltransferase). In normal postnatal rats, adrenergic cells were never observed in the SCG. However, numerous PNMT positive cells appeared following treatment with dexamethasone (DXM) on days 0-6 or corticosterone pellets on days 0-3. PNMT was localized to elements resembling SIF cells; they were small cells of variable diameter, scattered or in groups, often associated with blood vessels. In contrast, PNMT was never observed in principal ganglion cells. Unilateral ganglion decentralization did not prevent the appearance of the adrenergic phenotype after glucocorticoid treatment, indicating that preganglionic innervation was not necessary for PNMT appearance. Moreover, DXM (10^{-7}M) directly affected the SCG since the steroid elicited PNMT appearance in ganglia cultured for 4 days.

This discrete population of steroid-responsive cells was also present in the embryonic SCG. Treatment of pregnant rats with DXM (4mg/Kg daily for 4-5 days) evoked the appearance of PNMT in embryonic ganglia at 18.5 and 20.5 days of gestation. However, no PNMT positive cells were observed in sympathetic ganglia in 14.5 or 16.5 day old embryos from DXM treated mothers.

Since we observed that cessation of steroid therapy resulted in the disappearance of PNMT, postnatal rats were re-exposed to DXM to help define the fate of these cells. We employed 4 treatment groups: 1) saline days 0-6 and 24-30; 2) saline days 0-6 and DXM 24-30; 3) DXM days 0-6 and saline 24-30; 4) DXM days 0-6 and 24-30. In 30 day ganglia, PNMT appeared only in rats exposed to DXM on days 0-6 and 24-30. These results suggest that after initial DXM treatment, cells which can re-express PNMT persist into adulthood. Consequently, DXM withdrawal results in loss of PNMT, but not death of these cells. More generally, we conclude that glucocorticoids can influence the expression of the adrenergic phenotype in extra-adrenal tissue during a critical period extending from late gestation to early postnatal life.

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138.2 INFLUENCE OF GLUCOCORTICOSTEROIDS ON THE CHOICE OF TRANSMITTER TYPE OF SUPERIOR CERVICAL GANGLION NEURONS. I. S. McLennan*, I. A. Hendry*, C. E. Hill* (SPON: W. D. Chapple). Dept. of Pharmacol., John Curtin School of Med. Res., Aust. Natl, Univ., Canberra, A.C.T. 2601, Australia.

When intact immature superior cervical ganglia (SCG) are cultured, the neurons switch from an adrenergic to a cholinergic phenotype. This switching of phenotype is caused by a factor released by the non-neuronal cells (Patterson, A. Rev. Neurosci. 1, 1, 1978). If, however, physiological concentrations of glucocorticosteroids are included in the medium then the neurons are maintained in an adrenergic phenotype, as indicated by high levels of tyrosine hydroxylase (TH) and low levels of choline acetyltransferase (CAT) (McLennan et al., Nature 283, 206, 1980).

The mechanism by which glucocorticosteroids influence transmitter choice was studied by examining whether the glucocorticosteroid agonist dexamethasone (DEX) could irreversibly prevent the switching of phenotype. SCG from 2 day old rat pups were cultured in medium containing DEX (10^{-6}M) for various periods and then cultured for a further 14 days in medium lacking steroid. The ability of these ganglia to synthesize CAT was reduced after a 4 day, and abolished after a 7 day, culture in DEX. The loss of ability to switch phenotype parallels a similar age dependent change occurring in vivo. When SCG were cultured in the absence of steroid for 7 days, so as to make some of the neurons cholinergic and then subsequently cultured in DEX for 14 days, there was no significant elevation of TH or decrease of CAT in these ganglia, relative to ganglia cultured in the absence of steroid for 21 days. Thus DEX does not selectively destroy cholinergic neurons and is not acting as an adrenergic inducer. We therefore suggest that glucocorticosteroids influences transmitter type of the SCG in vitro by fixing the neurons in the phenotype being expressed at the time of exposure to the steroid.

138.3 HORMONAL CONTROL OF NEUROTRANSMITTER CHOICE IN CULTURED SYMPATHETIC NEURONS. K. Fukada. Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115.

Individual sympathetic neurons dissociated from superior cervical ganglia (SCG) of newborn rats have the capacity to become either cholinergic or adrenergic, depending on the culture environment (Patterson,P.H., Ann. Rev. Neurosci., 1:1, 1978). A factor produced by certain types of non-neuronal cells can influence this transmitter decision without affecting neuronal survival or growth. However, hormones in the sera used in the culture media have remained an uncontrolled variable in these studies. Recently, McLennan et al. (Nature, 283:206, 1980) have shown that glucocorticoids modulate transmitter choice of neonatal SCG in organ culture, suggesting a role for hormones in this aspect of neuronal development.

I have examined the effects of a variety of hormones on the development of sympathetic neurons after devising a serum-free medium for cardiac myocytes and fibroblasts (heart cells) according to Sato and coworkers (Bottenstein, J. et al., Methods in Enzymol. 58:94, 1979). Serum-free media supplemented with various hormones were incubated on heart cells, and the conditioned media (CM) from these cultures were tested for the ability to cause induction of neuronal acetylcholine (ACh) synthesis. Among the various hormones tested, epidermal growth factor (EGF) and glucocorticoids had large and antagonistic effects on the cholinergic induction : EGF stimulated the ACh induction more than 100-fold, while glucocorticoids decreased this stimulatory effect of EGF 10-20 fold. Thus the transmitter choice of SCG neurons can be controlled by certain hormones. However, it was not clear whether these hormones acted directly on the neurons or indirectly, via the heart cells, in causing cholinergic induction. Therefore, the effect of direct addition of EGF or glucocorticoids to neuronal cultures together with serum-free CM (without EGF or glucocorticoids) was tested. Since little effect on the transmitter choice was seen in such experiments, it was concluded that these hormones acted on the heart cells, controlling the ability of heart cells to produce effective CM.

These effects of EGF and glucocorticoids were relatively specific. The 24 hr incubation with these hormones for obtaining CM did not change the total DNA or protein content of the heart cells, or the amount of protein synthesized from ^{3}H-leucine.

These results suggest a further level of subtlety in the control of neurotransmitter choice in sympathetic neurons : specific hormones act on non-neuronal cells to selectively alter the production or liberation of a factor (or factors) that induces cholinergic development and reduces adrenergic development (supported by the M.D.A. and a NINCDS grant to P.H.Patterson).

138.4 ELEVATION OF MATERNAL GLUCOCORTICOID HORMONES ALTERS NEUROTRANSMITTER PHENOTYPIC EXPRESSION IN PRESUMPTIVE EMBRYONIC NEUROBLASTS. G.M. Jonakait, M.C. Bohn, M. Goldstein and I.B. Black. Div. of Developmental Neurology, Cornell Univ. Medical College, New York, N.Y. 10021 and Dept. of Psychiatry, NYU Medical Center, New York, N.Y. 10016.

We have been working with a population of presumptive neuroblasts, located in embryonic rat gut, which transiently expresses noradrenergic phenotypic characters. Immunoreactivity to tyrosine hydroxylase (T-OH) and dopamine-β-hydroxylase (DBH) as well as endogenous catecholamine (CA) fluorescence appear simultaneously in this population at gestational day 11.5 (E 11.5). Cells expressing these noradrenergic characters increase in number over the next 24 hrs, but by E 13.5 they have lost these noradrenergic traits. To characterize this population pharmacologically, pregnant rats were treated with reserpine (10 mg/kg, i.p.) on E 11.5, and embryonic guts were examined with both formaldehyde-induced CA fluorescence and immunohistochemistry 2 days later. Reserpine administration resulted in the persistence of both CA fluorescence and immunoreactivity to T-OH and DBH in embryonic gut cells. Since reserpine is known to elevate plasma glucocorticoids, we investigated the effects of these hormones on persistence of noradrenergic characters. Maternal hydrocortisone acetate administration (given as pellets implanted s.c. on E 11.0) reproduced the effect of reserpine on gut cells. In other experiments mothers were pretreated with dexamethasone (1 mg/kg) to inhibit the stress-induced rise of maternal plasma glucocorticoid hormones. This treatment, beginning one day before reserpine administration, prevented reserpine's effect of prolonging CA fluorescence in gut cells. In addition, pretreatment of mothers with mitotane (75 mg/kg, s.c., twice daily beginning E 5.5), a drug toxic to the adrenal cortex, inhibited the reserpine-induced persistence of CA fluorescence. Since 1) hydrocortisone mimicked reserpine; 2) inhibition of a stress-induced rise of plasma glucocorticoids blocked the action of reserpine; and 3) mitotane inhibited reserpine's effects, we conclude that the action of reserpine in prolonging expression of noradrenergic traits is mediated by the release of maternal glucocorticoid hormones.

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138.5 DISSOCIATED CELL CULTURE OF SIF CELLS: HORMONE-DEPENDENCE AND NGF ACTION. A. J. Doupe, P. H. Patterson, and S. C. Landis. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Small intensely fluorescent (SIF) cells constitute a minority cell population in many sympathetic ganglia, where they may act as interneurons. Like the sympathetic principal neurons that surround them, SIF cells are neural crest-derived, but it is unclear how they differentiate into a separate population. The number of SIF cells in a ganglion is influenced by glucocorticoid hormones: after hydrocortisone treatment both in vivo or in explant cultures, rat superior cervical ganglia (SCG) contain many more SIF cells than untreated controls (Eränkö, O. et al., Histochem. J. 4:49, 1972). The mechanism of this effect is unknown; increased survival, mitosis, or recruitment from another population of cells have all been suggested.

The use of dissociated cell cultures of SIF cells may help in the resolution of these questions. We have been able to obtain such cultures of SIF cells from newborn rat SCG by growth in 5 μM dexamethasone, with antimitotic agents, and without nerve growth factor (NGF). These cultures are virtually free of principal neurons and non-neuronal cells. The properties of the SIF cells include (1) small size (10-20 μm in diameter), (2) intense formaldehyde-induced fluorescence, (3) large granular vesicles of several types (100 nm diameter, characteristic of Type I SIF cells, and 300 nm diameter, characteristic of Type II SIF and adrenal medullary cells), (4) the capacity to generate action potentials, and (5) the synthesis and storage of dopamine, norepinephrine, and epinephrine (as judged by the incorporation of (^{3}H)-tyrosine and by HPLC analysis of endogenous amines). The characteristic fluorescence and ultrastructure develop over the first ten days in culture, by which time the cell number is stable and more than 90% of the cells are intensely fluorescent.

Adrenal medullary cells, although not NGF-dependent, can respond to NGF by neurite outgrowth (Unsicker, K. et al., PNAS 75:3498, 1978), and injection of NGF into rat embryos causes the medullary cells to be replaced by nerve cells (Aloe, L. et al., PNAS 76:1246, 1979). SIF cells in culture are also capable of responding to NGF by outgrowth of neuron-like processes, even after they have developed intense catecholamine fluorescence. Some also lose their intense fluorescence and acquire a neuronal ultrastructure.

These observations raise the possibility that there are cells in the SCG which have the potential to become either neurons or SIF cells, and that glucocorticoids and NGF can influence this choice. Also, the cultures may prove useful for the study of the function of SIF cells in the SCG. (Supported by the NINCDS, the Rita Allen Fndn., and the Ins. Med. Sci. Schol. Fund)

138.6 THE EXPRESSION OF AN ADRENERGIC PHENOTYPE IN FETAL ADRENAL MEDULLARY CELLS IS NOT INDUCED BY GLUCOCORTICOIDS. M. Brodsky,* G. Teitelman, D.H. Park, M. New, T.H. Joh and D.J. Reis, Laboratory of Neurobiology, Dept. of Neurology, and Dept. of Pediatric Endocrinology, Cornell Univ. Med. College, New York, NY 10021.

During embryogenesis of the rat, the enzymes tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH), which synthesize norepinephrine, first appear in cells of the developing adrenal medulla at day 16 (E16). It is not until E18 that phenylethanolamine-N-methyltransferase (PNMT), the enzyme synthesizing epinephrine, first appears (Teitelman et al., PNAS 76:509, 1979). We sought to determine whether the expression and/or subsequent increase of adrenal PNMT depends upon glucocorticoids secreted by the developing adrenal cortex.

At E16, when PNMT activity is nil, corticosterone (CS) is present in the adrenal (4.8 ng/adr). At E18, when PNMT activity is first detected (21.9±1.42 pmol/adr/h), CS levels have increased 100-fold to 500 ng/adr. To determine whether the appearance of PNMT is induced by the surge in CS biosynthesis, adrenals removed at E16 (16+0) were cultured without ACTH. Under such conditions, adrenal CS concentrations were similar on 16+0 and 16+2. However, PNMT activity appeared on E16+2 (5.33±0.43 pmol/adr/h) and increased to 22.09±2.39 by E16+5 where it remained until E16+9. CS was undetectable in the media added to the cultures. To determine whether CS modulates PNMT activity in vitro, adrenals removed at E16 were cultured 2-9 days with or without 10^{-5} M dexamethasone. Although dexamethasone did not change the time at which PNMT appeared, it increased PNMT activity three-fold. Immunochemical titration with a specific antibody to PNMT demonstrated that the increase in enzyme activity was entirely due to an increased accumulation of enzyme protein. In contrast to PNMT, TH activity, which increased from 0.42±0.06 nm/adr/h at E16+2 to 0.73±0.02 nm/adr/h on E16+5, was not modified by the addition of dexamethasone. We conclude that while a high level of glucocorticoids is not required for the initial expression of PNMT, the amount of enzyme protein present in fetal adrenal medullary cells is regulated by CS. Factors other than GC are required for the differentiation of adrenal medullary cells into those that synthesize epinephrine.

(Supported by NIH grant HL 18974)

139.1 CORRELATION BETWEEN CELL BIRTHDATES AND SYNAPTIC CONNECTIVITY IN A NEURONAL ARRAY. M. Flaster and F. Macagno. Dept. of Biol. Sciences, Columbia University, New York, N.Y. 10027

The order in which cells of a developing neuronal array are generated may play a role in their subsequent formation of correct synaptic contacts. For example, orderly ingrowth of axons, coordinated with the sequential or relative availability of target neurons in the array, would result in a particular connectivity pattern. The order of generation of targets could be responsible for their sequential availability, or the age of the targets might determine their relative affinity for the incoming fibers.

In the visual system of adult Daphnia magna the position and connectivity of the 176 photoreceptors and the 110 laminar target neurons are invariant. The normal order of optic fiber ingrowth into the embryonic lamina is also stereotyped and invariant, with axons that project to lateral laminar targets arriving first and axons that project to dorsomedial targets arriving last. In the present study we find that there is a correlation between the order of cell generation in the lamina and the order of optic fiber ingrowth and subsequent formation of synaptic connections.

Embryos were micro-injected with ^{3}H-thymidine at specified developmental times and were subsequently fixed at maturity. They were then sectioned serially at 1 micron and processed for autoradiography. Computer-assisted 3-dimensional reconstruction and grain counting were used to follow the progress of all 110 laminar cells through their final round of DNA synthesis. The presumptive lamina completes its final cycle of cell replication in less than 10 hours at 22°, extending from about 29 to 39 hours of development (total development time is about 60 hours at this temperature). S-phase for individual cells is less than 3 hours in duration. We found that final mitoses (birthdates) proceed in an orderly manner such that cells most laterally situated in the lamina have the earliest birthdates, while dorso-medial cells are born last. This birthdate pattern closely parallels the pattern of fiber ingrowth from the eye into the lamina. EM serial reconstruction of control embryos fixed at injection time demonstrate that birthdates for cells of a particular location precede fiber contact by at least an hour. The time period following final S and prior to axonal contact and initiation of laminar cell morphological differentiation appears to be about the same throughout the lamina regardless of cell position. How the apparent clock-like coordination between cell cycle withdrawal by target cells and the contacting of these cells by photoreceptor axons is achieved is not known. The developmental significance of the order of this set of events remains to be established.

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139.2

Withdrawn by Author

139.3 REINNERVATION OF TRANSPLANTED SYMPATHETIC GANGLIA. Dale Purves, Wesley Thompson and Joseph W. Yip. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO. 63110.

Sympathetic ganglion cells are innervated preferentially by preganglionic axons arising from different levels of the thoracic spinal cord (see Purves and Lichtman, 1978, for a review). In the present study we have asked whether the basis of this phenomenon is a property of ganglion cells by transplanting different sympathetic ganglia to a common site.

Fifth thoracic or superior cervical sympathetic ganglia from donor guinea-pigs were transplanted to the bed of an excised superior cervical ganglion in host animals. In this way the same set of preganglionic axons (the cervical sympathetic trunk) was confronted with ganglion cells from different levels of the sympathetic chain. After 3-5 months the reinnervated transplants were examined by recording intracellularly from individual ganglion cells while stimulating thoracic ventral roots in vitro.

Transplanted thoracic and cervical ganglion cells were reinnervated to about the same extent by the preganglionic axons of the host's cervical sympathetic trunk. Thus, the mean amplitude of postsynaptic potentials, the estimated number of inputs, and the number of spinal segments providing innervation to each neuron were similar. Neurons in transplanted fifth thoracic ganglia, however, were reinnervated more frequently, and more strongly, by axons arising from more caudal thoracic segments than were neurons in transplanted superior cervical ganglia. Stimulation of axons arising from the fourth thoracic spinal segment (T4), for example, elicited postsynaptic potentials that were, on average, twice as large in transplanted fifth thoracic ganglion cells as in transplanted superior cervical ganglion cells; conversely, T1 axons reinnervated neurons in the superior cervical ganglion about 2-3 times more effectively than they reinnervated fifth thoracic ganglion cells. This difference in the reinnervation of the fifth thoracic and the superior cervical ganglion is in the same direction as (although less pronounced than) the normal difference in the segmental innervation of these ganglia.

We conclude that preganglionic axons can distinguish (or be distinguished by) ganglia derived from different levels of the sympathetic chain. Our findings are consistent with the view that some property of ganglion cells biases the innervation they receive.

Supported by NIH grant NS-11699 and a grant from the Muscular Dystrophy Association.

Ref: Purves, D. and Lichtman, J.W. Physiol. Rev. 58:825 (1978).

139.4 SYNAPTIC COMPETITION AND SPROUTING DURING REINNERVATION OF FROG SYMPATHETIC GANGLIA. Daniel H. Feldman* (SPON: C.G. Reiness). Dept. of Physiol., Univ. California, San Francisco, CA 94143

In frog lumbar sympathetic ganglia, B cells and C cells can be distinguished by the propagation rate of antidromic spikes elicited in the postganglionic nerve trunk. The two types of ganglion cells are selectively innervated by separate populations of preganglionic axons; B fibers innervate B cells, and enter the sympathetic trunk at levels anterior to the sixth segmental ganglion, while C fibers innervate C cells and enter the trunk at the seventh and eighth segmental levels. Thus, fast excitatory synaptic inputs to B cell or C cell populations may be driven separately by stimulating the appropriate preganglionic nerve roots. This simple anatomical arrangement of selective inputs to two cell populations allows examination of the specificity of reinnervation of ganglia using intracellular recording techniques.

I reported that after surgical interruption of both B and C fibers, both groups of fibers eventually restore synaptic contact with their appropriate ganglion cell targets. Inappropriate synapses (B fiber → C cell) were formed during early reinnervation, but most of these were eliminated shortly after appropriate innervation had returned to most C cells (Feldman, '79, Neurosci. Abst. 5:625). Does the disappearance of inappropriate synapses depend on a competitive interaction with returning appropriate synapses, or would inappropriate synapses be eliminated even in the absence of a preferred synaptic input? To distinguish between these possibilities I employed a lesion designed to selectively delay the return of C fibers, thus providing a prolonged period of reinnervation by regenerating B fibers alone. In ganglia examined 6-10 weeks after lesion, 65% of C cells still were inappropriately innervated by B fibers. When C fibers were allowed to return, the frequency of inappropriate synapses was reduced to less than 10% by this time. Thus, inappropriate synapses are retained in the absence of preferred synaptic input, and selective elimination of these synapses appears to be due to competition with appropriate inputs.

In another study I cut only the preganglionic B fibers, thereby denervating only B cells. 1½-5 weeks after lesion, the intact C fibers had sprouted and formed synapses with 47% of the B cells. When the B fibers returned they formed synapses only with B cells, and the C fiber sprouts could no longer be detected physiologically. Thus, anomalous (C fiber → B cell) connections are induced by partial denervation, and these connections are eliminated when appropriate synaptic inputs return. Supported by NIH Grant #NS10792.

139.5 Synaptic Selectivity: A New Concept

S. Roper, W. Proctor, and B. Taylor*. Depts. of Anat. and Phsyiol Univ. Colo. Health Sciences Center, Denver, Colo. 80262.

A number of laboratories, including ours, have shown that parasympathetic neurons in the cardiac ganglion of the frog are capable of establishing and maintaining synaptic connections with inappropriate, or "foreign", cholinergic inputs. For example, preganglionic vagal axons can sprout and reinnervate ganglion cells after partial vagotomy (Nature, 259, 317, 261, 148, 1976). In addition, intrinsic synapses, which normally do not occur, develop between denervated ganglion cells (Nature, 268, 456, 1977). Furthermore, even spinal motoneurons can innervate denervated ganglion cells (PNAS, 76, 4695, 1979). Although an inappropriate nerve supply will establish synaptic contact with denervated parasympathetic cardiac ganglion cells, when the original axons (i.e. vagal preganglionic fibers) regenerate, they reinnervate the neurons and eliminate the foreign synaptic innervation. This has been interpreted as selective reinnervation insofar as there is a preferential reinnervation of the target by appropriate axons rather than by inappropriate ones.

An important feature is common to all the above examples of inappropriate synaptic reinnervation in the cardiac ganglion. Namely, inappropriate nerve terminals establish synaptic contact only on the axons of the target neurons. This is in contrast with reinnervation by the original, vagal, nerve terminals, where synaptic contact is restored both to the soma as well as the axon. The inability of foreign inputs to reinnervate the soma appears to reveal fundamental regional differences in the postsynaptic targets.

If this is a general property of nervous tissue repair, it leads to a concept of synaptic territoriality, in which certain territories on a denervated target cell (e.g. the axon, in the case of cardiac ganglion cells) can accept synaptic contact from a variety of sources, including inappropriate ones, whereas other regions (e.g. the soma, in the case of ganglion cells) are more selective and form synapses only with appropriate axons. Although the mechanisms whereby foreign inputs are excluded from one synaptic territory and not the other(s) are not known, the concept of synaptic territoriality may suggest promising experimental approaches to the study of neuronal plasticity.

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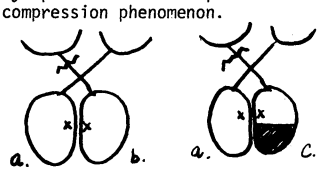
139.6 TARGET REGULATION OF SYNAPTIC DENSITY IN THE REGENERATED OPTIC PROJECTION TO A HALVED OPTIC TECTUM IN GOLDFISH. M. Murray, S. C. Sharma and M. A. Edwards. Dept. Anatomy, The Medical College of Pennsylvania, Philadelphia, PA and NY Medical College.

When a crushed optic nerve regenerates into a tectum whose caudal portion has been ablated, the retinal axons establish a compressed retinotopic projection upon the remaining half tectum within a few months (Gaze and Sharma, 1965). This projection to a reduced target should be accompanied by a substantial increase in synaptic density if each retinal axon makes its normal number of contacts but synaptic density should remain normal if the number of contacts is regulated by the postsynaptic structures. We examined synaptic density in the stratum fibrosum et griseum superficialis (SFGS, the major tectal target for retinal axons) in: a) control tecta; b) tecta 3-9 months after contralateral optic nerve crush; and c) tecta 6-9 months after optic nerve crush and contralateral caudal tectal ablation. Synaptic types, size and density were estimated by morphometric analysis of electron micrographs supplemented by HRP, RAG and degeneration studies. Counts of optic nerve terminals stained by orthograde HRP method indicated that at least 40% of SFGS terminals are retinal in origin. Early stages of regeneration into intact tectum are characterized by an increase in SFGS thickness, largely accounted for by the massive influx of an excess number of regenerating axon collaterals. By 4 months p.o. both SFGS thickness and axon numbers have decreased toward normal levels. Despite the larger number of retinal axons, synaptic density is subnormal and only approaches normal values by 4 months p.o. The increase in SFGS thickness in the reinnervated half-tectum however, persists and this is associated with a persistance of a large number of regenerating axons. Synaptic density nevertheless remains close to control values in the half tectum preparation. These results support the conclusion that synaptic number is regulated by the postsynaptic target rather than by the axon and that competition for limited *accomodation (e.g. postsynaptic sites or trophic factors)* is one characteristic of the compression phenomenon.



X = sample area

Supported by NIH grant NS13768.

140.1 TESTICULAR CONTROL OF THE FREQUENCY OF GONADOTROPIN RELEASING HORMONE SECRETION IN THE RHESUS MONKEY. T.M. Plant* (SPON: M.J. Kelly) Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pa. 15261.

The intermittent secretion of the gonadotropic hormones is considered to be occasioned by, and therefore to reflect, a corresponding pulsatile pattern of gonadotropin releasing hormone (GnRH) discharge by the hypothalamus. Thus, in intact adult male rhesus monkeys GnRH appears to be released with a frequency of approximately 1 pulse every 2-3 h (Plant, 1980). In order to study the role of the testis in determining this discontinuous pattern of hypophysiotropic stimulation, 4 adult male rhesus monkeys (8.5-10.2 kg BW) were fitted with indwelling cardiac catheters and housed in remote sampling cages which permitted continuous access to the venous circulation without restraint of the animals. Sequential blood samples were taken via the catheter every 10 min for 8-12 h, between 0700 and 2100 h, one to 4 days before and 1, 2, 4 and 8 days after bilateral orchidectomy. Plasma luteinizing hormone (LH) concentrations were determined by a previously validated RIA (Karsch et al., 1973). The pulsatile release of LH prior to orchidectomy occurred, as previously described, with a frequency of 1 pulse every 2 to 4 h. Two days after castration, however, the frequency of LH release had increased dramatically to approximately 1 pulse per h and by the 4th postcastration day frequencies as high as 1 pulse every 0.5 h were observed in some cases. Subsequent sc implantation of testosterone (T) containing Silastic capsules, which produced plasma T concentrations in the upper physiological range, resulted in a progressive reduction in the high frequency of pulsatile LH secretion characteristic of the open-loop situation: 24 h after initiation of T replacement the frequency of LH discharge had declined from approximately 1 pulse every 40 min to approximately 1 pulse every 70 min and after 96 h of steroid treatment, frequencies (1 pulse every 2 h) comparable to the intact situation obtained.

These findings suggest that, in the rhesus monkey, the testis decelerate the hypothalamic mechanism responsible for the timing of pulsatile GnRH release, and that this gonadal influence on the pattern of hypophysiotropic stimulation may be mediated by testicular testosterone secretion.

References: Karsch et al., Endocrinology 92, 1740, 1973; Plant, In Testicular Development, Structure and Function, (Eds.) A. Steinberger and E. Steinberger, Raven Press, New York, 1980.

140.2 BROMOCRYPTINE AND APOMORPHINE INCREASE 3H-ESTRADIOL UPTAKE BY SPECIFIC BRAIN AREAS AND PITUITARY IN THE RAT. W.G. Hope* and D.E. Woolley*. (SPON: V. Vijayan). Dept. of Animal Physiology, Univ. of Calif., Davis, CA 95616.

It is well known that catecholamines (CA) and estradiol (E_2) interact to regulate gonadotropin and prolactin secretion. For example, depletion of norepinephrine (NE) inhibited luteinizing hormone (LH) release in proestrous rats (Kalra and McCann, 1973), and dopamine (DA) infusion into the third ventricle evoked LH release more readily on diestrus-2 and proestrus than on other days of the cycle (Schneider and McCann, 1970). A possible neuroanatomical substrate for interaction of CA and E_2 has been suggested by the identification of estrocatecholaminergic neurons, which contain both E_2 receptors and a CA, in the basal hypothalamus and other brain areas (Stumpf and Grant, 1975). The purpose of the present study was to determine whether or not the interactions of CA and E_2 might be mediated via modulatory effects of CA on E_2 uptake by brain and pituitary. To do this Long-Evans rats were studied 3 days after ovariectomy. All rats received 3H-E_2 (1 µg/kg) iv. In addition, one group received 200 µg/kg diethylstilbestrol (DES) iv a few minutes before 3H-E_2 to help quantify the DES-blockable (receptor-mediated) uptake of 3H-E_2. Another group received bromocryptine (5 mg/kg) ip 1 hour before the 3H-E_2 and a final group received the bromocryptine plus DES. All animals were sacrificed 1 hour after 3H-E_2 administration. Bromocryptine pretreatment significantly increased receptor-bound 3H-E_2 levels in the nuclear fractions from pituitary, basal hypothalamus (BH) and septum. Binding was increased 30-70% in these areas. 3H-E_2 binding was also increased in anterior hypothalamus (AH), dorsal hypothalamus (DH), amygdala (AM) and cortex, but not significantly. In order to determine whether the effects of bromocryptine were mediated by its DA agonist properties, the experiment was repeated except that apomorphine, an established DA agonist, was substituted for bromocryptine. Apomorphine was injected ip in a dose of 1 mg/kg 15 min before, at the same time as, and 30 min after 3H-E_2 injection. Receptor-mediated nuclear binding of 3H-E_2 was significantly increased in the pituitary, AH, BH, DH and AM, though not in the septum or cortex. These findings suggest that CA neurotransmitters, perhaps DA especially, may influence the effect of E_2 on the brain and pituitary by increasing the uptake and/or binding of E_2. Further work is required to determine if the effects of these drugs are mediated primarily by increasing E_2 entry into cells, by increasing E_2 binding to its receptor, or by some other action. (Supported by NIH grant HD-12385-01.)

140.3 SEROTONIN-ACTIVE DRUGS MODIFY TEMPORAL PATTERNS OF PREOVULATORY LUTEINIZING HORMONE SECRETION IN RATS. R. F. Walker* (SPON: J.A. Winer). Dept. Anatomy & Sanders-Brown Center for Aging Research, Univ. Kentucky Med. Sch., Lexington, Ky. 40536

This study was designed to determine the influence of serotonergic neurotransmission on the dynamics of preovulatory LH secretion. Young female rats (Long-Evans) showing regular 4 day estrous cycles were housed in pairs in a 14:10 (lights on 0500h; off 1900h) photoperiod. Cyproheptadine (CP), methysergide (MS) (serotonin receptor antagonists) or quipazine (Q) (serotonin receptor agonist) was injected during the afternoon or evening of proestrus. Serial blood samples were collected from the tail vein of unanesthetized animals for determination of LH content following drug treatment. Serum LH concentrations peak in controls at approximately 1800h during the preovulatory "surge" under the photic conditions of the animal facility. When CP (7mg/kg) or MS (3mg/kg) were administered as a single injection (ip) at 1600h, the LH surge and ovulation were blocked. Continued daily injection of these drugs sustained the ovulatory blockade and caused vaginal smears to remain cornified. Since CP and MS have opposite secondary effects on dopamine receptors, the drug effects were probably due to blockade of serotonin receptors. If the first injections of CP or MS were given at 1700h, the LH surge was initiated but it terminated prematurely. Conversely, the LH surge was initiated early by an injection (sc) of Q at 1530h. When lights in the animal room were left on beyond 1900h on the evening of proestrus, serum LH levels remained high and serotonin turnover increased in the hypothalamus, compared with controls in the standard photoperiod. The stimulatory effect of extra light exposure on LH levels could be mimicked by an injection of Q at 1930h, ½ hour after lights were turned off. Serum LH values in Q treated rats were at surge values at 2000h while saline injected controls showed nearly basal levels at this time. Q injections given at 1530h or 1930h on diestrus 2, neither initiated nor sustained LH secretion. These data suggest that the temporal pattern of LH secretion during its preovulatory surge is determined, at least in part, by a period of serotonergic neurotransmission. Since Q was ineffective if given on diestrus, the response to this serotonin receptor agonist may be estrogen dependent, further suggesting that serotonergic neurotransmission may be a component of the positive feedback effect of estrogen on preovulatory LH secretion. (Supported by NIH AG05068)

140.4 A SEX-RELATED DIFFERENCE IN THE EARLY RESPONSES OF CENTRAL CATECHOLAMINERGIC AND LHRH NEURONS TO CASTRATION. L.V. DePaolo*, S.M. McCann and A. Negro-Vilar. Dept. of Physiology, Univ. Texas Health Sci. Ctr., Dallas, Tx 75235.

It is well known that acute pituitary gonadotropin responses to castration differ between male and female rats. While plasma FSH levels increase 4-8h after orchidectomy(ORDX) or ovariectomy (OVX), initial increments in LH occur 8-12h after ORDX, but are delayed for several days following OVX. Therefore, the present experiments were conducted to determine if alterations in turnover (T/O)rates of dopamine(DA) and norepinephrine(NE) as well as LHRH concentrations occur shortly after ORDX or OVX and if so, whether the observed changes may explain the diverse LH responses. Four-day cycling female rats were used on diestrus day 1. Animals were castrated(CAST) or sham operated at 0800h under ketamine anesthesia. Catecholamine(CA) T/O rates were estimated 3 and 8h after castration by injecting α-methyl-p-tyrosine (α-MPT, 190 mg/kg) through a chronic atrial cannula and measuring CA levels in rats decapitated 45 and 90 min after injection. Steady-state CA levels and LHRH concentrations in tissue and LH, FSH and prolactin levels in plasma were measured in separate groups of rats not receiving α-MPT. DA and NE contents were determined by radioenzymatic assay and LHRH contents by RIA in tissue punches of medial preoptic area(MPOA), suprachiasmatic nucleus(SCH), dorsal anterior hypothalamic area(DAHA), arcuate nucleus(ARC) and median eminence(ME). Significant increases ($p<.001$) in DA T/O rates occurred in the ARC 3 and 8h after ORDX when compared to T/O rates of this amine measured in sham rats. LHRH concentrations also were statistically higher($p<.005$) in the ARC of CAST rats at both time intervals after ORDX. These changes in ARC DA T/O rates occurred even though plasma prolactin levels did not differ between sham and CAST rats at either 3 or 8h after surgery. Similarly, the T/O rate of DA in the ME was faster($p<.05$) 3h after ORDX. Whereas ME LHRH concentrations were higher 3 and 8h after ORDX, the increased levels were significant($p<.005$) only at 8h. The T/O rates of NE in the DAHA(3h) and SCH(8h) of CAST male rats were elevated ($p<.001$ and $p<.05$, respectively) compared to sham rats. Contrastingly, CA T/O rates were generally unaffected or decreased following removal of the ovaries with the exception of an increased T/O of DA($p<.01$) in the ME 3h after OVX. Most importantly, no alterations in DA T/O rates or LHRH levels were observed in the ARC at any time after OVX. Likewise, no significant changes in LHRH levels were seen in any of the other areas studied at either 3 or 8h post-OVX. These studies reveal an important sex difference in the response of dopaminergic neuronal elements in the ARC to ORDX which may be related to the acute increases in ARC and ME LHRH levels and pituitary LH release in male rats after ORDX. (Supported by NIH grants HD05776 and HD09988).

140.5 IONTOPHORESIS OF MORPHINE INTO THE ARCUATE NUCLEUS: EFFECTS ON DOPAMINE CONCENTRATIONS IN HYPOPHYSIAL PORTAL PLASMA AND ON SERUM PROLACTIN CONCENTRATIONS. J.T. Haskins, G.A. Gudelsky, J.C. Porter, and R.L. Moss, Depts. of Physiology and Ob-Gyn, Univ. of Texas Southwestern Med. Sch., Dallas, TX 75235.

It is established that morphine and several opioid peptides stimulate the secretion of prolactin. Moreover, it has been suggested that this effect results from a suppression of dopamine release from tuberoinfundibular neurons into hypophysial portal blood, thereby removing the tonic inhibitory control of prolactin secretion. In the present investigation we have addressed the issue of whether morphine, instilled by iontophoresis in minute quantities into the arcuate nucleus of the hypothalamus, can inhibit the release of dopamine into hypophysial portal blood and stimulate the release of prolactin. Pituitary stalk blood was collected from pentobarbital-anesthetized diestrous rats for 3 consecutive and contiguous 30-min periods. During the second collection period, anodal current was used to iontophorese morphine (50 mM) or NaCl (150 mM) bilaterally into the arcuate nuclei. Iontophoresis of morphine at 1 μA for 10 min followed by 2 μA for 20 min resulted in no significant reduction in the concentration of dopamine in pituitary stalk plasma; however, when an iontophoretic current of 2 μA was used for 10 min followed by 5 μA for 20 min, there was a 60% decrease in the concentration of dopamine in stalk plasma. During the 30-min collection period following iontophoresis of morphine at this current, dopamine concentrations in stalk plasma were reduced 70%. After morphine application at a still higher current (2 and 10 μA), dopamine concentrations in stalk plasma were reduced 83% when compared to control values. Iontophoresis of NaCl at a current and polarity similar to that used with morphine had no significant effect on stalk plasma concentrations of dopamine. When rats were pretreated with the opiate antagonist, naloxone (5 mg/kg, ip), iontophoresis of morphine (2 and 5 μA) into the arcuate nuclei was no longer effective in reducing dopamine concentrations in pituitary stalk plasma. In still another group of animals, morphine was applied (2 and 5 μA) to the arcuate nuclei, and blood samples were taken from jugular cannulae before, during, and after iontophoresis. During and after iontophoresis, serum prolactin concentrations were 2 to 3 times greater than those seen during the pre-iontophoretic period. In view of these results, it seems reasonable to conclude that morphine can inhibit the release of dopamine and stimulate the release of prolactin by acting on nerve cells within the arcuate nucleus.

140.6 PROLACTIN AUGMENTATION OF THE K^+-INDUCED RELEASE OF ENDOGENOUS DOPAMINE AND NOREPINEPHRINE FROM SUPERFUSED MEDIAL BASAL HYPOTHALAMIC FRAGMENTS. M.M. Foreman and J.C. Porter, Depts. of Ob-Gyn and Physiology, Cecil H. and Ida Green Ctr. for Reprod. Biol. Sci., Univ. of Texas Southwestern Med. Sch., Dallas, TX 75235.

It has been demonstrated that the administration of prolactin can within 16-24 hr result in an increase in the turnover of dopamine (DA) within the median eminence (Hökfelt and Fuxe, Neuroendocrinology 9:100, 1972; Gudelsky et al., Neuroendocrinology 22:206, 1976) and in the concentration of DA in hypophysial portal blood (Gudelsky and Porter, Endocrinology 106:526, 1980). However, Clemens et al. (Brain Res 25:371, 1971) and Yamada (Neuroendocrinology 18:263, 1975) have observed an alteration in the electrical activity of hypothalamic neurons within minutes following the intravenous or iontophoretic administration of prolactin, respectively. Perkins and Westfall (Neuroscience 3:59, 1978) have found that prolactin can amplify the electrically induced release of tritium from hypothalamic fragments preloaded with [^{3}H]DA. The present investigation was undertaken to ascertain the effects of prolactin on K^+-induced release of endogenous catecholamines from superfused medial basal hypothalamic (MBH) fragments. DA and norepinephrine (NE) were quantified by a radioenzymatic assay procedure (Ben-Jonathan and Porter, Endocrinology 98:1497, 1976).

MBH fragments from adult male rats were placed in 120 μl chambers (3 MBH/chamber) and superfused at a rate of 70 μl/min at 37°C with a modified Krebs-Ringer bicarbonate solution (118 mM NaCl, 5.0 mM KCl, 1.15 mM $MgSO_4$, 1.15 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 11.1 mM glucose, 10 μM L-tyrosine, and 0.1% bovine serum albumin), which had been adjusted to pH 7.4 and equilibrated with 95% O_2/5% CO_2. After a 1-hr stabilization period, the fragments were superfused for 20 min with a Krebs-Ringer bicarbonate solution similar to that described above but which had been adjusted to 30 mM KCl and 93 mM NaCl. The addition of rat prolactin (50-5000 ng/ml) to the buffer containing 30 mM KCl was found to amplify significantly the K^+-induced release of both DA and NE in a concentration-dependent manner. The stimulatory effects of prolactin (200 ng/ml) on K^+-induced release of DA and NE were prevented by the addition of anti-rat prolactin gamma globulin (0.5 mg/ml) to the superfusion solution. The results of these experiments are supportive of the view that prolactin amplifies the acute release of DA and NE from the hypothalamus. The augmentation of catecholamine release by prolactin may be one mechanism by which prolactin influences hypothalamic neurosecretion.

141.1 SUBSTRATES OF A CEREBRAL ACTIVITY CODE PRECEDING SPEECH. D.H. York, T.W. Jensen and J.G. Rosenfeld. Dept. of Physiology, School of Medicine, University of Missouri, Columbia, MO 65212.

Previous studies in this laboratory have defined specific activity recorded from the scalp preceding speech which is related to the content of the speech production. The present study extends the initial findings and characterizes ten utterances of similar vocal tract configuration with variations in vowel consonant sequencing. Fifteen individuals consisting of right handed females with a mean age of 23.5 years who were either speech pathology or medical students served as subjects. A scalp electrode was placed over the midline interaural position, C_Z (10-20 International System) and referenced to linked ear lobes. A ground was placed on the right forearm. A differential recording of the EEG amplified 10^6 through a bandpass of 1-500 H_Z was recorded on one channel of a multichannel tape recorder. A second channel recorded a square wave coincident with the onset of voicing, sensed by a microphone placed in front of the mouth. A third and fourth channel were used to record EMG from various facial musclature including obicularis oris, obicularis oculi and masseter muscles. The subject sat in a reclined position with head supported at the neck in a semi-darkened room and maintained visual fixation on a white spot 1.0 meter distant. The subject was instructed to produce an utterance with normal inflection and tone at approximately 1/3 sec for 30 repetitions, following which a rest period was interposed before an additional voicing trial. When 100 repetitions of a word had been obtained a rest period followed, before a new word was attempted. Four or five words were voiced per session. Analysis of the data was undertaken by backward averaging the EEG activity using the voice onset trigger as reference. Sample periods of 200 msec, from 216-590 msec preceding voicing were analyzed by entering averaged waveforms into a microprocessor. Each peak was identified with a latency value such that all positive and negative going waves with a pulse width of 1.0 msec were identified. A second program then compared waveforms for 10 subjects and clustered peaks of identical latency (±1.5 msec) to find periods of common activity.

A temporal activity pattern of 28-44 peaks was defined for each utterance. The pattern for each word was unique, but consistent across subjects and significantly different from peaks generated randomly. Words of similar temporal production 'apple' versus 'apper' had portions of their activity pattern identical over the period 375-525 msec preceding voicing. However, marked differences were apparent in the period 216-300 msec, suggesting that the activity is temporally coded.

141.2 INFLUENCE OF TEMPORAL LOBE LESIONS ON RADIO-TELEMETERED ELECTRICAL ACTIVITY OF AMYGDALA TO SOCIAL AND VISUAL STIMULI IN PRIMATES. A. Kling, K. Sternberger*, V.W. Soo*, T. Willis* and M. Imran*. Departments of Psychiatry and Bioengineering, CMDNJ-Rutgers Medical School, Piscataway, N.J. 08854

Utilizing a chronic electrode in the amygdaloid nucleus electrical activity was recorded from 4 M. mulatta and 1 C. aethiops by radio telemetry during: 1) free-moving behavior, and 2) while restrained in a primate chair and viewing projected slides of varying subject matter. Three subjects were subsequently subjected to bilateral ablations of the temporal pole (TP) and two with lesions of the entire temporal neocortex (TL) and after complete recovery, the same conditions repeated. In each case, the depth electrode and radio-telemetry device was not disturbed by the superimposition of the neocortical lesions.

Following the ablation, all subjects showed varying degrees of tameness and hyperorality and in group setting marked decrements in social interactions. For all TP subjects, a marked reduction in amygdaloid electrical activity in all frequency bands (by Fourier analysis) occurred during all behavioral events. For the two subjects with TL lesions, one was similar to the TP subjects but in the other during solitary behavior showed a marked increase in amygdala activity when compared to its pre-operative recordings.

Analysis of electrical activity to viewing visual stimuli indicated a post-operative reduction in responses to the slides for the TP subjects while the reverse was true for the two TL subjects, namely an increase in the number of slides responded to as well as in the power spectrum.

These results suggest that neocortical inputs to the amygdala from TP are largely facilitating while inputs from the middle and posterior portions of temporal neocortex appear to be also inhibitory, especially with regard to processing of visual stimuli. The behavioral changes observed after temporal neocortical lesions in different social environmental settings may thus be related to alterations of amygdaloid function and processing of visual information.

141.3 AUDITORY BRAINSTEM RESPONSES IN AUTISTIC AND CONTROL CHILDREN. R.M. Edwards, P. Tanguay, J. Schwafel, R. Norman, N. Squires, and J. Buchwald. Depts. of Neuroscience and Psychiatry and Physiology, Brain Research Institute and Mental Retardation Research Center, U.C.L.A., Los Angeles, Calif. 90024.

Early infantile autism is characterized by a severe impairment in the cognition and use of language, although the hearing in these children has been described as normal. We have studied one aspect of language acquisition, the reception and transmission of sound, as a possible source of this language impairment, using an objective measure of function in the auditory brainstem pathway, the auditory brainstem responses (ABRs).

Seventeen autistic children, diagnosed using DSM-III criteria, and 17 age and sex matched children with no known disabilities participated in this study. ABRs to monaurally presented click stimuli of three intensities (45, 60, and 75 dB above normal hearing threshold) and three rates (20, 50, and 100/sec) were recorded from a vertex electrode referenced to the mastoid ipsilateral to the ear stimulated. The latencies from stimulus onset to the 5 vertex positive waves were measured and the central tranmission times for waves I to III, III to V, and I to V were calculated. The data were tested for significant group differences in wave latencies and transmission times between and within the two groups.

The latency and transmission time data were first inspected for outlying values (values ± 4 SD from the control means). Two of the autistic subjects, previously reported to have normal hearing, had a marked prolongation of wave I bilaterally. Their latencies at high intensity stimulation were similar to those for low intensities, consistent with a peripheral hearing loss. Their data were not included in subsequent analyses. Eleven of the remaining fifteen autistics had one or more outlying values which were generally longer than the normal means. None of the controls had outlying data.

Significant group differences were demonstrated in the central tranmission times under certain parametric conditions. The autistic subjects had longer III-V and I-V transmission times, but generally shorter I-III transmission times. While these trends were present in all the parametric data, they became significant with low stimulus intensity and fast rate.

ABRs, thus, indicate differences in the transmission of auditory information in autistic children compared to controls. Peripheral hearing impairments in two autistic children, previously thought to have normal hearing, were demonstrated; and prolonged transmission times of some auditory stimuli were found for the group as a whole.

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141.4 COHERENCE SPECTRAL STUDIES ON THE EEG ACTIVITY OF THE AMYGDALA, LIMBIC STRIATUM AND CEREBRAL CORTEX INDUCED BY REPEATED DOSES OF THE AMPHETAMINES. R. J. Morgan, Colorado State Univ., Fort Collins, CO 80521; C. C. Turbes, G. T. Schneider* and J. M. Simard*, Creighton School of Medicine, Omaha, NE 68178.

Recordings are made from cats with chronic electrode implants using hardwire and telemetry techniques. Analog data is collected with an FM tape recorder from the cerebral cortex, septal nuclei, basal-lateral nucleus of the amygdala and the limbic striatum of the brain.

Data are analyzed using a Varian V-72 minicomputer. The discrete time data is transformed using a Fast Fourier Transform (FFT) algorithm. Auto and cross spectral studies show that the first administration of d- and l-amphetamines shows a marked decrease in power at all frequencies investigated. This is most apparent at cortical levels.

With repeated daily administration of d- and l-amphetamine there is an increase in power in the 12 to 16 Hz and the 32 to 50 Hz activity, which never reaches the preamphetamine levels. There is a shift of the 40 Hz rhythm toward 48 Hz. These changes are associated with abnormal behavior.

Coherence functions between two brain areas consider the phase locking of rhythmic activity in terms of power at a given frequency. Coherence spectral estimates made on the EEG activity between the sigmoid gyrus and basolateral amygdala following the first amphetamines decreases the per cent coherence in the 5 to 10 Hz, 12 to 16 Hz and the 32 to 50 Hz activity. In the second and third treatments with the amphetamines there is an increase in the per cent coherence in the 5 to 10 Hz, 12 to 16 Hz, 32 to 50 Hz and the 70 to 100 Hz activity. There is also an increase in per cent coherence between these same brain regions after the second and third doses of d- and l-amphetamine at 180 Hz to 300 Hz. In the 180 Hz to 300 Hz activity, the per cent coherence ranged from 18% to 30% before the amphetamines. During the second and third treatments of d- and l-amphetamines, per cent coherence ranged between 54% and 67% at 180 Hz to 300 Hz. This is during periods of very abnormal non-stereotyped motor and hallucinatory behavior.

141.5 BEHAVIORAL CORRELATES OF SPIKE-WAVE DISCHARGE IN PETIT MAL EPILEPSY. M. M. Orren* and A.F. Mirsky (SPON: A. Siegel). Lab. of Neuropsychology, Boston Univ. Sch. of Med., Boston, MA 02118.

Seven patients with generalized spike-wave discharges in the EEG and clinical absences were tested on a variety of behavioral tasks in order to ascertain their functional capabilities during seizures. All patients or their parents gave their informed consent before participating in this study. Performance on simple motor, stimulus-response and discriminative tasks were monitored simultaneously with six to ten channels of EEG activity recorded from bilaterally placed parasagittal scalp electrodes. Task stimuli included spatially discrete and diffuse visual stimuli, pure tones and, in a few cases, shock to the median nerve. The response required was the same for all tasks. During some of the testing sessions, eye position was also recorded on videotape.

The data of individual patients were considered separately, by task, except where similarity of findings warranted pooling of results. Only seizures with a clear-cut electrographic spike-and-wave or wave-and-spike onset were included. The analysis focused on changes in task responding during successive pre-seizure and seizure segments, defined from the polygraphic record.

The patients varied considerably in their ability to perform a simple motor task during spike-wave activity. Complete arrest of responding, as well as nearly perfect performance were observed, depending on the patient. All patients, however, were significantly impaired during spike-wave discharge when the task involved stimulus-contingent responding, regardless of the modality tested and information content of the stimulus. The degree of impairment and its time course in relation to the spike-wave burst did depend on stimulus modality. Patients were more responsive to auditory and somatosensory than to visual stimuli. Analysis of the videotapes and comparisons of performance to diffuse versus discrete visual stimuli indicated that seizure-related eye movements were not responsible for the relatively poor levels of responding on visual tasks.

The results presented here suggest the complexity and idiosyncratic nature of the petit mal absence attack. During paroxysmal discharge differential, rather than uniform disruption of functional systems is often observed.

141.6 COMPARISON OF DEPTH PROFILES OF SPINDLES AND SPIKE AND WAVE DISCHARGES IN GENERALIZED PENICILLIN EPILEPSY OF THE CAT . M.Avoli*, G. Kostopoulos, P. Gloor and A. Pellegrini*. Dept.Neurology & Neurosurgery, McGill University, MNI, Montréal, Canada, H3A 2B4.

Recent work in our Laboratory has suggested that spike and wave discharges in generalized penicillin epilepsy are generated by the same thalamocortical volleys which normally induce spindles. The cortical response to such thalamocortical volleys appears to assume a spike and wave form when there is a mild increase in cortical excitability.In order to test this hypothesis we compared the potential gradients through the cortical layers of barbiturate spindles and of spikes of spike and wave discharges observed in this model. We used 2 techniques: (a) comparison of epicortical EEG with sequential microelectrode recordings from increasing depths at steps of 200 µm(22 animals) and (b) simultaneous recordings at 8 different depths through a thin film multicontact electrode probe (distances between contacts: 300 µm: Prohaska et al, EEG Journal 1977, vol.42, p.421-422) (12 animals). Data obtained by both techniques were analyzed by visual inspection as well as by computer assisted averaging triggered from peaks of spindles or spikes of the epicortical EEG. The methodological advantages and limitations of the two techniques are complementary. They yielded similar results. Depth profiles of both spindles and spikes of spike and wave discharges showed a polarity reversal within the superficial cortical layers (for spindles, mean: 255 µm, range: 100 to 700 µm; and for spikes, mean: 310 µm, range: 100 to 900 µm) and maximum amplitude at about 0.8 to 1 mm from the cortical surface. The surface negativity mirrored by positivity in the depth was most prominent in spindles while the opposite held for the main phase of the spikes. Exact mirror images were not always observed for spikes, especially when they consisted of more than 2 phases.

142.1 ONTOGENY OF OPIATE RECEPTORS: DIFFERENCES BETWEEN HIGH AND LOW AFFINITY BINDING SITES. An-Zhong Zhang and Gavril W. Pasternak, George C. Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

Previous reports of the ontongeny of opiate receptor binding examined total binding without addressing possible receptor subpopulations. We now report differences in the development of high and low affinity opiate binding in rat brain. Saturation studies over wide concentration ranges, performed simultaneously on young and adult rat brain membranes, were analyzed by Scatchard analysis and expressed as percent of adult levels. While the appearance of total ^{3}H-D-ala^{2}-met^{5}-enkephalinamide binding was quite similar to previous reports, major differences between high and low affinity binding were found. Low affinity binding was relatively constant from day 2 through day 18, ranging from 55% to 62% of adult levels. By contrast, high affinity binding increased dramatically from day 2 (16%) to day 18 (68%; $p<0.002$). Significant differences between levels of high and low affinity binding in 2 day old rats found with a variety of ^{3}H-ligands (morphine, 22% vs 72%; ethylketocyclazocine 26% vs 77%; naltrexone, 27% vs 62%; and dihydromorphine, 23% vs 60%) disappeared by 14 days of age. Morphine's analgesic potency increased almost 2-fold from day 2 (ED_{50}: 56mg/kg) to day 7 (ED_{50}: 30mg/kg) and 40-fold to day 14 (ED_{50}: 1.4mg/kg). The correlation between log ED_{50} and levels of high affinity binding was very good ($r=0.98$). Similarly, β-endorphin (1μg,icv) or D-ala^{2}-met^{5}-enkephalinamide (15 μg, icv) was analgesic in all 14 day old rats tested but in none of the 7 day old animals ($p<0.001$; $p<0.006$, respectively). Unlike analgesia, morphine's depression of respiratory rates were more pronounced in 2 day old rats (ED_{50}: 2.5 mg/kg) than in 14 day old rats (ED_{50}: 9.3 mg/kg). Although the complexity of development precludes definitive conclusions, the apparent association of analgesia with high affinity binding and respiratory effects with low affinity binding is supported by very similar conclusions using the irreversible narcotic antagonist naloxazone (Pasternak et al Science 208 514, 1980). The similar development of high affinity mu, kappa and delta agonist and antagonist binding suggests that they may represent the same site, as previously proposed (Pasternak, Society for Neuroscience, Abstracts, 1980, this volume).

142.2 OPIATE AND DIGITALIS RECEPTOR ASSAYS DISTINGUISH BETWEEN NEUROEXCITANT AND INACTIVE ORGANOCHLORINE INSECTICIDES AND BETWEEN ANESTHETICS AND CONVULSANTS. F. S. LaBella. Dept. of Pharmacol. and Ther., Fac. of Med., Univ. of Manitoba, Winnipeg, Manitoba R3E 0W3.

Unitary theories of general anesthesia do not account for the inactivity or convulsant activity of close structural or isomeric analogs of neurodepressants. Other evidence suggests the existence of specific receptor sites mediating the actions of hydrophobic compounds, both of the neuroexcitant and neurodepressant types. To our knowledge, there does not exist a simple, isolated system that can differentiate between these two types of agents. We found that the specific binding of 3H-naloxone to rat brain tissue in vitro was inhibited in a concentration dependent manner by the excitant organochlorinated insecticides (OCI), by ether (E) and octanol (OCT), and by the convulsant Indoklon (IND), CF3-CH2-OCH2-CF3, and its anesthetic isomer, Isoindoklon (ISO), (CF3)2-CHOCH3. Inhibition of opiate receptor binding was closely related to lipophilicity of the compound, irrespective of its pharmacological action. However, in the presence of 100 mM NaCl the inhibition of naloxone binding by E, OCT, and ISO was greatly potentiated, whereas that by OCI and IND was attenuated. 100 mM KCl was equally effective as NaCl on the action of anesthetics, but the effect of the excitant drugs was, in contrast to NaCl, unaffected by KCl. Specific binding of 3H-ouabain in the absence of Na, was depressed by anesthetics and enhanced by neuroexcitants. In the presence of NaCl, which by itself inhibits ouabain binding to brain, both anesthetics and excitants enhanced ouabain binding. DDE, a non-insecticidal analog of DDT, and the dimethyl derivative of the cyclodiene OCI, lindane, were inactive in the receptor assays. These observations point to a unique isolated system which responds consistently to anesthetic agents as a class and, in a different way, to neuroexcitant compounds. The system appears to act as a reliable in vitro predictor of neuroactivity of hydrophobic chemical compounds. These findings also delineate brain receptors and/or associated membrane moieties as possible specific sites of action of both neuroexcitants and neurodepressants. Furthermore, the critical site of action of both classes of agents may be at the same or closely adjacent functional membrane components. (Supported by the MRC of Canada, the Manitoba Heart Foundation and the Sellers Foundation. The author is an MRC Career Investigator).

142.3 MORPHINE AND OPIATE PEPTIDES: DIFFERENTIAL ACTIONS ON CALCIUM ACTION POTENTIALS. M.A. Werz and R.L. Macdonald. Neurosciences Program and Department of Neurology, University of Michigan, Ann Arbor, MI 48109.

Opiates have been found to depress the release of transmitter from dorsal root ganglion (DRG) neurons (Macdonald and Nelson, et al: Sci 199: 1449, 1978; Mudge, et al: PNAS 76: 526, 1979). Since transmitter release is calcium-dependent, we have studied the effects of opiate compounds on the duration of calcium-dependent action potentials of mouse DRGs grown in primary dissociated cell culture. Furthermore, since recent studies have suggested the existence of multiple opiate receptors and differential physiological functions for morphine and the enkephalins, we have investigated the dose-dependent actions of morphine, leucine-enkephalin (L-Enk) and methionine-enkephalin (M-Enk).

Spinal cords and attached DRG's, from 12.5-14 day old fetal mice, were mechanically dissociated into single cell suspension and grown in culture for three to six weeks prior to electrophysiological study. Action potentials in DRGs have both sodium and calcium components. The calcium component was increased by recording in Tris buffered saline containing the potassium conductance blockers tetraethylammonium (17mM) and 3-aminopyridine (5mM). DRGs were differentiated from spinal cord cells by morphological characteristics. Action potentials were elicited at regular intervals while opiate agonists, naloxone plus opiate agonists, or control buffer were applied by superfusion.

Dose-dependent effects of morphine were observed, with low doses (2.0 μM) decreasing 34% and higher doses (20 μM) increasing (54%) the duration of calcium action potentials. In contrast, L-Enk prolonged calcium action potentials from .6 to 20 μM. Preliminary data with M-Enk suggest that it prolongs at low doses but shortens at higher doses. Both effects, prolongation and shortening of calcium action potentials, were reversed by superfusion with either control buffer or naloxone plus agonist.

Low doses of morphine shortened the duration of calcium action potentials, consistent with depression of transmitter release by opiates. However, high doses of morphine and low doses of enkephalin prolonged the calcium action potential, consistent with a convulsant action (Heyer and Macdonald: Neurology 30: 375, 1980; see abstract Macdonald and Werz). Thus, we have observed dual opiate effects, either prolongation or shortening of calcium action potentials, dependent upon opiate agonist and dose. These data are consistent with neurochemical and behavioral studies suggesting the existence of multiple opiate receptors. Supported by NIH grant NS 15225 and RCDA NS 00408 (R.L.M.).

142.4 CLASSIFICATION OF OPIATES AND OPIOID PEPTIDES ACCORDING TO THEIR AFFINITIES, AGONIST AND ANTAGONIST ACTIVITIES TO MORPHINE AND ENKEPHALIN RECEPTORS. K.-J. Chang*, E. Hazum*, P. Cuatrecasas* (SPON: A.T. Dren) Dept. of Mol. Biol., Wellcome Research Laboratories, RTP, NC 27709.

The binding of many opiates and enkephalins to enkephalin (δ) and morphine (μ) receptors was compared by using three different binding assays; a) ^{125}I-[D-Ala^{2}, D-Leu^{5}]enkephalin, and ^{125}I-[D-Ala^{2}, N-Me-Phe^{4}, Met(O)^{5}ol]enkephalin and b) [^{3}H]ethylketocyclazocine to brain membranes, and c) [^{3}H]diprenorphine and [^{3}H]naloxone to neuroblastoma cell and brain membranes, respectively. According to their relative binding potencies and the effects of Na^{+} and GTP on the binding to these two receptors, opiates and enkephalins can be classified into 7 classes: (1) morphine-type μ agonists; (2) enkephalin-type δ agonists; (3) mixed agonists-antagonists; (4) putative κ agonists; (5) putative σ agonists; (6) nalorphine-type antagonists; and (7) opiate antagonists. Studies with [^{3}H]ethylketocyclazocine do not reveal a specific κ receptor distinct from those already described that bind morphine and enkephalins. The benzomorphan analogs, ketocyclazocine and ethylketocyclazocine (putative κ agonists), and N-allylnormetazocine (putative σ agonist) bind to morphine (μ) and enkephalin (δ) receptors with similarly high affinity. The potency of putative κ agonists, measured by competition of binding of the ^{3}H-labeled antagonist, is greatly reduced by the presence of Na^{+} and GTP, the "Na^{+} and GTP ratios" are similar to those of morphine and enkephalins. However, Na^{+} and GTP greatly reduce the potency of binding of putative σ agonists to enkephalin receptors but only slightly reduce the binding affinity to morphine receptors. These data suggest that putative κ agonists have agonist activity toward both receptors, whereas putative σ agonists behave as agonists for enkephalin receptors while having antagonist activity for morphine receptors. Mixed agonists-antagonists also show smaller difference in affinity to both receptors. These findings may have important implications for understanding the differences in the pharmacological effects of these drugs. A model is hypothesized to explain the complex central pharmacological effects of opiates and opioid peptides.

142.5 CONVULSANT ACTIONS OF OPIATES ON MAMMALIAN NEURONS IN PRIMARY DISSOCIATED CELL CULTURE. R.L. Macdonald and M.A. Werz. Dept. of Neurology and Neurosciences Program, Univ. of Michigan, Ann Arbor, MI 48109.

Opiates have been demonstrated to have convulsant as well as analgesic actions. While specific opiate-induced depression of transmitter release from nociceptive afferents may underlie analgesic effects, the convulsant mechanisms of action remain uncertain. Recent studies have shown that neuronal calcium conductance may be of importance in production of paroxysmal depolarizing shifts (PDS) (Wong and Prince, Science 204:1228, 1979), the intracellular event underlying interictal spikes in the EEG of patients with epilepsy. We have shown that convulsants such as bicuculline and penicillin prolonged calcium-dependent action potentials (Heyer and Macdonald, Neurology 30:375, 1980) of spinal cord (SC) and dorsal root ganglion (DRG) neurons in primary dissociated cell (PDC) culture. Furthermore, we have demonstrated that leucine-enkephalin (L-ENK) and high concentrations of morphine (20 μM) prolonged calcium action potentials while morphine at low concentrations (.6 to 20 μM) shortened calcium action potentials (see abstract, Werz and Macdonald) of neurons in PDC culture. Thus L-ENK and morphine might be expected to be convulsant at concentrations that prolonged calcium action potentials, and we have investigated opiate actions on neuronal activity of mouse spinal cord neurons in PDC culture.

Spinal cords and dorsal root ganglia were mechanically dissociated, plated as a single cell suspension and grown for four to six weeks prior to electrophysiological study. Spontaneous activity was recorded from SC neurons in phosphate buffered saline at 35-37°C.

Morphine had dose-dependent actions on spontaneous activity in SC neurons. Low concentrations (2 μM) had little effect on spontaneous activity but high concentrations (20-50 μM) produced high amplitude paroxysmal depolarizations (paroxysmal depolarizing events (PDE)), an in vitro equivalent of PDS. The PDEs were abolished by naloxone (50 μM). The opiate peptide L-ENK (20 μM) also produced PDEs.

Thus, we have demonstrated that opiates have dose-dependent and naloxone reversible convulsant actions on mouse SC neurons in PDC culture at concentrations that prolong calcium action potentials. Since these opposing opiate actions on calcium conductance are dependent upon opiate agonist and dose (see abstract, Werz and Macdonald), it is likely that their convulsant and analgesic actions are mediated by pharmacologically distinct receptors. Supported by NIH grant NS 15225 and RCDA NS 00408 (R.L.M.).

142.6 COMPARATIVE STUDIES OF [^{3}H]-5-HYDROXYTRYPTAMINE BINDING IN MEMBRANE PREPARATIONS OF RAT BRAIN AND RAT GUT. J. A. Heltzel and W. H. Vogel, Dept. Pharmacology, Thomas Jefferson University, Philadelphia, PA. 19107.

The specific binding of [^{3}H]-5-hydroxytryptamine (5HT) was measured in crude homogenates of whole rat brain minus cerebellum using a modification of the method outlined by Bennett and Snyder (1976). Homogenates of rat gut were prepared from strips of longitudinal intestinal muscle with attached myenteric plexus, as described by Creese and Snyder (1975). For brain and gut binding studies, the preparation consisted of a washed 50,000 x G pellet, using a tissue concentration of 40 mg/ml 50 mM TRIS·HCl containing 5 mM $CaCl_2$, pH 7.4.

In rat brain, 5HT binding met the following criteria: ligand saturability, tissue linearity, reversibility, rapid equilibration, and temperature dependence. Specific 5HT binding was evaluated using 10 μM unlabelled 5HT as a blank for nonspecific binding. Scatchard analysis in rat brain yielded an equilibrium dissociation constant (K_D) of 2.1 nM and the apparent number of binding sites (B_{max}) was 17.4 pmol/gram wet weight.

In rat gut, however, there were notable differences. Saturation of ligand binding could not be demonstrated up to a concentration of 40 nM [^{3}H]-5HT. The binding equilibrium was approached gradually, reaching completion by two hours. Once equilibrium was reached, the binding was slowly dissociable. Increasing tissue concentrations produced enhanced 5HT binding, but the relation was not strictly linear. Specific 5HT binding was measured using 0.5 μM unlabelled 5HT as the nonspecific binding blank. Under these conditions, Scatchard analysis in rat gut gave values for K_D = 4.0 nM and B_{max} = 13.6 pmol/gram wet weight.

Displacement of 5HT binding employed the serotonin agonists, tryptamine (TA) and 5-methoxytryptamine (5MT), and the serotonin antagonists, methysergide (MS) and N,N-diethyltryptamine (DET). Preliminary data suggest that the rank order of displacing potencies shows good agreement between gut and brain, although TA, MS, and DET are two orders of magnitude more potent in brain.

	IC_{50} Brain	IC_{50} Gut
TA	3×10^{-6} M	8×10^{-4} M
5MT	2×10^{-7} M	4×10^{-7} M
MS	3×10^{-7} M	2×10^{-5} M
DET	4×10^{-6} M	$> 1 \times 10^{-4}$ M

147.1 ORGANIZATION OF VISUOSPATIAL FUNCTIONS IN HUMAN NON-DOMINANT CORTEX: EVIDENCE FROM ELECTRICAL STIMULATION. G. Ojemann, I Fried*+, C. Mateer*, R. Wohns* and P. Fedio*#. Dept. Neurol. Surg., Univ. of Washington, Seattle, WA 98195.

Cortical localization of several visuospatial functions was assessed with the electrical stimulation mapping technique at 5-9 sites in the right, nondominant hemisphere of 10 patients undergoing craniotomies under local anesthesia for treatment of medically intractible epilepsy. Visuospatial functions measured include perception and memory for faces and angles, and emotional labeling of faces.

Perception of faces or angles was measured in all cases. Repetitive errors were evoked in 4 of 69 sampled sites, in 4 patients. These sites were at the parieto-occipital junction, except for one in inferior premotor frontal cortex. Post-distractional short-term memory for angles was measured in 5 cases; for faces in 2. Significant errors occured at 12 of 36 sites sampled for angle memory and 6 of 12 samples for face memory, at least one site in each patient. All but 2 of these sites are in posterior superior temporal gyrus or adjacent supra-marginal gyrus. With two exceptions, only stimulation during either input, or storage, or output phase of memory altered performance at a given site; sites where performance on one phase of the memory test was altered in different patients cluster together. When memory for both faces and angles was measured in the same patient, sites where this was altered overlap. Emotional labeling of face was measured at 56 sites in 8 patients. Significant alterations were evoked at 6 sites in 4 patients, all these sites are in posterior middle temporal gyrus. Facial recognition was intact at these sites: the stimulation effect was an alteration in the label usually used by that patient for the emotion portrayed by that face, but without any consistent emotional bias in the type of alteration. In each individual patient, changes in these different spatial functions were evoked from different cortical sites. No site showed evoked changes in more than one function, although changes in the same function (memory) occured at the same site with different spatial inputs (faces vs. angles). There is discrete localization of these visuospatial functions in the non-dominant hemisphere. That localization is at least as discrete, and perhaps with even less individual variability than is seen for language in dominant hemisphere.

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147.2 PATTERN REPRODUCTION AND POSTERIOR PARIETAL LESIONS IN THE MONKEY. Henry V. Soper, Edward H. Yeterian*, and William A. Wilson, Jr. Department of Psychology, University of Connecticut, Storrs, Connecticut 06268.

The integrity of the posterior parietal cortex is generally thought to be essential for normal processing of spatial information, but the deficits which have been observed may not reflect a simple inability to determine spatial relationships between objects. If, as in the human Balint's syndrome, parietal monkeys fixate on a focus, neglecting all peripheral information, there may be no deficit in appreciating spatial information per se. The present study was designed to determine if the posterior parietal cortex is involved in the ability to appreciate spatial relationships between objects, or if it is more likely that animals with this area removed tend to fixate more on a focus, possibly neglecting information away from the focus.

Six monkeys (Macaca mulatta) were presented with a 5 X 5 matrix of possible reward locations, one or two correct locations being illuminated during the cue presentation interval. Responding to a location illuminated it, and correct responses reproduced the cue pattern. The animals, initially experimentally naive, were tested until each had reached an asymptote in performance. Then they were divided into three equal groups. The animals in the posterior parietal group received lesions of the inferior parietal lobule, dorsal prestriate cortex, and the precuneal gyrus. One control group received lesions of the inferotemporal cortex, whereas the other received no lesions. The animals were tested for 100 sessions with no delay between the cue and response presentations. Then for 100 sessions a 2-sec delay was interposed, followed by 40 sessions with a 4-sec delay.

There was no difference between the groups on the 0-sec delay task, but introducing a 2-sec delay impaired the performance of the control groups much more than of the parietal one ($F's_{(2,3)}$ 13.1, 23.2, p's .05, .025 for the first and second 20-session blocks respectively). When the 4-sec delay was imposed the parietal performance was essentially unchanged, while the performance level of the other animals declined.

The results clearly demonstrate no impairment in processing spatial information per se following the removal of the posterior parietal cortex. These results do suggest that the parietal animals tend to fixate more on the focus of attention -- the reward locations -- than normal. The visual fixation appears to reflect that found in Balint's syndrome in humans with similar damage.

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147.3 CUTANEOUS DELAYED RESPONDING AND OTHER BEHAVIORS AFTER PREFRONTAL CORTICAL ABLATIONS IN CATS. R.B. Glassman, D.E. Cook*, and H.N. Glassman*. Dept. Psychol., Lake Forest College, Lake Forest, IL 60045.

Frontal damage causes deficits in delayed responding and other behaviors in various testing situations, usually involving visual stimuli. We report similar deficits in four frontally damaged cats trained to discriminate cutaneous cues.

The blindfolded cat, remaining on a small pedestal, was trained for food reward to stand on its hindlegs (up-response) when stroked on the upper lateral surface of either forelimb and to reach down below the pedestal surface with its mouth (down-response) when tapped in the same place (Physiol. Behav., 1977). Cats were then trained to delay discriminative responses for five seconds, while blocked by plates moved into position above the head and below the chin.

The following behaviors were seen after bilateral ablation of gyrus proreus: 1. Deficit in nondelay performance for 1-4 sessions; 2. During the same sessions, reluctance to complete up-responses to the learned cue, but performance of the same responses briskly and reliably to tactile stimuli to the head eliciting pursuit; 3. Early postoperative disorientation - stepping off pedestal and pacing, turning on pedestal, striking reflexly at the tactile cue, frequent meowing, remaining in up-response position with forepaws on top restraining plate; 4. After recovery from 1-3, deficit in delayed responding (near random level), associated with failure to maintain preparatory position during delay, gradually recovering to near normal over ten sessions. Two of the cats received ablation in stages; after the first, deficits were symmetrical and milder than after bilateral loss. Deficits were not usually seen in cutaneous, auditory, or visual orientation-localization, in movement, or in food motivation; however there was early postoperative reluctance to perform in a food retrieval test of forelimb motor control. Late postoperative nondelay testing with stimuli applied for the first time to the hindleg, revealed transfer as excellent as in normal cats.

Most of the findings fit the interpretation that proreal damage caused neither a sensory nor motor deficit, but a deficit in learned sensory-motor associations. Regional knowledge of response properties of single neurons helps to explain somesthetic or motor deficits seen after localized cortical damage. It is much more difficult to theorize about the microscopic neural phenomena that underlie the sensory-motor behavioral "connections" that were disturbed in the present experiment.

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147.4 SOMATOSENSORY INVOLVEMENT IN VISUAL NEGLECT. Robert Sinclair*, Simon Horenstein, and Arthur Toga*. Departments of Neurology and Psychology, Saint Louis University, Saint Louis, Missouri 63104.

Ablation of somatosensory areas II (SII) in the cat did not produce modification of a visually dependent learned task. As previously reported, ablation of auditory cortex in posterior ectosylvian gyrus (Ep) modified preference to double simultaneous visual stimulation (DSS) without affecting lateral preference to unilateral stimulation. Clinical cases of sensory neglect are usually trimodal. Somesthetic stimulation elicits visual orienting responses, and in the major sensory areas association fibers reciprocally connect all known subdivisions. This suggests that lesions of somatosensory cortex might also produce visual neglect.

A common corticocortical projection point (Vss) between auditory Ep and visual cortex exists in mid suprasylvian gyrus. SII does not demonstrate a similar connection with visual cortex. It does project to somatosensory area I (SI) as well as to part of auditory area I (AI), and has thalamic connections similar to the visual system. A third auditory area which is described in the upper and anterior portions of the ectosylvian gyrus (SIIA) exhibits overlap of auditory and somatosensory evoked responses.

The organization of the somatosensory system exhibits many similarities to the auditory and visual systems with the exception of visual-auditory projections to Vss. The purpose of the present study was to determine whether damage to SII results in visual neglect. This might indicate the degree of significance of lesions of Vss in causing sensory neglect.

Three cats were appetitively conditioned in a specially designed "Y" maze (Toga, A.W. et al., Psychological Reports, 40:1071-4, 1977) equipped for multiple modalities of stimulation. On unilateral stimulation animals were required to move into the sole lighted arm of the maze and on DSS to move into either of two in order to obtain food reward delivered from an identical feeding cups at the end of each. Each animal established its own lateralization pattern to DSS. Response to unilateral stimulation was usually appropriate to the side stimulated. After training, subpial resection of SII in the anterior ectosylvian gyrus was performed and animals tested from the 1st to 10th postoperative days. After intracardiac saline-formalin perfusion, brains were removed for anatomic study. Lesions imposed no apparent postural bias. There was no statistically significant effect on visually dependent discrimination.

Results suggest visual-auditory projections to Vss represent a unique relationship between these primary sensory areas. While Vss lesions may produce somesthetic neglect, subcortical connections alone may be responsible for the somesthetic involvement in trimodal neglect following parietal lobe damage in human cases.

147.5 ENVIRONMENTAL CONSTRAINTS ON THE MOTOR ABILITIES OF DENEOCORTICATE RATS. I.Q.Whishaw, A.J.Nonneman and B. Kolb. Department of Psychology, University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

The motor abilities of deneocorticate (all neocortex removed) rats have been subjected to careful analysis in a number of recent studies. Prominent among the rat's chronic disabilities are: 1. Lack of forepaw inhibition during swimming, 2. Inability to chain together long sequences of grooming behavior, and 4. Reduced exploratory activity in an open field.

In the present study we reexamined the motor abilities of deneocorticate rats, and in addition, we varied the environmental condition under which the tests and observations were made. Performance varied markedly with test conditions.

In swimming tests deneocorticates swam ineffectively and showed no forepaw inhibition in warm water (37.5°C), but swam more vigorously than control rats and also showed forepaw inhibition in cold water (18°C).

Deneocorticates failed to sit up and manipulate food when given large (4 gm) pieces of food, but they ate small pieces of food in the normal way. Unlike controls, however, they had difficulty holding on to, and chewing, small food pellets.

When the grooming of deneocorticate rats was scored when they were wet and in a novel environment they were profoundly impaired. When grooming was scored when the rats were dry and in their home cage, grooming sequences approximated those of normal rats. However, deneocorticates still neglected the caudal part of their body more than control rats.

When activity tests were given in a lighted open field, deneocorticate rats were less active than control rats. When the tests were given in the dark they were more active than control rats and left the walls of the open field more than control rats.

Environmental conditions also placed constraints on many other behaviors including drinking water, eye blinking, righting responses, and motor activity in activity wheels.

The results show that the motor behavior of deneocorticate rats is more subject to environmental variation than is true for normal rats. This suggests that a general function of the neocortex is to release an animal from environmental constraints, and give it more control of its motor activity in widely divergent environmental conditions.

147.6 RELATIONSHIP BETWEEN BEHAVIORAL CORRELATES OF UNIT ACTIVITY IN THE ANTEROMEDIAL CORTEX AND THALAMUS IN THE RAT. T. L. Martin and H. M. Sinnamon, Laboratory of Neuropsychology, Wesleyan University, Middletown, CT 06457.

Previous work in this laboratory (Kanki et al, Neurosci. Abst. 5:276, 1979) had identified a population of neurons in the anteromedial cortex of the unrestrained rat which possibly code rewarding and aversive motivational properties of various stimuli by decreases and increases in activity, respectively. The activity of these cells decreased during rewarding brain stimulation, drinking saccharin, and sniffing-exploration; activity increased during withdrawal from various objects and often after removal of a drinking tube. The purposes of this study were first, to further describe this class of cells by testing them with wider range of aversive stimuli, and second, to determine whether the thalamus also showed this biphasic pattern.

Water deprived rats were presented with various stimuli: drinking tubes containing .1% saccharin, 50% condensed sweetened milk, 10% quinine or nothing; scent-marked Petri dishes; a conspecific of opposite sex; tactile probing of face and body; a startling click; a black-white grid moving in the visual fields; an aversive odor; aversive prodding by means of a metal rod; and repetitive bright flashes from an overhead strobe. Unit activity was recorded differentially through a pair of 25 µm stainless steel wires lowered in a bundle of four wires by means of a chronically implanted microdrive.

So far, 34 thalamic recording sites in and around the dorsomedial (DM) nucleus have been tested. Within the medial part of DM activity in 13 sites was correlated with head movements (n=9), visual stimulation (n=1), orienting (n=1), or tactile stimulation of the snout (n=1); activity in one site had no observable relation to behavior. Of the 9 sites tested in the lateral DM, increases in activity were associated with head movement (n=5), locomotion (n=1), or forepaw movement (n=1); two sites had no detectable correlate with behavior. The activity of 8 sites in the nucleus parafasicularis (PF), was correlated with tactile stimulation of the vibrissae (n=4), sniffing (n=1), movement of the forepaw (n=1), or head movement (n=1); the activity of one unit could not be related to behavior. Two cells within the anterior thalamic complex demonstrated increases in unit activity during the presentation of aversive stimuli, while another was sensitive to the animal's place within the testing chamber. One site in the posteromedial nucleus was associated with mouth movements. These preliminary results suggest that the DM is less likely to be involved in the coding of motivational properties that it is with the processing of head movement information.

147.7 Unilateral Thalamic Damage and Motor Behavior of Rhesus Monkeys R. K. Deuel and T. W. Anderson.* Departments of Pediatrics and Neurology, Washington University School of Medicine, St. Louis, MO 63110.

In primates, bilateral destruction of thalamic nuclei, notably medialis dorsalis (nMD), has been reported to cause behavioral deficits that are known to follow removal of specific frontal cortical grey sectors. To test whether unilateral nMD lesions result in hemineglect and other behavioral symptoms that follow unilateral frontal cortical damage, we implanted yttrium-90 pellets unilaterally in five mature (4.0-5.3 K) naive rhesus monkeys, using Olszewski's stereotoxic atlas as a guide.

On standardized, scored neurological exams given at intervals from 20 - 1,184 days postoperatively, preference for the hand ipsilateral to the lesion (I hand) was found, with impaired dexterity of the hand contralateral to the lesion (C hand). These deficits were stable over at least two years, and even worsened in two animals. Mild somatosensory, but no visual field, deficits were found. Postoperative learning of a contingent arm and hand response by the C limb was normal, whereas there was severe impairment in learning a motor response with C fingers.

Histological verification of the lesion revealed destruction of nMD in all animals, with marked additional nuclear destruction that varied among animals. The internal capsule was invaded in two animals.

Unilateral nMD lesions result in deficits in fine motor learning and performance, but not visual field deficits. Spontaneous recovery, such as occurs after frontal cortical lesions, was not observed. Rather, motor deficits were unchanged even after prolonged training of the affected limb.

147.8 IMPAIRED HEAT STRESS-INDUCED SALIVA SECRETION IN VMH LESIONED RATS. F. W. Flynn*, L.A. Evey* and J.C. Mitchell. Dept. of Psychology, Kansas State University, Manhattan, Ks. 66502.

A previous study demonstrated that ventromedial hypothalamic (VMH) lesions reduce basal saliva secretion in rats (Flynn et al., Physio. Behav., 1980, 24, 451-455). The present study examined the salivary response of "static" VMH and control rats to heat stress. Saliva secretion of five control and five VMH rats was observed at four different ambient temperature settings: 26° (room temperature), 31°, 36°, and 41°C. Rats were exposed to a temperature setting for 30 min and then saliva secretion was measured. Salivation was measured by inserting a preweighed cotton swab into the rat's mouth and holding it there for 1 min. The swab was then reweighed to determine the amount, by weight, of saliva secreted. Rats were adapted to this procedure prior to the heat stress tests. Body temperature was measured rectally following 30 min exposure to a temperature setting.

Rats with lesions of the VMH displayed less saliva secretion and lower body temperature than controls. VMH rats required both a higher ambient temperature and a higher body temperature than controls for saliva secretion to increase significantly.

While VMH lesioned rats appeared to have no gross impairment of a heat loss mechanism, the lesions did alter the initiation and amount of saliva secreted. These changes in saliva secretion indicate that the lesions interfere with autonomic control of the salivary glands.

There is conflicting literature on temperature regulation by VMH rats. Our observation of hypothermia is consistent with Han (Am. J. Physiol., 1968, 215, 1343) but conflicts with Mayer and Greenberg (Am. J. Physiol., 1953, 173, 523) who reported hyperthermia in VMH rats at room temperature. However, Mayer and Greenberg reported that when exposed to 40°C, VMH rats had lower body temperature than controls. The reason for the conflicting reports of temperature regulation by VMH rats is not immediately apparent.

147.9 HIPPOCAMPAL SUPERSTITION: A CASE OF CA-MEDIATED STEREOTYPY. L. D. Devenport, J. A. Devenport* and F. A. Holloway. Dept. Psychiat. & Behav. Sci., U. Okla. Health Sci. Ctr., Oklahoma City, OK 73190.

Rats are quite resistant to the development of superstitious behavior, but this resistance is lost after bilateral lesions of the hippocampus are made (Devenport, Science 205:721, 1979). Subsequent analysis has revealed that although adventitious response-reinforcer conjunctions definitely contribute to the response topographies observed (Devenport, Behav. Neur. Biol., in press), hippocampal superstition is primarily a condition of relative behavioral invariance which is exacerbated by the non-contingent delivery of reinforcers (Devenport & Holloway, J. Comp. Physiol. Psychol, in press).

Perhaps the hippocampus opposes the action of dopaminergic (DA) systems involved in the production of behavioral stereotypy. The shift toward DA dominance following hippocampal lesions would be further potentiated by noncontingent reinforcement (Fouriezos et al., J. Comp. Physiol. Psychol. 92:661, 1978). This possibility was tested in two ways.

In one, sham- and hippocampal-lesioned rats were maintained in clear plastic cages where activity was automatically monitored. Stereotypy (0-6 scale) was rated "blindly" across days of free-feeding, total deprivation, and daily refeeding (an amount sufficient to maintain body weights at 80%). Groups did not differ significantly during free-feeding, but deprivation, and most especially, scheduled refeeding strongly boosted the activity and stereotypy scores for hippocampals. Shams showed only slight increases. When stable, stereotypy scores taken 1 hr. before refeeding were about 3.3 for hippocampals, 0.5 for shams. Administration of haloperidol (0.1 mg/kg, s.c.) to hippocampals reversed their activity and stereotypy to the sham level. Administration of d-amphetamine (2 mg/kg, i.p.) to shams was sufficient, in combination with the refeeding regimen, to elevate their respective scores to undrugged hippocampal levels.

In the second experiment, hippocampals and shams were tested in operant chambers under an interval schedule of pellet delivery. Some were trained to lever press, others received pellets noncontingently. Noncontingently-maintained hippocampals developed robust superstitious behavior but other groups did not. Haloperidol administered to hippocampals eliminated all evidence of superstition but affected contingently-maintained performance only moderately. D-amphetamine had opposite symmetrical effects in shams.

Our evidence suggests that the hippocampus opposes catecholamine-mediated stereotypy, superstition being a particular instance.

147.10 EFFECTS OF SEPTAL AND HIPPOCAMPAL LESIONS ON PERFORMANCE OF A SHOCK ESCAPE TASK. Sarah E. De Rossett* and William G. Drew*. (SPON: Ralph E. Miller). Behav. Neurophys. Lab., Dept. of Psych., Univ. of Ky. Col. of Med., Lexington, Ky. 40536

In an attempt to distinguish between the behavioral effects of septal and hippocampal lesions, young adult male Long-Evans hooded rats were given either septal or hippocampal lesions, allowed to recover, and then tested twenty days post-operative on performance of a shock escape task previously shown to be sensitive to the effects of septal, but not hippocampal, lesioning. Individual animals were placed in standard operant conditioning chambers and 90 sec later, a 1.5 mA scrambled footshock was delivered to the cage floor. Shock was terminated when the animal pressed one of two bars present within the chamber. Daily sessions consisted of ten trials. Behavioral measures included: (1) total number of bar presses made during the session, (2) number of times shock onset occurred while the animal had the bar depressed, (3) total amount of time during the session in which the bar was depressed, and (4) the latency with which the animal terminated the shock. Testing was conducted for five days, after which the animals were tested on the reversal of the task (i.e. the other bar present within the chamber functioned to terminate shock). Subsequent to behavioral testing, all animals were sacrificed and lesion sizes were determined.

Repeated measures analysis of variance revealed that septal, hippocampal, and sham-operated controls all learned to terminate the shock during both phases of testing, increasing their efficiency in doing so over sessions. No significant treatment effect was seen. The total number of bar presses made during each session did not differ between any of the groups during either phase of testing. The number of times the animal had the bar depressed when shock onset occurred increased across the five days of initial testing, while remaining relatively constant during the reversal. No significant differences existed between the groups, however, during either phase. Total bar time increased across days for all the groups during the first phase of testing, remaining relatively unchanged during the reversal. No significant differences existed between any of the groups during either phase. Thus, none of these measures differentiated the groups.

The fact that animals with hippocampal lesions perform this task comparably to controls corroborates previous unpublished findings. The demonstration that septal animals improve their performance across sessions, however, stands in marked contrast to previous work by Gotsick et al. (Phys. Behav. 6:199, 1971). These data suggest that strain and age differences, post-operative recovery time, as well as contextual cues may be responsible for the discrepancy.

147.11 FUNCTIONAL MAPPING OF HYPOTHALAMIC PATHWAYS ELICITING AGGRESSION AND ESCAPE BEHAVIOR IN THE CAT: A RADIOAUTOGRAPHIC STUDY. S. Fuchs, A. Siegel, and H. Edinger. Depts. of Physiology and Neurosciences, College of Medicine & Dentistry of N.J.--N.J. Medical School, Newark, New Jersey, 07103.

This study was designed to identify the efferent pathways associated with quiet biting attack, affective defense, and escape behavior in the cat. Behavioral responses noted above were elicited from the hypothalamus subsequent to electrical stimulation utilizing moveable electrodes which were advanced through guide tubes anchored to the skull. Under anesthesia, the electrode was removed and replaced with a Hamilton syringe which was lowered to the exact position previously occupied by the electrode. An injection of 0.2 µl ^{3}H-leucine was then placed into the site of the electrode tip. Following survival times of 1-4 days, animals were sacrificed and the brain tissue prepared for autoradiography.

Following injections placed into the anterior lateral hypothalamus and associated with quiet biting attack, labeled axons and terminals were traced to the dorsal septum, diagonal band, preoptic area, supramammillary region, ventral tegmental area, midline thalamus, and lateral habenular nucleus. Labeled axons arising from affective display sites situated in the ventromedial nucleus were traced principally in a rostral direction and terminated in greatest quantities in the bed nucleus of the stria terminalis and medial preoptic area. Fiber pathways associated with affective display and which project as far caudally as the midbrain central gray and ventral tegmentum appear to arise from the borderline region of anterior medial hypothalamus and preoptic area. Fibers associated with flight behavior seem to arise from the dorsomedial hypothalamus and have widespread projections to the substantia innominata, preoptic area, diagonal band, medial amygdaloid nucleus, supramammillary region, central gray and central and ventral tegmental fields. Additional studies currently under investigation utilizing ^{14}C-2-deoxyglucose tracing method have appeared to replicate the findings reported above. For example, stimulation of affective display points in the ventromedial nucleus results in subsequent activation of the anterior medial hypothalamus, medial preoptic area, bed nucleus of stria terminalis, but not of any structures caudal to the site of stimulation.

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147.12 **EFFECTS OF STRIA TERMINALIS AND AMYGDALOID LESIONS ON SEX AND AGGRESSION OF MALE GOLDEN HAMSTERS.** **Mike S. Perkins*, Mary N. Perkins and B.N. Bunnell. Department of Psychology, University of Georgia, Athens, GA 30602**

Since corticomedial amygdaloid (CMA) and stria terminalis (ST) lesions produce ejaculatory deficits in rats, the effects of such lesions were examined in hamsters. Adult male hamsters were given 4 Preop sexual satiation tests. Hormonally treated ovariectomized lures were introduced to males adapted to an arena. Two pairings with submissive male hamsters were given in which test and stimulus animals had access to their home cages and a neutral area. Bilateral radiofrequncy lesions were placed in the ST, amygdaloid-hippocampal area (AHA) or both the CMA and AHA (CMA-AHA). Electrodes were lowered into the brain for sham operations (Sham) but current was not applied. Two social tests were conducted between Postop satiation tests given on days 3 and 10. Relative to the last 2 Preop tests, Sham and ST lesions failed to affect ejaculation frequency (EF). Increases in EF following

Group	n	Mean Ejaculation Frequency				Aggres. Resp.	
		Preop 3 & 4	Postop 1 & 2	Preop High	Postop High	Preop	Postop
Sham	5	11.4	11.8	14.6	13.6	47	26
ST	5	12.9	13.1	15.8	15.0	41	0
AHA	3	12.2	17.0	14.0	20.7	45	26
CMA-AHA	4	14.1	17.1	15.0	19.8	44	7

amygdaloid (AMYG) damage (AHA plus CMA-AHA groups) were especially pronounced during one of the two Postop tests despite substantial declines in female receptivity with increased EF. Comparison of Preop and Postop tests having highest EF dramatically illustrates increased EF following AMYG damage. Despite changes in EF, there were decrements in achieving individual ejaculations. Ejaculatory latencies increased during the middle of tests after AHA lesions and during the middle and later components following CMA-AHA lesions. Intromissions with thrusting (tI) typically occur toward the end of tests, but the percentage of ejaculatory series having tI increased markedly after AMYG damage (Preop=11%, Postop=49%). All groups showed Postop reductions in mean aggressive responses but decreases were similar in Sham and AHA lesion groups. ST lesions abolished aggression whereas CMA-AHA lesions caused a substantial decline. In summary, AMYG damage either facilitated or attenuated mating depending on the behavioral measure. ST lesions did not affect copulation but abolished aggression. Affects of AMYG damage on aggression depended on lesion placement. Finally, the AMYG and ST may have different roles in controlling sexual behavior of rats and hamsters.

147.13 EFFECTS OF ELECTROLYTIC AND KAINIC ACID SEPTAL LESIONS ON HYPERREACTIVITY AND PENTOBARBITAL NARCOSIS. S.M.Simasko*, P.W.Kalivas* and A.Horita*(SPON:R.M.Quock). Dept. of Pharmacology and Dept. of Psychiatry and Behavioral Science, School of Medicine, University of Washington, Seattle,WA 98195.

Electrolytic septal lesions have been shown to cause a hyperreactivity syndrome (Albert and Richmond,Physiol.Behav. 15:339-347,1975) and a prolongation of pentobarbital narcosis (Harvey,et.al.,J.Pharmacol.Exp.Ther.144:24-36,1964).Electrolytic lesions destroy both cell bodies and passing fiber tracts thus preventing identification of the cells responsible for the resulting effects.Kainic acid has been shown to selectively destroy neurons receiving glutaminergic input while preserving the majority of passing fibers (Herndon and Coyle, Science 198:71-72,1977). To determine wether the effects of electrolytic septal lesions are caused by cell body or fiber tract destruction we have investigated the effects of kainic acid induced septal lesions. Male S.D. rats (280-330) were given kainic acid lesions (0.5µg/1µl/20min.) or radiofrequency electrolytic lesions (10 mamps/30sec) of the septum. Two control groups received intraseptal saline (1µl/20min) or bilateral intraventricular kainic acid (0.25µg/0.5µl/10min/hemisphere). All lesions were histologically verified after the experiment. All rats were tested 3 days prior to surgery for hyperreactivity (a standard test of poking,tapping,etc. with reactivity of response rated by an observer blind to the treatment of animal),and on days 3,7,and 10 post surgery. Animals with electrolytic and kainic acid septal lesions were significantly more reactive than both control groups on day 3 post-surgery. Hyperreactivity decreased to control levels by day 10. Following the last hyperreactivity test the rats were given I.P. injections of 45 mg/kg pentobarbital. Loss of righting reflex and narcosis duration were measured. Time to loss of righting reflex took greater than 5 minutes in 67% of animals receiving intraseptal kainic acid while less than 30% of the control and electrolytic lesioned animals required more than 5 minutes. Narcosis duration was greater than 150 minutes in 100% of electrolytic lesioned animals. In contrast, narcosis duration was less than 150 minutes in all other treatment groups. These results suggest that septal lesion hyperreactivity is mediated by neurons originating in the septum. Prolongation of pentobarbital narcosis by electrolytic lesions require destruction of non-kainic acid sensitive neurons or involves fiber tracts passing through the septum.

147.14 FUNCTIONAL CORRELATES OF THE AMYGDALA AND ASSOCIATED STRUCTURES IN THE RAT BRAIN: A ^{14}C-2-DEOXYGLUCOSE ANALYSIS. Robert E. Watson, Jr., Allan Siegel, Henry Edinger, Jennifer J. Poulakos,* Raymond Troiano* and Saul Weiner*. Depts. of Physiology and Neuroscience, N.J. Medical School, Newark, N.J. 07103.

In an attempt to further characterize the nature of the functional organization of the amygdala, patterns of uptake of ^{14}C-2-deoxyglucose (2DG) were assessed following electrical stimulation of various sites within the amygdala and associated structures in the rat. The experimental paradigm consisted of electrical brain stimulation delivered continously for periods of 30 sec on and 30 sec off for 45 minutes following injection of 2DG. Brains were removed and processed for autoradiography.

It was noted that a specificity existed regarding nuclei, which when stimulated, resulted in metabolic activation of the hypothalamus. Amygdaloid and related structures producing such activation included the medial, basomedial and cortical nuclei and the medial entorhinal cortex and bed nucleus of the stria terminalis. These nuclei differentially activated regions of the hypothalamus. Stimulation of the basomedial amygdaloid nucleus resulted in an activation pattern restricted more to the lateral hypothalamus while stimulation of the cortical and medial amygdaloid nuclei activated medial hypothalamus. Stimulation of the bed nucleus of the stria terminalis resulted in a more homogeneous distribution of label over both lateral and medial hypothalamic areas.

The overall patterns of subcortical activation following stimulation of these structures were similar. In these instances, label was noted in the stria terminalis, the bed nucleus of the stria terminalis, medial aspect of nucleus accumbens, lateral aspect of the lateral septal nucleus and substantia innominata. All amygdaloid nuclei were labeled over at least a portion of their rostro-caudal extent.

In contrast, stimulation of the lateral, basolateral, central and anterior amygdaloid groups as well as pyriform or lateral entorhinal cortices did not result in demonstrable metabolic activation of any area of hypothalamus. Stimulation of those amygdaloid nuclei which did not produce hypothalamic activation generally resulted in labeling of the substantia innominata and bed nucleus of the stria terminalis, with the exception of the anterior amygdaloid area which failed to label the bed nucleus.

Two important conclusions resulting from this work include: 1) electrical stimulation of only the medial, basomedial and cortical amygdaloid nuclei result in metabolic activation of the hypothalamus, and 2) a prevalent activation of nucleus accumbens and substantia innominata was noted following electrical stimulation of most amygdaloid nuclei. (Supported by NIH Grant NS 07941-11.)

147.15 ACUTE HYPERKINESIA AND GASTRIC ULCERATION INDUCED BY HYPOTHALAMIC LESIONS: EFFECTS OF DRUGS AFFECTING CATECHOLAMINE SYSTEMS. J.N. Nobrega and N.I. Wiener. Psychology Department, York University, Downsview, Ontario, M3J 1P3, Canada.

Hypothalamic lesions can induce a number of autonomic and behavioral effects in the first 24 hr post-surgery, including pronounced motor hyperactivity and stomach ulceration. Two series of experiments were conducted to investigate the possible involvement of norepinephrine and dopamine systems in the acute gastric and motor effects of hypothalamic lesions. Rats received anodal electrolytic lesions (1 mA/ 30 sec) in the medial hypothalamus followed 1 1/2 hr later by s.c. injections of drug or vehicle solutions. Total motility scores (stabilimeter counts) were recorded at 6 and 24 hr after the lesion. Stomachs were examined for ulcers 24 hr after the brain lesion.

In the first series of experiments, chlorpromazine (3, 10, 30 mg/kg) and haloperidol (1, 4.5, 9 mg/kg) significantly attenuated lesion-induced hyperactivity. Clozapine (3, 12, 18 mg/kg), phentolamine (3, 12, 36 mg/kg) and propranolol (3, 12, 36 mg/kg) had less pronounced effects on lesion-induced hyperactivity. Chlorpromazine and haloperidol significantly reduced lesion-induced ulceration. Phentolamine aggravated lesion-induced ulceration, while clozapine and propranolol had inconsistent effects across the doses tested. The combination of phentolamine + propranolol (12 mg/kg of each) induced a several-fold increase in the number and total length of lesion-induced ulceration.

In a second series of experiments, post-lesion injections of amphetamine (2, 6, 12 mg/kg) and apomorphine (1, 5, 15 mg/kg) induced qualitative changes in lesion-induced hyperactivity (from rearing and running to gnawing and biting) but did not result in higher stabilimeter scores at 6 or 24 hr post lesion. Isoproterenol (10, 15, 30 mg/kg) attenuated lesion-induced hyperactivity and pargyline (100 mg/kg) potentiated the hyperactivity after the first 6 hr. Amphetamine, apomorphine, isoproterenol and pargyline all caused significant reductions in the number and total length of lesion-induced gastric ulcers.

These results suggest that increased dopaminergic activity, perhaps due to increased DA turnover in terminals of lesioned pathways, may play an important role in lesion-induced hyperkinesia. On the other hand, decreases in central and/or peripheral noradrenergic activity may be a prominent factor in gastric ulceration induced by hypothalamic lesions.

147.16 AFFERENTS TO THE MESENCEPHALIC TEGMENTUM IN THE RAT. R. Waltzer*, P. Saxton, J. Siegel, T. Masino*, and N. Forrest*. Inst. for Neuroscience and Behavior, Univ. of Delaware, Newark, DE 19711.

The bulbar brainstem is a locus of both ascending and descending inhibitory influences. Previous experiments (Neurosci. Abst. 3, 213, 1977; Neurosci. Abst. 5, 204, 1979) in rat and cat injecting horseradish peroxidase (HRP) into the gigantocellular tegmental field (FTG) of the medulla have shown a consistent diffuse projection from the rostral mesencephalic reticular formation (MRF). In an effort to anatomically explore the influences converging upon the MRF injections of HRP were made in the mesencephalic tegmentum dorsal to the rostral tip of the red nucleus.

The use of tetramethylbenzidene (TMB) as a chromagen in these experiments made it possible to limit the amount of HRP injected to 0.02µl and therefore localize the injection to the target area. Despite the small injections labelled cells were seen from the rostral foretip of frontal cortex to the solitary nucleus of the caudal medulla, showing widespread afferent projections converging upon the MRF. By the HRP technique not only areas with robust projections but diffuse pathways from a few isolated cells were seen, revealing two patterns of afferent projections upon the MRF.

Cells projecting to the MRF were found bilaterally throughout the bulbar and pontine RF as well as contralaterally in the MRF. The ipsilateral neocortex displays two patterns of projections; one which is robust from the frontal cortex, and one which is diffuse from the cortex overlying the diencephalon and mesencephalon. In the thalamus, the centromedian nucleus was seen to project robustly to the MRF. Isolated labelled cells were also seen in structures known to be synchronogenic, i.e. the ipsilateral preoptic-basal forebrain area, dorsal raphe, and contralateral solitary nucleus. In addition, labelling was found in regions which serve to relay sensory information. These structures include the ipsilateral medial and lateral geniculates and the superior colliculus; the contralateral dorsal and ventral nuclei of the lateral lemniscus and the principal sensory and spinal trigeminal nuclei; and the bilateral parabrachial and vestibular nuclei.

The pathways found in this study from structures affecting the level of arousal or relaying sensory input provide further anatomical information about sources of influence upon the MRF. Moreover, these diffuse projections from synchrogenic structures suggest an anatomical pathway for inhibitory control of arousal.

147.17 AN ANALYSIS OF OPEN FIELD HYPERACTIVITY FOLLOWING ELECTROLYTIC MEDIAN RAPHE LESIONS IN THE RAT. D. Wirtshafter, K. E. Asin and E. W. Kent. Dept. Psychology, Univ. Ill. at Chicago Circle, Chicago, IL 60680.

A number of authors have reported that electrolytic lesions of the median raphe nucleus (MR) produce dramatic hyperactivity. In the current study, therefore, we examined in detail the behavior of MR lesioned rats in two open field situations.

In a standard open field, MR lesioned animals entered many more squares over a 5 min period than did sham operated controls. Measurment of the amount of time spent locomoting demonstrated, however, that this hyperactivity resulted entirely from MR lesioned animals spending a greater amount of time moving. Actual rate of motion (i.e. number of squares entered/time spent moving) was almost identical in MR lesioned and control rats. Whereas control animals frequently stopped to investigate parts of the field, MR lesioned animals rarely did so. Frequency of rearing was similar in the two groups, demonstrating that this behavior is controlled by different mechanisms than locomotion. Statistical analysis of the patterning of movement showed that MR lesioned animals moved about the field in a significantly more stereotyped fashion than did controls. This observation may be related to the stereotyped behaviors shown by MR lesioned rats in a variety of other situtations including tests of spontaneous alternation and 8-arm radial maze performance.

In our second experiment the open field was divided into two halves, in one of which a number of objects were placed. Both control, and to a significantly lesser extent, MR lesioned rats showed a preferance for the 'interesting' side of the field. Both control and MR lesioned animals spent more time per square in the 'interesting' than in the 'uninteresting' side, but this effect was significantly smaller in MR lesioned animals. Number of squares entered per unit time by MR lesioned animals in the 'interesting' side was about equvalent to that of controls in the 'uninteresting' side.These results demonstrate that the activity of MR lesioned animals is less sensitive to environmental complexity than is that of controls'and suggests the possibility that the hyperactivity of MR lesioned animals may result, at least in part, from their paying less attention to environmental stimuli than do controls.

Evidence for an attentional defecit was also obtained in a study of simultaneous discrimination learning. MR lesioned rats showed normal acquisition of a black/white discrimination, in agreement with our previous findings, but were severely impaired on the acquisition of a light grey/dark grey discrimination. It is possible that an attentional defecit may be able to account for many of the behavioral effects of damage to the MR and related limbic structures such as the hippocampus and septum.

147.18 ACTIVITY OF SINGLE NEURONS IN THE BASAL FOREBRAIN DURING SOCIALLY MOTIVATED APPROACH AND OTHER LOCOMOTION. J. W. Mink, D. B. Adams, and H. M. Sinnamon. Dept. of Psychology, Wesleyan Univ., Middletown, CT 06457

The purpose of this study was to search for a correlation between the socially mediated approach behavior of a male rat and the neural activity of the basal forebrain. The basal forebrain is considered here to include the medial and lateral preoptic areas, the bed nucleus of the stria terminalis, the lateral septum, the nuclei of the diagonal band, and nucleus accumbens.

Neural activity was recorded differentially through pairs of 25 micrometer stainless steel electrodes, implanted chronically. Stimulation electrodes were implanted ipsilateral to the recording electrode in the medial forebrain bundle (MFB) and lateral midbrain central gray (CG) to allow anatomical and electrophysiological identification of behaviorally tested neurons. Activity of neurons was measured during several standardized conditions: 1) during behavioral immobility; 2) while the water-deprived male rat ran in a running wheel to approach a water reward; 3) during running in the opposite direction so that the male subject approached an estrus female rat; 4) during exposure to olfactory, tactile, visual, and auditory stimulation; 5) during escape from the experimenter's grasp; 6) during single pulse stimulation to the MFB and CG.

So far, 40 neurons have been recorded in six animals. The recorded cells fall into 10 behaviorally defined categories. A total of 19 cells were found to be maximally active during drinking (N=5), approach to the female (N=2), orienting behavior (N=3), locomotor activity (N=4), escape from the experimenter (N=2), tactile stimulation (N=1), or when the rat was located in a specific place in the testing apparatus. Twenty-one cells were classified as uncorrelated fast (N=6), uncorrelated slow (N=8), or uncorrelated phasic (N=7). Responses to MFB or CG stimulation were found in six behaviorally tested cells and in five other cells that could not be sufficiently isolated for behavioral testing. Preliminary findings support the suggestion that the basal forebrain is involved in socially motivated approach.

147.19 ANODAL HYPERPOLARIZATION BLOCK TECHNIQUE PROVIDES EVIDENCE FOR ROSTRO-CAUDAL CONDUCTION OF REWARD RELATED SIGNALS IN THE MEDIAL FOREBRAIN BUNDLE. Peter Shizgal, Catherine Bielajew, and Ivan Kiss. Dept. Psychol., Concordia U., Montreal, Quebec.

Recent experiments (Shizgal et al., J.c.p.P., 1980, 94, 227.) suggest that longitudinally oriented, reward related fibers link the lateral hypothalamus (LH) and ventral tegmental area (VTA). This inference is based on a behaviorally derived measure of collision effects obtained with concurrent, two electrode stimulation. Although the collision technique provides measures of anatomical linkage and conduction velocity, it cannot determine the behaviorally relevant direction of conduction in the directly stimulated pathway. The present experiment was designed to obtain such information.

Subjects were male rats that showed evidence of collision during concurrent, unilateral self-stimulation of the LH and VTA. To test for the behaviorally relevant direction of conduction, we gathered four sets of strength-duration curves per rat using the following anode-cathode combinations: 1) skull screw anode, LH cathode; 2) VTA anode, LH cathode; 3) skull screw anode, VTA cathode; and 4) LH anode, VTA cathode. A constant behavioral output, self-stimulation paradigm was used throughout.

The results from the first two subjects suggest that reward related action potentials in directly stimulated MFB neurons propagated rostro-caudally. Strength-duration curves for the two VTA cathode conditions were parallel. In contrast, the curve for condition 2 converged on the curve for condition 1 as pulse duration was increased. This would be expected if the VTA anode in condition 2 produced a hyperpolarization block between the synaptic terminals and the point of current exist at the LH cathode. At short pulse duration, the block should decay before the action potentials triggered by the LH cathode arrive at the VTA. At longer pulse durations, current should continue to enter through the VTA electrode as the action potentials arrive and hence, some conduction failures should occur.

With the LH cathode, the direction of current flow appeared to have a large effect on substrate excitability. At the shortest pulse duration (100 μsec), the current threshold using the VTA anode was only about 60% of the threshold for the skull screw anode condition. Thus, the blocking effect at the longer pulse durations was manifested in a convergence of the initially lower strength-duration curve for condition 2 with the curve for condition 1.

The LH-VTA conduction time estimates from the collision test were consistent with the pulse durations at which the putative block reached maximum strength.

147.20 THE CONTRIBUTION OF NIGRAL EFFERENTS TO SUBSTANTIA NIGRA SELF-STIMULATION. R.M. Clavier, C.R. Gerfen, and D.H. Henkelman*. Dept. of Anatomy, U. of British Columbia, Vancouver, B.C. V6T 1W5

The substantia nigra (SN) has repeatedly been used as a locus to elicit intracranial self-stimulation (ICSS). However, the neural elements critical to SN ICSS have not been identified. The suggestion that SN ICSS results from stimulation of the A-9 dopaminergic (DA) cell group located in the substantia nigra pars compacta (Corbett and Wise, 1980) is not supported by findings that SN ICSS persists following generalized lesions of DA systems with 6-hydroxydopamine (6-OHDA) (Clavier and Fibiger, 1977, Cooper, et al., 1978). The latter two studies did not rule out DA involvement in SN ICSS since the facilitation of the behavior by d-amphetamine (1.0 mg/kg) was reversed after the lesions. In the present study 6-OHDA (5 ug in 2.5 ul 0.9% saline with 0.3 mg/ml ascorbic acid) was injected via a combined electrode-cannula into the SN ICSS site to limit the lesion to the A-9 DA cell group. 15 rats in the present study were shown to have had complete A-9 lesions as revealed by catecholamine (CA) histofluorescence. The A-10 DA cell group ipsilateral to the lesion was spared in all 15 animals. The nucleus accumbens and medial caudate nucleus showed normal CA fluorescence while the lateral two-thirds of the caudate was devoid of CA fluorescence. All 15 animals recovered to prelesion SN ICSS rates by 10 days after the lesion and most recovered to over 80% of prelesion rates within 3-4 days. Of the 10 rats that showed prelesion potentiation of SN ICSS with 1.0 and 0.5 mg/kg d-amphetamine, 5 animals showed the same degree of facilitation to the drug after A-9 destruction. However, the other 5 animals responded to 1.0 mg/kg d-amphetamine with a significant (85%) reduction of SN ICSS bar pressing. 0.5 mg/kg amphetamine administration did not significantly alter these animals' ICSS rates. A possible anatomical correlation is suggested in that the latter 5 electrode placements were caudal to the former 5 sites.

Since the above data suggest that SN ICSS persists in the absence of A-9 DA neurons the possible contributions of non-DA SN efferents to nigral ICSS was investigated. Electrolytic lesions of the following areas that receive non-DA SN efferents were ineffective in altering the behavior: the parafascicular thalamic nucleus (bilateral), the paralamellar mediodorsal thalamic nucleus (bilateral), the ventromedial thalamic nucleus, the superior colliculus, the central grey, and the pedunculopontine nucleus. Interestingly,lesions that involved the midbrain reticular formation led to a persistent (21 days) and significant (80%)reduction of SN ICSS (N=4). Contralateral lesions involving the same region had no effect on the behavior. We are currently investigating this area and its relationship to SN ICSS.

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147.21 EXCITABILITY CHARACTERISTICS OF THE SUBSTRATES FOR FRONTAL CORTEX AND LATERAL HYPOTHALAMIC SELF-STIMULATION. S. Schenk*, P. Shizgal, and C. Bielajew*. (SPON: J. Stewart). Concordia University, Dept. of Psychology, Montreal, Quebec, Canada.

The present experiment was designed to compare the excitability characteristics of the substrate(s) supporting frontal cortex (FC) and lateral hypothalamic (LH) self-stimulation. This is part of a long-term mapping project aimed at characterizing the pathways underlying the rewarding effects of electrical brain stimulation. These pathways may then be identified using electrophysiological recording techniques.

Male Charles River rats, weighing 300-400 grams were stereotaxically implanted with two monopolar stimulating electrodes aimed at the LH and FC. Following recovery from surgery, rats were trained to self-stimulate at both electrode placements; depression of a lever produced a 500 msec. train of cathodal, rectangular pulses, .1 msec. in duration. Stabilization consisted of delivering trains of pulses and varying the number of pulses per train in a descending series. The number threshold (NT) was defined as the number of pulses that supported a one-half maximal rate of responding.

The refractory period test consisted of a series of NT determinations. In the first and last determinations, trains of equally spaced single pulses were used; trains of pulse pairs were delivered in the remaining NT determinations. Both the first pulse (the C-pulse) and the second pulse (the T-pulse) of each pair were delivered to the same electrode. The interval between the pulses (the C-T interval) was varied from 0.15 to 15.0 msec. A T-pulse effectiveness ratio was computed for each C-T interval. This was determined by comparing the paired pulse NT with that for single pulse stimulation.

At the short C-T intervals (<.6 msec) the value of the T-pulse effectiveness was at a minimum at both electrode placements. However, at the LH placement, there was a gradual increase in effectiveness between 0.6 msec and 1.2 msec., when a leveling off in effectiveness was observed. In contrast, the T-pulse effectiveness at the FC placement was minimal for as long as 1.6 msec.; asymptotic levels were not reached until a C-T interval of 5.0 msec.

These data suggest that either (1) different neurons with different excitability cycles are being stimulated by FC and LH stimulation; or (2) different parts of the same fibers are being stimulated by FC and LH stimulation. We are currently applying the collision technique (Shizgal et al., JcpP, 1980, in press) in an attempt to distinguish between these two possibilities.

147.22 RESPONDING IS AN IMPORTANT FACTOR IN BRAIN-STIMULATION REWARD. A.Ettenberg, A. Laferrière*,P.M. Milner* and N. White. Dept. of Psychology, McGill University, Montreal,Qué.,Canada,H3A 1B1.

A major tenet of reinforcement theory is that when two responses lead to the same reinforcement, an animal will choose the least effortful response. There is, however, a growing literature the results of which are particularly difficult to reconcile with the concept of "least-effort". In a variety of situations, animals prefer food or water reinforcers when they are self-administered (earned) as opposed to when they are provided ad lib (free). A problem exists however, concerning the notion of response-independent or noncontingent reinforcement. With natural reinforcers, for example, animals must perform a consummatory response and therefore such reinforcement cannot truly be considered noncontingent. The use of rewarding brain-stimulation (BSR), on the other hand, can overcome this difficulty since it can be applied without the subjects having to perform a consummatory, or any other contingent response. While a few experiments have been done which touch on this question of free vs. earned reinforcement, they are methodologically weak. A properly conducted study would provide important insights into the theoretical notions of reinforcement as well as its neural substrates. The present experiments, therefore, investigated this phenomenon using two novel experimental paradigms.

In one set of experiments, male albino rats were trained to lever-press for 0.5 sec trains of 60Hz sine wave lateral hypothalamic stimulation. Stimulation consisted of 90 sec periods after which the BSR-lever was retracted and experimenter-administered or imposed stimulation (EAS) was presented at the same rate and current parameters as during the self-stimulation. The rats could demonstrate a preference for self-administered BSR by pressing a reset lever located on the opposite wall of the test chamber. This action terminated the EAS and reinstated the BSR-lever for an additional 90 sec. The results show that rats prefer to respond for BSR than to have the same stimulation imposed by the experimenter. This was true even when a signal preceded each train of EAS or when subjects had a great deal of previous EAS experience. In another experiment, conditioned taste preferences were observed following novel-taste/self-stimulation pairings but not following various novel-taste/EAS pairings. Together these data suggest a response mechanism involvement in the rewarding nature of intracranial stimulation. The importance of this finding for distinguishing between drug-induced reward and performance deficits in self-stimulation behaviors is discussed.

147.23 PROTOCOL TIMING AND DATA ACQUISITION BY MICROCOMPUTER. Rodney B. Murray* and Ronald J. Tallarida* (SPON: E.B. Geller). Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia, PA 19140.

This paper describes a technique that may improve the accuracy of data collection in behavioral and pharmacologic testing. This technique could be used when a precise injection and observation schedule must be geared to the kinetics of one or more drugs, such as in the time-dependent method for studying drug-receptor affinity (Tallarida, et al., JPET 206:38, 1978). For example, it would not be unusual to design a pharmacologic experiment that employs 4 groups of 5 animals each. If each animal was to be tested for two different responses during a control period and 5 times after drug administration, 240 separate tasks would have to be performed. These programs were designed to minimize the required time and the confusion during such an experiment.

The authors' programs were written in BASIC with the exception of a short machine language subroutine used to generate tones to prompt the user. The programs were run on an inexpensive microcomputer (TRS-80 Level II with 16K RAM). The entire microcomputer with video monitor, tape recorder, and small amplifier/speaker unit were mounted on a portable typewriter table. The operator is instructed with the use of prompts on the video display to enter the parameters of the particular experimental protocol. Prompting messages on the video display and audible tones alert the operator to perform the proper task at the correct time. The tones generated by the computer vary in pitch and in number according to the selected protocol. In addition, the operator may enter data directly into the computer for processing and/or storage on magnetic tape.

This technique is currently being used in our in vivo study of narcotic agonists and antagonists. Program listings are available from the authors.

147.24 SEIZURES EVOKED BY ELECTRICAL STIMULATION OF SUBCORTICAL STRUCTURES IN COLLARED LIZARDS. R. A. Sugerman* (SPON: R. I. Peters). Dept. Biol. Sci., Wichita State Univ., Wichita, KS 67208.

In mammals chemical and electrical stimulation are often used to evoke seizures. Chemicals like strychnine and penicillin have been found to readily evoke seizures in turtles, snakes, and lizards. The use of electrical stimulation of the brain in reptiles for evoking seizures is a technique which has not often been utilized. Volanschi and Servit (Exp. Neurol., 24:137-146, 1969) in a brief statement reported that diffuse electrical stimulation evoked seizures. The following study was conducted to determine the current threshold levels which are required to evoke seizures from the lizard's brain and eye and compare the optimal stimulation parameters for lizards and mammals.

The animals were anesthetized using either hypothermia or Nembutal (20 mg/Kg) and monopolar insect pin electrodes were implanted into the fore- and midbrain in arrays of 1 to 7 electrodes or as a single electrode in a movable apparatus. Constant current stimulation was provided using biphasic square wave pulse pairs of a 2 msec duration, a frequency of 5 or 50 Hz, and current between 0.1 and 1600 μA for the brain; and up to 10 mA in the vitreous body. Stimulation sites were marked using the Prussian blue technique. The brains were fixed in formalin, embedded in paraffin, and serially sectioned in the frontal plane.

Electrical stimulation of the brain in collared lizards evoked seizures from subcortical structures of the fore- and midbrain at current thresholds between 15 to 1600 μA. Areas in or near the ventral diencephalon had the lowest thresholds while much higher thresholds were localized in the anterior forebrain and midbrain. Thresholds for evoking seizures from sites in or near the amygdaloid complex are higher than sites in the hypothalamus. In mammals, the amygdala has a lower threshold for evoked seizures than the hypothalamus. The stimulation parameters for evoking seizures in lizards and mammals are essentially the same for the CNS. At considerably higher thresholds seizures could be evoked from direct stimulation of the eye.

148.1 PLASTICITY OF THE TECTOFUGAL PATHWAY DURING VISUAL CONDITIONING. J. Wall, J.M. Wild, J. Broyles*, C.M. Gibbs* and D.H. Cohen. Dept. of Neurobiology & Behavior, SUNY, Stony Brook, N.Y. 11794.

Visually conditioned heart rate change in the pigeon has been developed as a vertebrate model system for cellular studies of associative learning. In delineating the relevant neural pathways we showed that the ascending visual pathways transmit the conditioned stimulus information. Described here is an initial evaluation of neuronal activity along these pathways during conditioning, including (a) the retinal ganglion cells and (b) the thalamic and telencephalic neurons of one involved pathway, the tectofugal system.

Upon isolating the activity of a single neuron in a behaviorally naive animal, 10 6-sec pulses of whole field illumination were given. Presented next were 40 such lights followed by .5-sec foot-shocks (conditioning), 40 unpaired lights and shocks (sensitization) or 40 additional lights alone (habituation). The small unconditioned cardioacceleration to the light was enhanced by conditioning and attenuated by non-associative procedures.

Retinal output was assessed by recording from single optic tract fibers. We previously showed that with whole field illumination almost all such cells discharge at light onset, cease discharging during sustained illumination, and discharge at light termination. A large number of response parameters were statistically evaluated over training and none changed; maintained activity was also unchanged. We thus conclude that retinal output remains invariant during associative and non-associative training.

To analyze the tectofugal pathway we began at its telencephalic target, ectostriatum, since negative results would suggest the pathways's sub-telencephalic relays are also unaffected. Most ectostriatal cells respond to whole field illumination, distributing into various response classes. At least two such classes show enhancement of light-evoked responses during conditioning and attenuation or no change during non-associative treatment.

Given this, we undertook analysis of the thalamic relay, nucleus rotundus, to pursue the most peripheral site of change. All rotundal cells responded to whole field illumination and showed significant enhancement of their light-evoked responses over conditioning; during non-associative procedures responses either remained unchanged or attenuated. Maintained discharge was unchanged in both rotundus and ectostriatum.

The tectofugal pathway thus undergoes training-induced modification. Germane in this regard is that the tectofugal neurons of this study also generally responded to the foot-shock, unlike the retinal ganglion cells. Study of the optic tectum must now be initiated to identify the most peripheral sites of such change along the tectofugal pathway. (Supported by NSF grant BNS7919166 and NIH grant PO1 NS14620.)

148.2 PLASTICITY OF THE THALAMOFUGAL PATHWAY DURING VISUAL CONDITIONING C.M. Gibbs* and D.H. Cohen, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, N.Y, 11794.

Visually conditioned heart rate change in the pigeon has been developed as a vertebrate model system for cellular studies of associative learning, and as part of this development we showed that the ascending visual pathways transmit the conditioned stimulus information. Another report at this meeting (Wall et al.) describes our initial analysis of these pathways during conditioning, and it was found that the retinal ganglion cell discharge does not change but that neuronal discharge in both the thalamic and telencephalic components of the tectofugal system is modified.

To evaluate the generality of these findings we initiated study of another of the involved visual pathways, the thalamofugal system. We began with the principal optic n. (OPT), the avian homologue of the lateral geniculate, since this provided an opportunity to examine a retinorecipient cell group. OPT consists of various subnuclei; in these preliminary experiments we sampled cells primarily in the n. dorsolateralis anterior, pars lateralis.

Upon isolating the activity of a single neuron in a behaviorally naive animal, 10 6-sec pulses of whole field illumination were given. Presented next were 40 such lights followed by .5-sec foot-shocks (conditioning) or 40 unpaired lights and shocks (sensitization). The small unconditioned cardioacceleration to the light was enhanced by conditioning and attenuated with unpaired stimuli.

Approximately 90% of the OPT neurons responded at short latency to whole field illumination with a range of 26-55 msec. (The latency range of retinal ganglion cell responses is 19-54 msec.) Several response classes were formulated on the basis of the short latency responses, including units with increased discharge, decreased discharge, or biphasic responses. Discharge patterns after the initial response were complex and are not yet fully analyzed.

With respect to the short latency responses, these changed over conditioning in > 80% of the units, generally augmenting. In contrast, non-associative training resulted in either no change or, more commonly, response attentuation. Maintained activity of OPT neurons did not change, and approximately 90% responded to the foot-shock, generally at latencies > 150 msec.

These findings extend the tectofugal results and indicate that at least two of the pathways implicated in transmitting the conditioned stimulus information show associative change. They further suggest that such change may occur at retinothalamic synapses. (Supported by NSF grant BNS7919166 and NIH grant PO1 NS14620.)

148.3 NEURONAL ACTIVITY IN THE AUDITORY SYSTEM DURING DIFFERENTIAL CONDITIONING IN RABBITS. K. Foster*, E. Orona*, R. Lambert* and M. Gabriel. (SPON: W. Riffee). Dept. Psychol., Univ. Texas, Austin, TX 78712.

Past studies (e.g., Gabriel, Miller, and Saltwick, 1977) have demonstrated that neurons in cingulate cortex of rabbits develop discriminative activity to the auditory stimuli used for differential behavioral conditioning. The discriminative activity developed at an early stage of conditioning, prior to the development of discriminative behavior. However, neurons in the reciprocally connected anteroventral nucleus of the thalamus developed discriminative activity in a late stage of training, after the advent of significant behavioral discrimination. The present study represents a preliminary report of research directed towards establishing whether early and late developing discriminative activity occurs in the cortical and thalamic components of the auditory projection system.

The results indicated that auditory system neurons manifested both early and late developing discrimination. However, the early developing effects occurred primarily within structures of the secondary projection pathway (dorsal and medial divisions of the medial geniculate nucleus of the thalamus, and the secondary auditory cortex), whereas late developing discrimination occurred within the classical projection pathway (ventral division of the medial geniculate nucleus and the primary auditory cortex; chi square, df = 1, = 6.07, $P < .05$). Unlike the effects seen in the cingulate cortical-anteroventral thalamic system, no differences in the development of neuronal discrimination were observed between the cortical and thalamic components of the auditory pathways.

Reversal of the CS-UCS contingencies, so that the original CS+ became the CS- and the original CS- became the CS+, led to an immediate reversal (during the first session of reversal training) of discriminative neuronal activity in both primary and secondary auditory structures (chi square, df = 1, = 5.26, $P < .05$). (In contrast, cingulate cortical and anteroventral thalamic neurons manifested activity appropriate to the original task during the first three sessions of reversal training.) These data suggest distinctive functional contributions of the auditory and the cingulate cortical-anteroventral thalamic systems to behavioral conditioning. (Supported by NIMH Grants 26276 and 31351 to M.G.)

148.4 MODIFICATION OF AUDITORY CORTEX SINGLE UNIT ACTIVITY DURING PUPILLARY CONDITIONING. W. Hopkins* and N. M. Weinberger. Dept. Psychobiology, Univ. of Calif., Irvine, CA 92717.

Previously, differential plasticity has been found in morphologically distinct subdivisions of the medial geniculate body during pupillary conditioning; magnocellular neurons (MGm) develop discharge plasticity in contrast to ventralis cells (MGv) which are not plastic (Ryugo & Weinberger, Beh. Biol., 1978, 22, 275). The present study investigated primary auditory cortex which receives convergent input from MGm and MGv. Single unit activity was recorded during acquisition of the classically-conditioned pupillary dilation response in chronically-prepared cats under neuromuscular paralysis and artificial respiration (Oleson et al., Beh. Biol., 1972, 7, 829). Subjects were trained with tone or white noise signalled pawshock. A sensitization phase, consisting of non-paired presentation of these stimuli, preceded pairing and controlled for nonassociative factors. Data were obtained from one neuron in a single training session during which the pupillary conditioned response was generally established. Subjects were routinely involved in several training sessions at weekly intervals.

When compared with the sensitization phase, the evoked activity of 17 of 32 neurons (53%) was significantly altered during conditioning. Background rate was changed significantly in 11 neurons (32%). Furthermore, background, but not evoked, activity developed a change in sign (usually decrease to increase) from sensitization to conditioning. Evoked and background activity changes often varied independently for a given neuron, as previously reported (Kitzes et al., Exp. Neurol., 1978, 62, 678; Perkins & Disterhoft, Neurosci. Abst., 1979, 5, 218).

Recent studies of single unit activity in MGm and MGv during pupillary conditioning have revealed no discharge plasticity in MGv but plasticity in virtually 100% of MGm neurons (Weinberger, DuPont Symp., June 1979, Wilmington). Furthermore, MGm neurons show an increase in either evoked or background activity but not both. The present findings indicate that many auditory cortical neurons are altered during the conditioning procedure. Further, they do not merely reflect the dynamics of either MGm or MGv cells during conditioning, but rather exhibit effects which could be due to the combined influence of both of these thalamic sources of input.

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148.5 ASSOCIATIVE CHANGE IN NEURONS OF INTERMEDIATE AND DEEP LAYERS OF SUPERIOR COLLICULUS OF BEHAVING RAT DURING DIFFERENTIAL APPETITIVE CONDITIONING. D. Birt and M. E. Olds. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

We have previously shown that neurons in a region near the caudal tip of rat medial geniculate show associative short latency response changes during a differential appetitive conditioning and reversal paradigm in which different frequency tones are used as the conditioned stimuli. The horseradish peroxidase technique has been used to identify afferents to this region so that other portions of the neural circuitry involved in these associative changes may be identified and studied. This technique shows that one source of afferent input to this region is the intermediate and deep layers of the ipsilateral superior colliculus (SC). In the present experiment multiple unit auditory evoked responses were recorded from electrodes chronically implanted in deep and intermediate layers of SC of behaving rats during a similar conditioning paradigm.

Most units responded phasically to a wide range of auditory frequencies and were also responsive to somatosensory stimuli, particularly those applied to the contralateral face and anterior portion of the body. The majority of units showed a selective increase in response to the tone frequency paired with pellet presentation during conditioning. These increases started as early as the period 14-28 ms after stimulus onset and persisted throughout the interval between tone onset and pellet presentation. The early latency unit response changes were only very loosely correlated in magnitude and latency with learned movements of the head and body toward the pellet dispenser, while longer latency unit changes were highly correlated with movements of the head and body. These results support a role of the intermediate and deep layers of SC in the neural circuitry which evaluates and responds to the behavioral significance of sensory stimuli.

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148.6 NEURONAL RESPONSES RELATED TO VISUAL RECOGNITION. E.T. Rolls, W. Caan,* D. Perrett,* and F.A.W. Wilson.* Dept. of Exp. Psychology, Oxford Univ., Oxford, England.

To analyse the neural basis of long-term memory of visual stimuli, recordings were made from single neurons in monkeys performing a visual recognition task of the type impaired in anterograde amnesia in man. Each visual stimulus was shown usually only twice per day, once as novel, and after 0-17 other intervening items in the recognition task, on a second trial as familiar, when the monkey could lick to obtain fruit juice if he recognized the stimulus correctly. Close to the fornix in the region where it descends through the far anterior thalamus into the septal region, a population of neurons was found which responded to the visual stimuli only when they were familiar. The neurons responded with latencies of 130-190 ms to the familiar stimuli compared with latencies of 400-500 ms for the lick responses made by the monkey. These neurons could fail to respond if the monkey licked incorrectly to a novel stimulus, and were not phasically activated during ad lib licking, so that their responses were not due to the lick responses made by the monkey in the recognition task. Further, in a different, visual discrimination, task a number of these neurons were found to respond both to the familiar rewarded visual stimulus to which the monkey always licked, and to the familiar aversive visual stimulus to which he did not lick. This not only shows that the responses of these neurons are independent of the monkey's lick responses, but also shows that in an association memory task these neurons respond on the basis of recognition memory, providing firm evidence for a dissociation of these types of memory, and their neural encoding. In further experiments it was shown 1) that the responses of individual recognition-related neurons became smaller as the number of intervening stimuli and the retention interval increased, 2) that these neurons differed in the number of intervening stimuli over which recognition-related responses could occur, 3) that if a stimulus was shown twice, this could enhance the magnitude of the recognition-related response on a later trial, and enable these neurons to respond to a stimulus over even 140 intervening stimuli, and 4) that these neurons responded selectively to familiar visual stimuli even when the monkey was not performing a task.

148.7 CORRELATES OF HIPPOCAMPAL UNITS IN RATS PERFORMING A WORKING MEMORY TASK. S.J. Mitchell and D.S. Olton. Dept. of Psychology, The Johns Hopkins Univ., Baltimore, MD 21218

Rats were trained to solve a spatial working memory task on an elevated 3-arm radial maze, the arms of which could be raised like drawbridges. At the beginning of each trial, one of the arms was lowered; the rat ran to the end of it and drank some milk there. When the rat returned to the center of the maze, he was confined there while milk was placed at the end of some other arm. The arm with milk, and the arm previously visited were then lowered. The correct response for the rat was to go to the arm with milk and avoid the arm already chosen. Because the arm lowered at the beginning of each trial changed from trial to trial, the only way the rat could determine which arm ought to be chosen when two arms were presented was to remember which arm had been chosen earlier in that trial. Rats with bilateral fimbria-fornix lesions are unable to solve this task.

Following training, each rat had a pair of blunt cut insulated wires implanted in one hippocampus (HPC) and a device for holding a microelectrode assembly implanted above a hole in the skull over the other HPC. Screws in the frontal bone and the interparietal bone served as the indifferent electrode and the ground, respectively, for the rat. A microelectrode assembly was screwed into the holding device prior to a recording session. With the rat in the center of the maze, the microelectrode was lowered to HPC. The signal was led through field effect transistors attached firmly to the rat's implant and then through standard amplifiers to the audio channel of a videorecorder. When a cell was isolated, the rat's behavior during the spatial memory task and during a free period was filmed on the video channel of the videorecorder and the activity of the cell recorded simultaneously on the audio channel. After the cell's activity was recorded, slow waves were recorded from the microelectrode and compared to slow waves from the other HPC electrodes to determine the placement of the microelectrode.

The mean rate of activity of each unit was determined for each location on the maze and each period of the memory task. Units had correlates to particular locations, and to locations combined with behaviors thought to be associated with the memory components of the task.

148.8 HIPPOCAMPAL UNIT ACTIVITY RELATED TO SENSORY, MOTOR AND EEG EVENTS DURING ODOR DISCRIMINATION LEARNING. J. Phillips*, H. Eichenbaum, & M. Kuperstein*, Dept. Biol., Wellesley Coll., Wellesley, MA 02181 & Dept. Psychol. & Brain Sci., M.I.T., Cambridge, MA 02139.

Recent analyses of the behavioral correlates of hippocampal neuronal activity emphasize either unit responses to sensory and motor events during learning (Olds et al., J. Neurophysiol. 35: 202, 1972; Thompson et al., Amer. Psychol. 31:209, 1976), or unit activity patterns related to unlearned behaviors and EEG (Ranck, Exper. Neurol. 4:461, 1973). In an initial attempt to relate these findings, an odor discrimination task previously used to describe synchronization of sniffing cycle and hippocampal rhythmic slow activity (RSA; Neurosci. Abstr. 5:900, 1979) was applied to an analysis of hippocampal unit activity.

Sniffing, RSA and dentate single unit activity were recorded in rats performing 400 trials on a go, no-go, water-rewarded discrimination of 4 S+ and 4 S- odors presented individually. In post hoc analyses, unit activity was correlated with trial periods: 1) approach to the stimulus port, associated with high amplitude RSA, 2) stimulus sampling, associated with synchronized sniffing and low-amplitude RSA, and 3) reward consumption associated with the absence of RSA.

Nearly every unit fired maximally at a preferred phase of RSA, and altered its firing rate reliably during trials. Most units increased firing abruptly at the onset of stimulus sampling, had greater responses to S+ than S- stimuli, and some responded differentially to odor quality. Other units fired maximally during the reward period; some during reward consumption only; others after non-reward only. Other units fired in bursts in phase with RSA and were most active during approach and after non-reward, and were minimally active during drinking; at the onset of stimulus sampling, a long burst was succeeded by groups of 1-3 action potentials in phase with RSA and sniffing cycle.

These results are consistent with observations of Olds and Thompson who also found that units in the hippocampus are differentially responsive to stimuli of learned significance. In addition, the behavioral correlates of many units observed in this situation are similar to those described in freely behaving rats by Ranck. This training paradigm combines useful aspects of different strategies in the study of neuronal-behavioral correlates.

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148.9 RATE-DEPENDENT SHIFTS IN HIPPOCAMPAL SLOW WAVES DURING CLASSICAL CONDITIONING. S.D. Berry and R.F. Thompson, Dept. of Psychology, Miami Univ., Oxford, OH 45056 and Dept. of Psychobiology, Univ. of California, Irvine, CA 92717

Learning-dependent changes in hippocampal slow wave activity were assessed in 16 New Zealand White rabbits during classical conditioning of the nictitating membrane (NM) response. The conditioning paradigm consisted of a tone conditioned stimulus (CS, 350 msec, 85 dB, 1 Khz) and corneal air puff unconditioned stimulus (US, 210g/cm^2, 100 msec duration) The US was given 250 msec after CS onset. Subjects were given 13 blocks of training trials per day, each block consisting of 8 paired (CS-US) trials and 1 test (CS alone) trial. The intertrial interval averaged 60 sec. Training for each subject continued until it had reached a criterion of 8 conditioned responses (CRs) in 9 consecutive trials, or for a maximum of 4 sessions. A CR was defined as NM extension of more than 0.5 mm prior to US onset or (on test trials) within 500 msec of CS onset.

Hippocampal slow waves were recorded on magnetic tape from a multiple unit electrode located in the CA1 pyramidal cell layer of the dorsal hippocampus. Two-minute samples were recorded prior to the start of training and at the end of the testing session in which criterion was reached. Slow waves were analyzed on a PDP-12 computer that calculated frequency histograms based on the period between successive positive-going zero crossings. The values in the histogram were transformed into a frequency index, consisting of the ratio of high (8-22 Hz) to low (2-8 Hz) frequency waves. This index, when computed from a baseline slow wave sample, was previously reported to predict rabbit NM conditioning rate (r=.72; Berry, S.D. and Thompson, R.F., Science, 200:1298, 1978).

In this paper, we report that, although the post-criterion frequency index was not significantly related to the rate of conditioning (r= -.013), the shift in slow wave index from pre-training to post-criterion was highly correlated with the number of trials taken to reach criterion (r= -.86, $p < .01$). Faster-learning subjects displayed more high frequency activity after training than before, reflected by an increase in frequency index. Slower-learning animals shifted in the opposite direction, displaying relatively more low frequency activity after training. An additional 4 subjects, given a control treatment (explicitly unpaired tones and air puffs), showed no consistent shift across 2 days of testing.

These data indicate that hippocampal slow waves in the rabbit display frequency changes across training that are more related to the rate of NM conditioning than to training per se.

148.10 EFFECTS OF SEPTAL LESIONS ON SENSORY EVOKED POTENTIALS IN THE DENTATE GYRUS OF THE CHRONIC RAT. M.O. West, J.H. Robinson* and S.A. Deadwyler*. Dept. Physiol. & Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103

Averaged evoked potentials (AEPs) were recorded from the perforant path synaptic zone of the dentate gyrus of rats conditioned to respond in the presence of a tone stimulus on an operant stimulus discrimination paradigm. As previously reported (West et al. Fed. Proc. 39(3): 1753, 1980), the N_1 negative component (20-30 ms latency, 20-30 ms duration) of the AEP was mediated by the perforant path projection from entorhinal cortex; N_1 varied inversely with the conditioned status of the animal and therefore appeared to be related to stimulus novelty. The N_2 negative component (70-80 ms latency, 50-80 ms duration) was recorded in the synaptic zones of perforant path and commissural/associational projections to the dentate gyrus, exhibiting maximum amplitude in the inner molecular layer; N_2 may therefore have resulted from the activation of intrahippocampal circuits. N_2, in contrast to N_1, was never observed in unconditioned normal animals and varied directly with the conditioned status of the animal; N_2 reached maximum amplitude during well-established stimulus discrimination performance but was attenuated or absent during extinction of the conditioned behavior. The N_1 component of AEPs recorded from animals sustaining lesions of the medial septal nucleus did not differ from N_1 in normal animals during any stage of conditioning. However, in contrast to normal animals, a prominent N_2 component was consistently observed in unconditioned septal animals; AEPs obtained from unconditioned septal animals thus resembled AEPs from conditioned normal animals (i.e., both N_1 and N_2 components were present). A further difference between AEPs from normal vs. septal animals was observed during extinction of the conditioned behavior: whereas the N_2 component of the AEP was attenuated or nonexistent in normal animals during extinction, a prominent N_2 component was recorded from septal animals during behavioral extinction (no deficits were observed in the behavior of septal animals in either acquisition or extinction of the conditioned behavior). These results suggest that activation of septal input to the hippocampus by a sensory stimulus serves to suppress activity in intrahippocampal circuits until the stimulus acquires behavioral significance.

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148.11 HABITUATION OF HUMAN LIMBIC SENSORY RESPONSES. Charles L. Wilson, Michael Wang*, Thomas L. Babb, Eric Halgren and Paul H. Crandall. Brain Res. Inst., Reed Neurological Res. Cntr., and Dept. of Surg. (Neurol.), Sch. Med., UCLA, Los Angeles, CA 90024.

Investigators concerned with sensory processing in the animal limbic system have found that a high proportion of units in the amygdala and hippocampal formation: 1) are responsive to simple sensory stimuli, 2) are polysensory, and 3) display rapid habituation with repeated presentation of stimuli (Brown and Buchwald, Exper. Neurol., 40: 608-631, 1970; Lidsky et al., Exper. Neurol., 44: 130-134, 1974; Vinogradova, In: Isaacson and Pribram (Eds.), The Hippocampus, Vol. II, pp. 3-70, 1975). Limbic units recorded in patients with chronically implanted 40 μM platinum fine wires for localization of temporal lobe seizure foci, were tested with simple auditory and visual stimuli. Out of over 500 single and multiple units recorded from electrode placements verified by X-ray localization as being within amygdala, hippocampus, or entorhinal cortex, the 11% responding to diffuse flash or binaural click stimuli presented at a rate of 0.5 Hz were analyzed to determine whether they showed response decrements indicative of habituation. Habituation of neural responsiveness was assessed by measuring changes in amplitude of excitatory or inhibitory components of PSTH's evoked by initial vs. later portions of a train of sensory stimuli. Of the 60 units responsive to sensory stimulation, 95% responded to photic stimuli, 12% to auditory stimuli, and 7% were bimodally responsive. Out of 158 amygdala units, just three were responsive and only one showed habituation. Of 154 hippocampal units tested, 20 were responsive, 40% of these showing response decrement during stimulus presentation, while 44 out of 210 entorhinal cortex units were responsive with 35% of these showing habituation. Thus human limbic units differ from those of lower mammals in that a smaller proportion are influenced by sensory stimuli, but a substantial number of those which do respond show habituation. With response latencies ranging between 27 and 225 msec, habituation occurred in only 2 of 11 units having latencies equal or less 30 msec, while over 50% of 29 units with latency between 31 and 100 msec showed habituation. Further aspects of response decrements to sensory response will be related to the functional significance of phylogenetic changes in hippocampal formation and amygdala.

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148.12 CINGULATE CORTICAL AND ANTERIOR THALAMIC CORRELATES OF REVERSAL LEARNING IN RABBITS. Michael Gabriel, Edward Orona*, and Kent Foster*. Dept. Psychol., Univ. Texas, Austin, TX 78712.

Multiple-unit activity of the cingulate cortex and the anteroventral (AV) nucleus of the thalamus in rabbits was examined during reversal training following differential conditioning of a locomotory (wheel rotation) avoidance response. The positive and negative conditional stimuli (CS+ and CS-) were pure tones (1 kHz and 8 kHz) and the unconditional stimulus (UCS) was a footshock (1.5 ma.) delivered through the grid floor of the wheel.

During the first session of reversal training, neuronal activity appropriate to the original task (i.e., a greater neuronal response to the CS- than to the CS+) occurred in the deep laminae (V & VI) of cingulate cortex. Activity in the superficial laminae (I-IV) manifested the original discrimination in certain records, and the reverse discrimination in others. These effects diminished and ceased during subsequent sessions. However, the short-latency (25-msec.) deep laminar discrimination appropriate to original learning, persisted throughout training to the reversal criterion. Activity of the AV nucleus underwent a gradual transition, in parallel with behavioral reversal learning, from activity appropriate to the original task to that appropriate to the reversal task. Extra footshocks presented following the attainment of behavioral criterion educed the neuronal discrimination appropriate to the original task in the deep laminae, and they educed discrimination appropriate to the reversal task in the superficial laminae.

These results considered together with the data of original learning lead us to propose that the encoding of the associative significance of the CSs during original learning occurs in the deep laminae of cingulate cortex, but continuing operation of the code during performance of the well-learned behavior, and any recoding that may be called for such as reversal of the code, are processes which occur at the level of the AV nucleus. While recoding occurs in the AV nucleus the deep laminae manifest persistent retention of the activity appropriate to the original task. The persistent original code may interfere with the attainment of behavioral reversal. Finally the present results and other findings (Gabriel, M., Miller, J. D., Saltwick, S. E. Physiol. Psychol., 4: 124, 1967) suggested that the superficial laminae are sites of convergent input from the AV nucleus and from the primary auditory system. However, the influences from these regions are manifested in superficial laminae only under conditions fostering high arousal. (Supported by NIMH Grants 26276 and 31351 to M.G.)

148.13 ACQUISITION OF NEURONAL ACTIVITY OF THE PREFRONTAL CORTEX AND THE MEDIODORSAL THALAMUS DURING DISCRIMINATIVE CONDITIONING. Edward Orona*, Kent Foster*, Richard W. Lambert*, and Michael Gabriel. (SPON: D. Albrecht). Dept. Psych., Univ. Texas, Austin, TX 78712.

Multiple-unit activity was recorded in the prefrontal cortex (PFC) and the reciprocally interconnected mediodorsal thalamic nucleus (MD) during discriminative conditioning of a locomotory (wheel rotation) avoidance response. The conditional stimuli were pure tones (1 kHz and 8 kHz) and the unconditional stimulus (UCS) was a footshock (1.5 ma.) delivered through the grid floor of the wheel. Rabbits received daily sessions of differential conditioning, in which the CS+ (followed in 5 sec by UCS onset) and the CS- (never paired with shock) were presented in a random sequence. Assessments of neuronal discriminative activity were made for specific behaviorally-defined stages of conditioning, so to compare directly these regions to a previously studied limbic corticothalamic system (the cingulate cortex and anteroventral thalamus; Gabriel, M., Miller, J.D., & Saltwick, S.E., J. Comp. Physiol. Psychol., 91: 423, 1977). The earliest discrimination in PFC appeared during the first session of conditioning, prior to behavioral discrimination. The magnitude of the effect was greatest then and during the session of first significant behavioral discrimination. When the stringent criterion of discriminative performance was met, the magnitude of PFC discrimination was reduced relative to the prior stages. In contrast, the MD demonstrated its largest discriminative effects at the criterial stage.

This sequential pattern of discriminative effects bears a striking similarity to the previous findings obtained in the cingulate cortex and anteroventral thalamus. However, the present data indicated a difference between the two systems: the proportion (.63) of records in PFC which manifested discriminative activity in the first session of conditioning exceeded significantly that proportion (.33) of the cingulate records. Also a greater proportion (.64) of the MD records manifested neuronal discrimination in the early (precriterial) sessions of conditioning, relative to the proportion (.33) of anteroventral thalamic records manifesting early discrimination. Thus the PFC-MD system may develop discriminative neuronal activity more rapidly during the course of training than the cingulate cortical-anteroventral thalamic system. These findings suggest the PFC and MD may be part of a rapid "working memory" system, involved in the initial-most encoding of associative significance of cues during behavioral learning. (Supported by NIMH Grants 26276 and 3151 to M. G.)

148.14 MULTIPLE UNIT ACTIVITY OF THE ABDUCENS NERVE IN THE ANESTHETIZED AND PARALYZED RABBIT. N. E. Berthier* and J. W. Moore. Dept. of Psychology, University of Mass., Amherst, Ma. 01003.

The unconditioned nictitating membrane response (NMR) of rabbit to stimulation of the paraorbital region of the face was investigated using electrophysiological recording from neurons of the abducens (6th cranial) nerve. Rabbits were anesthetized with chloralose and urethane, paralyzed with gallamine, and artificially respirated. Tungsten or glass microelectrodes were inserted into the 6th nerve using a ventral approach. Electrical stimulation (1.5 mA, 5ms duration) to the area surrounding the ipsilateral eye evoked a response of 5 ms latency. The latency of the motor volley suggests that this reflex is di- or trisynaptic. The number of activated units increased as a function of stimulus intensity and duration. Increases in stimulus duration but not intensity increased the duration of the efferent volley. Repetitive stimulation at intervals of .3 to 30 s produced consistent habituation, i.e., fewer activated units and reduced duration of the response volley.

Auditory stimuli and tactile stimulation of the paraorbital region are typically employed as conditioned and unconditioned stimuli, respectively, in the unanesthetized-unparalyzed rabbit NMR preparation. In the present study, action potentials from the 6th nerve were evoked by tactile stimulation of the ipsilateral paraorbital region and by an intense auditory stimulus ($>$ 100 dB SPL). These findings indicate that the neural elements mediating the unconditioned NMR remain intact in the acute anesthetized preparation. Neurophysiological investigation of the mechanisms of classical NMR conditioning in this preparation are indicated.

149.1 Response of Cutaneous Receptors to a Pruritic Stimulus. R. P. Tuckett* (Spon: P. R. Burgess). Department of Physiology, University of Utah, Med. Ctr., Salt Lake City, Utah 84108

The purpose of this study was to apply a potent itch-producing substance, cowhage (Mucuna pruriens), to cutaneous receptors and, by a process of elimination, determine which might be involved in signaling the sensation of itch to the central nervous system.

All types of myelinated fiber receptors have been sampled in cat (type I, type II, G_1 hair, intermediate hair, G_2 hair, F_1 field, intermediate field, F_2 field, D-mechanoreceptor, and delta nociceptor). None showed any response to application of cowhage. Whole-nerve recordings from small cutaneous nerves, in which individual delta fibers potentials could be distinguished, showed no change in activity after application of cowhage to the entire receptive field of the nerve.

Most unmyelinated-fiber receptor populations have also been sampled. Six C-mechanoreceptors, four thermal cooling receptors and one thermal warming receptor did not respond. A subcutaneous receptor was also unresponsive.

Twenty polymodal nociceptors were tested with cowhage and all but four responded. Polymodals can be activated by noxious mechanical and thermal (heat) stimuli. While firm rubbing of the skin and heating the skin (to what are noxious levels in humans, 46-52°C) produced a vigorous discharge from most polymodal receptors I studied, these stimuli do not produce itching sensations in normal human subjects. Hence, the nervous system must have a mechanism to differentiate among the variety of modalities to which polymodal receptors respond. (Supported by Grant NS15102 from USPHS).

149.2 NEUROTENSIN CELL BODIES IN SUBSTANTIA GELATINOSA. V. Seybold and R. Elde. Dept. of Anatomy, Univ. of Minnesota Medical School, Minneapolis, MN 55455.

The immunohistochemical localization of neurotensin immunoreactive (N-IR) terminals in substantia gelatinosa (SG) of the rat spinal cord has been confirmed by several laboratories. By combining the peroxidase-antiperoxidase (PAP) procedure of immunohistochemistry and thick sections (50-100 µm),it has been possible to describe two kinds of N-IR cell bodies within SG which may give rise to the terminals within the same region.

Six, 300 gm, male albino rats were used in these studies. Under anesthesia, PE-10 tubing was inserted in the cisterna magna and directed 9 cm down the subarachnoid space of the spinal cord to the lumbar enlargement. Colchicine (25 µg in 10 µl) was injected through the tubing, and the tubing was withdrawn. Two days later, the animals were sacrificed by vascular perfusion with 4% paraformaldehyde. 50 -100 µm frozen sections were cut in a transverse plane or a longitudinal plane along a radial axis through one dorsal horn. Free floating sections were processed for neurotensin immunoreactivity using the PAP procedure. The neurotensin antiserum used in this study does not cross-react immunohistochemically with other peptides localized in SG to date.

In transverse sections, two bands of N-IR fibers and cell bodies are observed in cross-section: one at the outer border and one at the inner border of SG. Cell bodies were 5 µm in diameter in this plane and 15-20 µm in length in the longitudinal plane. In the longitudinal plane, primary dendrites emanate from the rostral and caudal aspects of the cells, but the two bands of cells differed in arborizations of their dendrites. The secondary dendrites of N-IR cells in the inner band began branching closer to the cell body and tertiary dendrites had a greater medial and lateral extension in comparison to N-IR cells in the outer band. Interestingly, N-IR axons were not observed in these sections.

Based on comparisons of dendritic arborizations, the N-IR cells in outer SG are similar to the islet cell and the N-IR cells in inner SG are similar to the II-III border cell described by Gobel (J. Comp. Neur. 180:395, 1978).

V. Seybold is supported by a fellowship from the PMA Foundation; R. Elde is supported by an award from the McKnight Foundation.

149.3 NISSL, WEIL AND GOLGI ANALYSIS OF THE PERIAQUEDUCTAL GREY IN RAT, CAT AND MONKEY. Ingrid A. Abols* and Patrick W. Mantyh* (Spon: J. deGroot) Dept. of Anatomy, Univ. of Cal., San Francisco, Ca.94143

In a comprehensive analysis of the periaqueductal grey (PAG), Nissl and Weil stained sections through the midbrain were examined in 10 rat, 6 cat, 3 macaque and 9 squirrel monkey cases. In addition, Golgi material was obtained in 5 rat, 2 cat, 5 macaque and 3 squirrel monkey cases, by processing 3 mm blocks according to Golgi Cox or Golgi Kopsch methods.

Nissl and Weil stained material did not reveal any cyto- or myelo- architectural borders within the PAG, except for an apparent cell poor region immediately surrounding the cerebral aqueduct. No significant difference in cell size or shape was found within various regions of the PAG in any of the animals examined.

Golgi impregnated material revealed the PAG to be composed of a heterogeneous group of cells which do not appear to be regionally segregated according to size, shape or dendritic arborization. Most cells observed had extensive dendritic arborizations in the coronal plane with lesser, but still extensive arborizations in the sagittal plane. The cell somas ranged in size from 10-30um and had shapes ranging from small spherical to large pyramidal. Also noticeable in the Golgi sections was a dense plexus composed of both neuronal processes and glia in the border immediately surrounding the aqueduct. The lateral border, or annulus, of the PAG, formed chiefly by the mesencephalic root of V seen in Nissl or Weil material, is not readily seen in Golgi impregnated material. The stratum profundum of the superior colliculus and the adjacent ventrolateral tegmentum appeared similar in cytoarchitecture to the PAG. Both PAG neurons and neurons from these surrounding areas have dendritic arbors that interdigitate with each other without respect to borders defined by Nissl cytoarchitecture or more apparent myeloarchitecture features. Some PAG cell dendrites extended beyond the stratum profundum into the stratum intermedium of the superior colliculus. No striking differences in the organization of the PAG or its constituent cell morphology was observed between rat, cat, macaque or squirrel monkey.

The PAG in the animals examined appears to be composed of a population of cells with a wide range of soma size and shape that have extensive dendritic arbors, which often extend into adjacent parts of the tectum or tegmentum. Therefore cytoarchitectural and connectivity (Mantyh, Neurosci. Abstr. '80) features of the PAG do not reveal apparent anatomical correlates to the variety of functional subdivisions suggested by immunohistochemical and physiological studies. (Supported by H.J. Ralston NS-11614 and W.R. Mehler NASA task 199-05-02-07).

149.4 EFFECT OF LESIONS OF NUCLEUS RETICULARIS GIGANTOCELLULARIS ON MORPHINE- AND STIMULATION-PRODUCED ANALGESIAS IN THE RAT PERIAQUEDUCTAL GRAY. J.S. Mohrland, D.Q. McManus* and G.F. Gebhart, Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242

Focal brain stimulation (FBS) and microinjection of morphine into the periaqueductal gray (PAG) region of the mesencephalon results in a profound analgesia. That analgesia has been shown to involve an inhibitory spinopetal pathway. The lack of a substantial anatomical projection from the PAG to the spinal cord implicates more caudal brainstem nuclei in this descending system. Nucleus reticularis gigantocellularis (NGC) of the medullary reticular formation is known to be involved in transmitting nociceptive information, and recent evidence suggests it is also involved in PAG-induced analgesias. In the present study NGC lesions were used to evaluate the role of this nucleus in analgesias produced by PAG morphine and FBS.

Cannula/electrode assemblies were constructed to allow for stimulation and morphine microinjection at essentially the same PAG locus. At least one week following stereotaxic implantation of the assemblies into the PAG of rats; the analgetic efficacy of morphine (5µg base in 0.5µl artificial CSF) and FBS (40-120µA of 64 Hz, 0.5 msec biphasic pulse pairs for 5 min) was evaluated using standard hot plate and tail-flick tests. Electrolytic lesions were then made bilaterally into NGC; approximately two weeks later the analgetic efficacy of the PAG manipulations (vide supra) was again evaluated.

Control (pre-morphine) hot plate and tail-flick reaction times were not altered by NGC lesions. The lesions also failed to alter the increase in reaction times produced by FBS (both tests) and by morphine on the hot plate test. NGC lesions did, however, significantly attenuate the analgesia produced by PAG morphine administration as measured by the tail-flick test, an effect not observed in sham-treated animals. These results further implicate NGC in analgesia resulting from microinjection of morphine into PAG. They also suggest a separation of analgesia produced by FBS from that by morphine microinjection.

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149.5 AN ANALYSIS OF REGIONAL SUBDIVISIONS AND NEURONAL POPULATIONS IN THE RODENT PERIAQUEDUCTAL GRAY. A. J. Beitz. Dept. of Anatomy, Univ. of South Carolina, Sch. of Med., Columbia, S.C. 29208.

Nuclear volume, neuronal and glial numbers, neuronal and glial density, neuronal shape and neuronal orientation were studied in the rat periaqueductal gray (PAG). A statistical analysis of the data obtained from measurements of neuronal lengths, widths and areas indicated that the neuronal population of the PAG is heterogeneous and cannot be divided into subpopulations based on cell size. The cells which comprise this midbrain region are extremely small in size. The mean area of the total cell population is 80.28μm^2 while the mean length and width are 13.77μm and 6.86μm, respectively. The nuclear volume occupied by the PAG as calculated from cross-sectional areas of the nucleus was 8.16 x 10^{-3} cm^3. The average neuronal density calculated from a series of coronal sections is 1.65 x $10^7/cm^3$ while the glial cell density is 6.44 x $10^7/cm^3$. The neuronal cell density was found to gradually increase from caudal to rostral portions of the central gray. In an attempt to accurately delineate the nuclear subdivisions which comprise this region, the PAG was divided into 19 regions on a purely subjective basis using the criteria of cell size, shape and orientation and cellular packing density. Data concerning the length, width, area and orientation of the individual neurons which comprised each region was entered into a computer and the regions were then statistically compared utilizing both a cluster analysis system and box plots. The results of this computer analysis indicated that the PAG can be divided into four nuclear divisions: (1) A dorsal division in which the cells display no particular orientational preference; (2) a dorsolateral division in which the majority of cells are oriented at a 30-70^o angle with respect to the cerebral aqueduct; (3) a ventrolateral division in which the majority of cells are oriented at 110-150^o angle with respect to the aqueduct; and (4) a medial division composed of small cells that surround the aqueduct, the majority of which maintain an orientation parallel to the aqueduct. The medial division contains the smallest neurons found within the PAG while the ventrolateral subdivision is characterized by the presence of large cells. These results support the existence of medial and dorsal subdivisions within the rodent PAG as originally described by Hamilton (1973) in the cat and, in addition, provide new evidence that dorsolateral and ventrolateral subdivisions are also present in the PAG.

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149.6 A GOLGI ANALYSIS OF THE RODENT PERIAQUEDUCTAL GRAY. S.M. Prichard and A.J. Beitz. Dept. of Anatomy, Univ. of South Carolina, Sch. of Med., Columbia, S.C. 29208

The neurons of the midbrain periaqueductal gray (PAG) of the rat were studied with several variants of the Golgi technique. Three general classes of cells have been identified in the PAG: (1) fusiform cells; (2) triangular cells, and (3) stellate cells. The fusiform cell type can be divided into two major subclasses: a small bipolar neuron which is the most prominent cell type encountered in the PAG being present in all four subdivisions, and a large fusiform cell which is most prominent in the ventrolateral subdivision of the PAG. The small bipolar neurons are characterized by an orientation which parallels the transverse or oblique plane of section and their distal dendrites often display prominent dendritic excrescences. Many of the small bipolar cells of the dorsal and dorsolateral subdivisions direct their axons toward the overlying inferior and superior colliculi. The small bipolar neurons and the large fusiform neurons of the ventrolateral subdivision, however, project the majority of their axons into the neighboring reticular formation. The triangular cells are a major constituent of the dorsolateral subdivision. These cells typically give rise to 3 or 4 primary dendrites which branch several times to form an ellipsoidal dendritic territory. The dendrites of these cells are highlighted by the presence of numerous dendritic spines, especially along their distal extremities. The final neuronal class, the stellate cells, also can be divided into two basic varieties: (1) an ovoid shaped large multipolar neuron (LMN), and (2) a spherically shaped smaller multipolar neuron (SMN). The ovoid shaped LMN's are characterized by a prominent elongated cell body which gives rise to 5 or 6 primary dendrites which branch only once or twice to form an ellipsoidal dendritic arborization. The spherical LMN's are characterized by a round cell perikaryon from which 4 or 5 primary dendrites originate. Moreover, these cells are most commonly found in the dorsal and dorsolateral subdivisions. The results of this study indicate that the rodent PAG is comprised of five basic neuronal types as identified in Golgi impregnated material. Moreover, this study supports the existence of four subdivisions within the PAG of the rat as defined in Nissl preparations (Beitz, 1980). The morphological variations defined above may underlie the functional heterogeneity of this region.

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149.7 RAPHE STIMULATION INDUCES PRIMARY AFFERENT DEPOLARIZATION OF BOTH LOW-THRESHOLD MECHANOSENSITIVE AND NOCICEPTIVE TRIGEMINAL AFFERENTS. James W. Hu and Barry J. Sessle. Faculty of Dentistry, University of Toronto, Canada M5G 1G6.

The nucleus raphe magnus (NRM) and periaqueductal gray (PAG) have been implicated in endogenous opiate-related mechanisms of suppression of nociceptive transmission. In the trigeminal (V) system, we previously reported that PAG and NRM conditioning stimulation inhibits reflex and single brainstem neurone responses to noxious oral-facial stimuli; we noted however that the responses of low-threshold mechanosensitive neurones also can be suppressed. Since these findings suggest that raphe influences may not be selective for nociceptive transmission, and since it has been suggested that the raphe influences may act presynaptically, we wished to determine if conditioning stimulation of PAG and NRM could presynaptically influence V afferents that were functionally identified as being either low-threshold mechanosensitive afferents or nociceptive (tooth pulp;cutaneous) afferents. The effect of stimulation in histologically verified sites in PAG and NRM was tested in anaesthetized or decerebrated cats on the antidromic excitability of single V primary afferents recorded in the V ganglion. The afferents were antidromically excited from V brainstem nuclei oralis or caudalis, and an increase in antidromic excitability was used as the measure of primary afferent depolarization (PAD) which is generally considered to reflect presynaptic inhibition. Facial nociceptive afferents responded to cutaneous noxious mechanical stimuli and some also were excited by noxious radiant heat. They could be antidromically excited from V nucleus caudalis, and had mean conduction velocities of 0.84±0.18 m/sec (n=20; C fibres) and 5.16±3.66 m/sec (n=5; A delta nociceptive fibres). The low-threshold mechanosensitive afferents in contrast responded only to non-noxious tactile stimulation of very restricted mechanoreceptive fields, could be antidromically excited from oralis or caudalis and had conduction velocities of 41.4±13.6 m/sec (n=48). The pulp afferents also could be antidromically excited from either oralis or caudalis, and had conduction velocities of 36.4±14.7 m/sec (n=10). NRM or PAG conditioning produced PAD in all 4 pulp afferents tested, in agreement with our earlier findings (Nature, 1978). PAD also occurred in 5 of 8 facial nociceptive afferents. However, PAD could also be produced in the same animals in 41 of 42 low-threshold mechanosensitive afferents. These findings suggest that PAG and NRM may exert a presynaptic regulation on V somatosensory relays but that the effect is not selective for nociceptive transmission.

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149.8 RAPHE, RETICULAR AND PERIAQUEDUCTAL INFLUENCES ON SENSORY RESPONSES OF DORSAL HORN NEURONS IN THE CAT. Bruce Gray * and Jonathan O. Dostrovsky, (SPON: J. Duffin), Department of Physiology. Univ. of Toronto, Toronto, Canada, M5S 1A8.

Previous work indicates that electrical stimulation of the periaqueductal gray (PAG) or nucleus raphe magnus (NRM) produces analgesia and blocks the nociceptive responses of spinal dorsal horn neurons. It is generally accepted that the PAG-NRM decending system(s) controls the transmission of nociceptive information at the spinal level. However recent results indicate that the inhibitory effects of the descending systems may not be limited to nociceptive neurons and furthermore that the effective regions in the brainstem do not appear to be limited only to the PAG and NRM but that adjacent reticular formation regions can also be quite effective. Thus it was decided to compare the effects of electrical stimulation of the PAG, NRM and nucleus gigantocellularis (NGC) on both nociceptive and non-nociceptive neurons.

Experiments were performed on chloralose anesthetized adult cats. Single unit recordings were obtained from the lumbar spinal cord using carbon fiber microelectrodes. Bi-polar stimulating electrodes were stereotaxically implanted in the PAG, NRM and NGC and the stimulation sites subsequently verified histologically. A total of 44 neurons were studied. These were identified according to standard criteria as low threshold mechanoreceptive (non nociceptive), wide dynamic range, and high threshold mechanoreceptive by a careful sensory examination. To test for inhibitory effects from the brainstem the neurons were excited at just suprathreshold levels by electrical skin stimulation or by a mechanical transducer which delivered tactile impulses. The brainstem conditioning stimuli were delivered 130 ms. prior to the peripheral stimulus and consisted of a 100 ms. 500Hz train of 0.1 ms. pulses. 90% of the cells tested could be inhibited by stimulation of either PAG, NRM, or NGC at currents below the maximum used (400 μA). In any given cat the thresholds for NRM and NGC were similar. PAG threshold currents were, on average, higher than NRM or NGC currents but, as with the latter sites, were similar for the different cell classes. Surprisingly no consistent differences in threshold currents were required for inhibition of the 3 different types of neurons.

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149.9 MARGINAL ZONE PROJECTION TO NUCLEUS SUBMEDIUS IN THE MEDIAL THALAMUS OF THE CAT: A POSSIBLE PAIN CENTER. A.D. Craig, Jr. and H. Burton. Depts. of Anatomy-Neurobiology and Physiology-Biophysics, Washington Univ. Sch. Med., St. Louis, MO 63110

Previous studies of spinothalamic projections have disclosed terminations in medial thalamus mainly in the central lateral nucleus (CL), and more sparsely in the central medial (CeM) and parafascicular (Pf) nuclei. However, with the use of the anterograde transport of horseradish peroxidase (HRP) apparent terminal labeling can be observed in several additional loci (Craig & Burton ('79) Soc. Neurosci. Abst. 5:705). In medial thalamus, diffuse and/or isolated labeling occurs in the paraventricular, parataenial, paracentral, mediodorsal, ventromedial, and reuniens nuclei. A relatively compact and dense field of label occurs in the submedius nucleus (Sm), which lies just ventrolateral to CeM; this projection has been confirmed autoradiographically. The spinal zone of Sm is ca. 800 µm long; the cervical zone appears to be caudal to the lumbar zone. The portion of Sm caudal to the spinal zone, extending ca. 1mm, is labeled following anterograde transport from the medullary dorsal horn (n. caudalis). Labeling homologous to these results has also been observed in the Sm of the monkey following spinal HRP injections.

Small HRP injections (35-100 nl, 50% solution in 0.8% poly-L-ornithine) in and around Sm have been made in the cat. Analysis of retrogradely labeled spinal and medullary cells indicates that the projection to Sm arises from lamina I cells, probably exclusively. Spinothalamic terminations in surrounding nuclei appear to arise overwhelmingly from laminae VII-VIII cells, in accordance with the results of previous retrograde studies (e.g. Carstens & Trevino ('78) JCN 182:151; Willis, et al. ('79) JCN 188:543). The retrograde results support the somatotopography described above, as well as the proportionately larger projection from n. caudalis. Labeled spinal marginal zone cells are consistently located at the dorsolateral apex of the dorsal horn, while medullary cells occur in clusters but are more evenly distributed mediolaterally in lamina I.

These results indicate that a discrete and compact spino- and trigemino-thalamic projection to Sm arises from the marginal zone. Recordings obtained from Sm neurons suggest that these cells respond vigorously to noxious thermal and mechanical stimuli, which is consistent with previous physiological results indicating that many spinothalamic lamina I cells are nociceptive-specific. This suggests that Sm may be functionally specific for pain.

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149.10 CONTRASTING FUNCTIONS OF VENTROBASAL AND MEDIAL-INTRALAMINAR THALAMIC NEURONS IN THE AWAKE SQUIRREL MONKEY. K. L. Casey and T. J. Morrow. Depts. of Physiology and Neurology, Univ. Mich. Med. Sch., Ann Arbor, MI 48109.

Previous studies indicate that ventrobasal (VB) thalamic neurons encode discriminative features of somatic stimuli, but the role of spinothalamic input to the medial and intralaminar (M-IL) thalamus remains unclear. Accordingly, we are comparing the responses of VB and M-IL neurons to natural stimuli and to electrical stimulation in the spinal lemniscus (SL) of awake, partially restrained squirrel monkeys. A chronic microelectrode system (Morrow, T. J., Brain Res. Bull. 5:91-93, 1980) is used in conjunction with stimulating electrodes implanted bilaterally in the SL at midbrain levels. Single pulse stimulation of the SL and natural somatic, auditory and visual stimuli are used to test unit responses.

Each of fifty-eight VB units had irregular resting discharge frequencies ranging from 5 to 15 Hz and showed little or no variation with the monkey's level of alertness. Thirteen units recorded from the ventral posterolateral nucleus were reliably driven by SL stimuli below 20 Hz and had latencies ranging from 0.8 to 2.0 ms. All these cells had small contralateral receptive fields and were excited by light tactile stimuli or hair movement; none were affected by noxious mechanical or thermal stimuli. The remaining VB units either lacked an apparent sensory or motor correlate or discharged in relation to specific limb movements.

In contrast, the discharge of 97 M-IL units ranged from 1 to 40 Hz and varied considerably in rate and pattern with the monkey's behavioral state. The resting and evoked activity of some units was markedly attenuated or completely eliminated during induction of second stage anesthesia with halothane. Sleep or drowsiness was associated with a slow bursting spontaneous discharge. The 9 units securely driven by SL stimuli (latencies: 3.5 to 15 ms) and 10 units not responding to SL pulses all gave brief burst responses to brisk but innocuous taps or deep stimuli delivered to any limb. Responsiveness declined with repeated innocuous stimuli. Noxious somatic or sudden non-somatic stimuli which elicited behavioral alerting and movement evoked maximum unit responses. The activity of these neurons did not correlate with ocular or somatic movement. Other M-IL units showed marked changes in excitability and stimulus specificity associated with changes in behavioral orientation and attention.

These results suggest that, in contrast with the discriminative function of VB neurons, the activity of M-IL neurons encodes some aspect of the arousal function of somatic and other sensory events.

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149.11 EVALUATION OF ELECTROCUTANEOUS PAIN: (1) COMPARISON OF OPERANT REACTIONS OF HUMANS AND MONKEYS AND (2) MAGNITUDE ESTIMATION AND VERBAL DESCRIPTION BY THE HUMAN SUBJECTS. C.J. Vierck, Jr., O. Franzen* and B.Y. Cooper. Dept. of Neuroscience, University of Florida, Gainesville, FL, 32610.

The purpose of the study is to evaluate by means of human psychophysical methods the intensive and affective aspects of AC and square wave electrical shocks used in monkey experiments. By using operant reaction measures for human and monkey subjects and also various direct psychophysical techniques of estimating the intensive and affective aspects of the same stimuli the present study relates the animal data to the human domain. The stimuli applied to the lateral calf of the leg consist of short trains (38-50 msec) at a rate of 4 Hz. The train repetition rate was chosen in order to minimize a very pronounced adaptation that was found to occur with continuous stimulation.

The psychophysical functions are best described by a two-limbed function and they show a high correlation with the verbal sensory descriptors.

Human and monkey subjects yielded similar monotonic functions relating force and latency of shock-escape to stimulation intensity in the pain range. These results permit a more accurate estimation of the alteration of pain reactions produced in experimental animals by CNS intervention.

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149.12 A COMPARISON OF OPERANT AND REFLEXIVE MEASURES OF MORPHINE ANALGESIA. B. Y. Cooper and C. J. Vierck, Jr. Dept. of Neuroscience, University of Florida, Gainesville, Florida, 32610.

The behavior of monkeys (macaca speciosa) in a shock escape task was examined. Measures were taken during the receipt of shock and during intertrial periods. In this manner we assessed both pain responsiveness and anticipation. Measures of escape vigor (bar pull force and latency), intertrial activity (vocalization, movement and bar pulls) and pain-induced reflexes were made. By using an ascending and descending pattern of shock stimuli, we were able to examine pain-induced analgesia mechanisms as well. We found that bar pull escape latencies and forces were strongly related to shock intensity, while pain-induced reflexes and intertrial measures showed only a weak relationship.

The effects of morphine on pain (.5, 1, 2 and 3 mg/kg) were clearly refected in all measures. These measures indicated large differences in individual responses to pain and susceptability to morphine analgesia. Treatment with PCPA or disulfiram showed a preferential blocking of morphine analgesia on the reflex measure. The disparities found between operant and reflexive pain measures are under further study.

149.13 ELECTROANALGESIA-INDUCED LATENCY SHIFT IN PULP-DRIVEN AFFERENT UNITS OF CAT TOOTH. Patrick J. Reynolds, Ann Kloka*, Richard B. Tacke* and R. Wayne Fields. School of Dentistry, University of Oregon Health Sciences Center, Portland, Oregon 97201.

In a previously reported study (Reynolds et al., Abstr. Soc. Neurosci. 5:615, 1979) of afferent unit activity from cat tooth pulp during administration of electroanalgesia (EA) current, a phenomenon of unknown origin and significance was observed. The unit discharge latency following rectangular pulse (0.1 msec) electrical test stimulation was seen to increase in some, but not all, units following administration of EA current. The phenomenon was transient and variable in most aspects, with these few common features: it was observed only as an increased latency (slowed conduction) from the pre-EA control value; it was observed only when threshold was elevated from pre-EA control; it occurred sometimes during, but most often after, a given EA administration protocol; it was always of a much briefer duration than the corresponding EA-induced threshold elevation for any unit.

The magnitude of the shift ranged from about 50% to over 400% in cases that were documented. Its transient nature made it difficult to document in some cases, where it appeared more as a "jitter" in latency, shifting between higher and lower values at alternate 1 second test intervals. In retrospect it cannot be ruled out as having occurred at a lesser magnitude (a few percent) in the 4 of 18 units where it was not apparent, since the time base resolution was not fine enough to detect such small change.

One explanation for the phenomenon is spread of stimulating current to an alternate, slower conducting, intrapulpal branch of the observed afferent unit (Matthews and Holland, Brain Res. 98:354-358, 1975). An often observed monotonic decrease in latency toward control over a 60-120 sec. period would argue against this hypothesis.

An alternative explanation would be a hyperpolarization-induced decrease in conduction velocity in the intrapulpal segment of the afferent unit. The potential occurence of large latency shifts should be considered carefully in the reporting of conduction blockade in peripheral nerve fibers.

An analysis of radiographs of several of the teeth from this study is underway to detect a possible relationship between EA dose and magnitude of latency shift. No other systematic quantitative correlate of the phenomenon is yet apparent.
(Supported by NIH Grant DE 04281)

149.14 ANALGESIA FOLLOWING MICROINJECTION OF CYCLIC NUCLEOTIDES AT SITES IN THE CAUDAL BRAIN STEM. R. A. Levy, B. D. Goldstein, M. M. Elyjiw* and H. K. Proudfit. Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago, IL 60680.

Cyclic nucleotides cause analgesia following intracerebroventricular injection in rodents (Cohn et al., Science 199:319, 1978). Since cyclic nucleotides are believed to act as second messengers at noradrenergic and cholinergic synapses, it is possible that this effect reflects an action at such synapses located in pain modulating pathways. The nucleus raphe magnus (RM) of the caudal brain stem,which controls nociceptive threshold via its descending projections to the spinal cord, appears to be modulated by inhibitory noradrenergic and excitatory cholinergic inputs. We have tested the involvement of cyclic nucleotides in this modulation by determining their capacity to alter nociceptive threshold following microinjection at sites within the RM.

The dibutyryl derivatives of cyclic guanosine and adenosine 3':5'-monophosphate (db cGMP and db cAMP) were injected, in 0.5 µl saline, at caudal brain stem sites with a 28 gauge cannula inserted through a chronically implanted guide sheath. Nociceptive threshold was assessed prior to and following injection of 20 µg db cGMP or 10 µg db cAMP using the tail flick, paw pinch, and hot plate tests.

Injection of db cGMP at midline sites, near or within the RM, caused no significant alteration in nociceptive threshold in any of the three tests. This suggests that endogenous cGMP does not play a role in either NE-mediated inhibition or ACh-mediated excitation of RM neurones. However, injection of db cGMP at sites located about 0.5 mm lateral to the midline, within the nucleus gigantocellularis (GC), caused a marked analgesia in all three tests. Since cGMP has been implicated as a second messenger for cholinergic transmission, the analgesia produced by injection of db cGMP at GC sites suggests such a role for endogenous cGMP at cholinergic synapses associated with pain modulatory pathways in the lateral/caudal brain stem.

Microinjection of db cAMP at sites within the RM caused a marked increase in nociceptive threshold in all three tests. This effect may reflect a facilitation of the cholinergic excitatory input to the RM by an enhancement of ACh release, analogous to the action of cAMP at the neuromuscular junction (Standaert et al., JPET 199:553, 1976). This analgesic effect of db cAMP also suggests that endogenous cAMP is not a second messenger at the inhibitory noradrenergic synapse onto the RM, as it is at noradrenergic synapses at other CNS sites; if cAMP played such a role then microinjection of db cAMP would be expected to inhibit the RM, thereby producing hyperalgesia instead of the observed analgesia. (Supported by GM 25998-01A1).

149.15 NEUROPHARMACOLOGICAL INVESTIGATION OF TRIGEMINAL NEURALGIA G.H. Fromm, A.S. Chattha*, C.F. Terrence*, and J.D. Glass. Dept. of Neurology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

We have previously found that drugs effective in relieving trigeminal neuralgia (carbamazepine, baclofen, phenytoin) facilitate inhibitory mechanisms as well as depressing excitatory mechanisms in the spinal trigeminal nucleus of cats. The facilitation of inhibitory mechanisms seemed to be at least as important as the depression of excitatory mechanisms, and suggested that a failure of inhibitory mechanisms may play a significant role in the pathogenesis of trigeminal neuralgia.

We have now investigated the effect of carbamazepine on various types of neurons in both the nucleus oralis and the nucleus caudalis of the trigeminal complex. Neurons were classified as relay neurons if they responded to stimulation of the trigeminal lemniscus with an unvarying short latency and followed repetitive stimulation at 200 Hz. Neurons were classified as type A interneurons if they responded transsynaptically to stimulation of the trigeminal lemniscus, and as type B interneurons if they did not respond to stimulation of the trigeminal lemniscus. We studied the effect of carbamazepine on excitatory synaptic transmission by observing its effect on the neuron's response to maxillary nerve stimulation. We studied carbamazepine's effect on inhibitory mechanisms by investigating its effect on the segmental inhibition produced by delivering a conditioning stimulus to the maxillary nerve prior to the test stimulus.

The administration of 5-20 mg/kg carbamazepine depressed the response to maxillary nerve stimulation of all the neurons studied in both nucleus oralis and nucleus caudalis. Carbamazepine also markedly facilitated the segmental inhibition of all the B interneurons in nucleus oralis. This facilitation was considerably greater than the depression of excitation. The segmental inhibition of relay neurons and of A interneurons in nucleus oralis was usually decreased by low doses of carbamazepine (5 mg/kg) and increased by high doses of carbamazepine (20 mg/kg). The segmental inhibition of all neurons in nucleus caudalis was also decreased by low and increased by high doses of carbamazepine.

The most striking effect of carbamazepine in these experiments was its facilitation of the segmental inhibition of B interneurons in nucleus oralis. This segmental inhibition appears to be presynaptic, mediated by primary afferent depolarization, and it represents a negative feedback type of inhibition. Facilitation of this negative feedback mechanism could very effectively decrease the response of the trigeminal nucleus to abnormally increased firing of the trigeminal nerve, and so prevent the paroxysms of tic douloureux without affecting the response to normal levels of activity.

149.16 EEG CHANGES INDUCED BY SYSTEMATIC ADMINISTRATION OF MORPHINE IN THE RAT.[+] J. D. Bronzino, W. Forbes, M. Kelly*, C. Cordova*, N. Oley and P. J. Morgane. Trinity College, Hartford, CT 06106

In a study of the effects of morphine on the electrographic activity of the brain, power spectral and amplitude analysis techniques have been applied to quantify EEG parameters not readily discernible by visual observation. Recent evidence Rieger et al., Pharmakopsychiat. 12: 94-101, 1979) suggests that the development of EEG amplitude histograms may be an important method that can be used to distinguish and quantify alterations in the EEG. In addition, we (Bronzino et al., Proceedings of IEEE-GEMB, 1980) have demonstrated that several indices namely the standard amplitude, skewness and kurtosis are sensitive and reliable measures of these amplitude histograms. In the present studies, this technique was used to quantify the EEG effects brought about by systematic injection of morphine. Adult Sprague-Dawley rats (n=10) were implanted with chronic indwelling bipolar recording electrodes in the frontal and parietal cortices. After a considerable recovery period, systematic injections of morphine (5mg/kg, 15mg/kg and 30mg/kg) as well as a control injection of Ringer's solution were given a week apart. Power spectral analysis and amplitude analysis techniques were then utilized to quantify the impact of these pharmacological manipulations upon the cortical EEG of the rat.

In agreement with the work of others (Khazan, N., In: Methods in Narcotics Research, pp. 173-209, 1975; Steinfels, G. F. et al., Neuropharmacology 19: 69-74, 1980; Yeung, J. C. et al., Neuropharmacology 17: 525-532, 1978) we have found that morphine produced a dose-dependent behavioral antinociception and sedation associated with EEG slowing and synchronization. However, the availability of compressed spectra arrays and the development of amplitude histograms have enabled us to better visualize the transition from desynchronization through spindling to a continuously synchronized EEG pattern. In addition, the measures of skewness and kurtosis follow the transition process and provide a finer resolution than visual inspection. The impact of systematic morphine injection upon these EEG measures are presently being utilized to compare and evaluate the effect of direct cerebral injection of morphine to the region of the nucleus tractus solitarius - area postrema upon cortical EEG.

[+] National Institute of General Medical Science Grant GM 27226-01

149.17 BENACTYZINE-INDUCED REVERSAL OF PHYSOSTIGMINE AND COLD-WATER ANALGESIA, J.A. ROMANO* J.M. KING. USA Biomedical Laboratory, Aberdeen Proving Ground, MD 21010 (SPON: L. OATMAN).

The cholinergic system has been implicated in the production of morphine analgesia. Physostigmine potentiates the antinociceptive effects of morphine whereas atropine and hemicholinium attenuate morphine analgesia on the tail flick test (Ann. N.Y. Acad. Sci., 1976, 281, 262). The attenuation of morphine effects was shown to involve central muscarinic pathways (Psychopharm., 1977, 52, 7). Physostigmine but not neostigmine is a potent analgesic in its own right and its effects are blocked by anticholinergic compounds (JPET, 1969, 169, 17).

The evidence for opiate involvement in stress produced analgesia is threefold: (1) administration of endorphin produces analgesia (2) hypophysectomy blocks antinociception of restraint stress, suggesting a role for pituitary endorphin, and (3) cross-tolerance also occurs between endorphin and morphine administration (Life Sci., 1977, 20, 1259).

The role of the cholinergic system in stress- and anticholinesterase produced analgesia was explored using three behavioral measures of pain: tail flick (TF), jump flinch (JF), and hot plate (HP). HP temperature was calibrated at 56.4°C and TF intensity at 69.1°C. One baseline measure was taken on JF and HP, three on TF. Benactyzine HCl (Ben), a drug which has known central anticholinergic properties, was used in each test (1.8 mg/kg, i.p.) to block either physostigmine salicylate (.65 mg/kg, i.p.) (Physo) or cold water analgesia (3.5 min at 2°C).

	HP(sec)		JF(mA)		TF(sec)	
	Pre	Post	Pre	Post	Pre	Post
Physo	13.5±2.4	17.0±1.8	.52±.06	.78±.07	7.8±1.7	21.0±3.2
Physo+ Ben	12.6±1.6	15.7±2.0	.56±.06	.57±.05	7.1± .9	6.6± .8
CWS	14.1±1.8	19.1±1.5	.60±.09	.80±.25	4.6± .3	11.4±1.3
CWS+Ben	14.2±2.5	18.2±2.5	.65±.04	.48±.02	4.5± .4	6.2± .6
Ben HCl	14.1±1.5	10.7±1.6	.53±.07	.63±.07	6.6± .4	6.4± .7
Control	13.3±1.2	14.4±1.6	.45±.06	.57±.06	7.2±1.0	6.3± .5

Ben was given 4 min before either the administration of physo or CWS. Analysis of variance indicated that Ben blocked the analgesia produced by either Physo or CWS on both TF ($p < .01$ and .02, respectively) and JF ($p's < .01$), but not on HP. These data are in agreement with those of Yaksh (Brain Res., 1979, 160, 180) that spinally mediated reflexes may be dependent upon non-endorphin-supra-spinal descending neurotransmitter systems.

149.18 ENHANCEMENT OF PHYSOSTIGMINE ANALGESIA BY MORPHINE: DEPENDENCE ON DOSE AND TEST SYSTEM. J.M. King and J.A. Romano*. USA Biomedical Laboratory, Aberdeen Proving Ground, MD 21010.

Physostigmine produces analgesia and potentiates the analgetic effect of morphine in the tail flick test (L. Harris, Fed. Proc., 1970, 29, 28). This enhancement has not been found using shock threshold measures (H. Herken, et al., Arch. Exp. Path. Pharm., 1957, 230, 313), and elevated central acetylcholine antagonizes morphine analgesia on the hot plate test (L. Botticelli, et al., Comm. Psychopharm., 1977, 1, 519). Different drug sensitivities have been found for tail flick and hot plate (T. Yaksh, Brain Res., 1979, 160, 180). Thus, the nature of the physostigmine-morphine interaction may be influenced by the analgesia measure employed. For the tail flick, animals received 3 predrug and 3 postdrug trials; with the hot plate 1 trial of each type was run. Stimulus intensities were 69.1°C for tail flick and 56.4°C for hot plate. Tail flick difference scores were analyzed using ANOVA's; hot plate data were analyzed using ANOCOVA procedures. For tail flick and hot plate, animals received simultaneous morphine SO4 (0, 1, 2, or 4 mg/kg) and physostigmine salicylate (0, .32, or .65 mg/kg). In both tail flick and hot plate procedures 9 animals received each of the 12 drug combinations. All drugs were given IP in a volume of 1 cc/kg. Drug effects were measured 30 minutes following injection. Tail flick latencies were recorded automatically. Foot thumps and paw licks were used as criterion responses in the hot plate test. Mean predrug latencies were 5.4 seconds for tail flick and 11.4 seconds for hot plate. In the tail flick, physostigmine and morphine yielded significant analgesia, and were additive in combination.

DRUG DOSES		Tail Flick latency change (sec)				Hot Plate corrected latency (sec)			
MORPHINE(mg/kg)		0	1.0	2.0	4.0	0	1.0	2.0	4.0
PHYSO-	0	-0.9	0.7	0.7	2.8	11.0	11.6	13.1	13.5
STIGMINE	0.32	7.1	5.7	10.0	11.7	11.8	12.0	16.2	16.5
(mg/kg)	0.65	9.2	11.4	10.1	12.1	20.5	17.8	22.4	13.4

In the hot plate, 2 & 4 mg/kg morphine were additive with .32 mg/kg physostigmine, as were 2 mg/kg morphine and .65 mg/kg physostigmine; however, 4 mg/kg morphine antagonized the analgesia produced by .65 mg/kg physostigmine. Thus, the degree and type of physostigmine-morphine interaction is determined by (a) the doses of each drug, and (b) the test system employed to measure analgesia. This latter determinant may reflect a difference in the level of mediation for tail flick and hot plate responses within the nervous system.

149.19 DIFFERENTIAL ROLES FOR ACTH AND VASOPRESSIN IN PAIN MODULATION OF BRATTLEBORO AND NORMAL RATS. M.M. Wallace, R.J. Bodnar, D. Badillo-Martinez*, J. Kordower*, T. Puleo*, G. Nilaver and E.A. Zimmerman. Dept. of Psychology, Queens College, Flushing, NY 11367 and Dept. of Neurology, Columbia University, College of Physicians and Surgeons, NY, NY 10032, USA.

Acute exposure to severe environmental stressors produces anti-nociceptive responses which adapt with repeated presentation. While certain stressors utilize endogenous opioid pathways, others, such as cold-water swims (CWS), appear to act independently and are mediated, in part, by the hypothalamo-hypophyseal axis. Hypophysectomy and destruction of the medial-basal hypothalamus diminish its analgesic effects in normal rats. Brattleboro rats genetically deficient in vasopressin and impaired in adrenocorticotrophic hormone (ACTH) release, also display attenuated analgesic responses to CWS, yet show normal analgesic reactivity to morphine. To analyze the role of vasopressin and ACTH in anti-nociceptive responses, the present study assessed whether central punctate administration of these peptides altered basal pain threshold responses as well as analgesic responsivity to stress in Brattleboro and normal rats. Flinch-jump pain thresholds were measured in cannulated rats 30, 60 and 90 min following intraventricular injections of 5 ul of either arginine vasopressin (AVP: 5 ug), ACTH (0.5 ug) or a vehicle control according to a Latin Square design. Relative to the vehicle control, pain thresholds were elevated by AVP in Brattleboro rats and lowered in normal animals across the entire test sequence. A smaller effect was observed following ACTH: pain thresholds were elevated 30 and 90 min afterwards in the Brattleboro and lowered 90 min afterwards in the normal animals. The same doses of AVP, ACTH or vehicle were then administered 30 min before CWS and 60 min prior to pain threshold tests. Preliminary data show that CWS analgesia dramatically increased in Brattleboros following ACTH and to a lesser degree by AVP. Peptide supplements failed to affect CWS analgesia in the normals. These data suggest an integral role for these peptides in modulation of nociceptive reactivity in Brattleboro rats. (Supported by NIH Grant NS 1449, NYSHRC Grant 1518 and NIH GRSG 5-S05-PR-07064.)

149.20 INTERACTION BETWEEN ANALGESIA AND CENTRAL BLOOD PRESSURE REGULATION. B. Delbarre, D. Casset-Senon*, G. Delbarre. Lab. Chir. Exp., Fac. Méd., 37032 TOURS CEDEX, FRANCE.

Recently, ZAMIR et al. (Brain Res., 160, 170-173, 1979) found changes in pain sensitivity in renal hypertensive rats. IGARASHI et al. (Neurosci. Lett., 12, 189-193, 1979) found a convergence of sensory input from tooth pulp, optic chiasma, sciatic nerve onto locus coeruleus neurons which are known also to play an important role in the central blood pressure (BP) regulation. All these facts suggest an interaction between brain circuits involved in central blood pressure regulation and pain perception.

To test this hypothesis, we have studied the action of drugs known for their analgesic effects on BP. In unanaesthetized cats, intraventricular injections of met-enkephalin (20 µg/kg), morphine (20 µg/kg), substance P (1 µg/kg) and Db.c.AMP (75 µg/kg) increase significantly BP, while naloxone (10 µg/kg) induces a long lasting fall of BP. In spontaneous hypertensive rats (SHR), naloxone (5 mg/kg S.C.) decreases significantly BP, two and three hours after administration. At the same dose, this drug decreases significantly the nociceptive threshold two hours after administration in SHR but not in Wistar Kyoto rats.

All these facts suggest that neurotransmitters involved in central blood pressure regulation may be related to those mediating pain.

149.21 RAPHE AND RETICULAR FORMATION NEURONS: CONTRASTING RESPONSES CORRELATED WITH THERMAL PAIN AND BEHAVIOR IN CATS. S. F. Eisenhart, Jr. and K. L. Casey. Neurosciences Program and Depts. of Physiology and Neurology, Univ. Mich., Ann Arbor, MI 48109.

There is evidence that the caudal reticular formation mediates both the activation and suppression of pain behavior. Some neurons in nucleus reticularis gigantocellularis (NGC) and nucleus reticularis magnocellularis (NMC) may mediate the activation of pain mechanisms; in contrast, nucleus raphe magnus (NRM) neurons have been most consistently implicated in pain suppression mechanisms. We are testing this hypothesis by recording the activity of single neurons from the caudal brainstem of awake, behaving cats.

Cats are trained to eat while partially restrained so that thermal pulses (7 sec duration, 20°C/sec rise time, 38 to 55°C) can be delivered to the shaved outer thighs. Hindlimb movements, interruption of eating, and vocalizations terminate the thermal stimulus and show a marked increase in frequency following thermal pulses above 52°C. These behaviors are significantly reduced if stimuli are delivered coincident with eating, thus providing an opportunity to correlate modulation of behavioral responses with changes in unit activity.

The NRM cells identified thus far show increases in discharge which are correlated with stimuli eliciting escape responses. For example, a cell with a resting discharge of 3.2 Hz discharged at 9.5 Hz following a 55°C pulse which did not elicit escape, but discharged at 26 Hz when the same stimulus elicited escape. The NRM cells show markedly increased activity 1 to 2 sec before an escape response; they do not discharge in relation to movements independent of the stimulus. Neurons in NGC and NMC recorded thus far have distinctly different response properties; in general, their activity is best correlated with motor behavior which is not necessarily related to the thermal stimulus or to escape behavior. However, some cells in the medial NGC have shown decreased activity only during noxious thermal pulses eliciting escape.

These results support the hypothesis that neurons in the medial caudal brainstem reticular formation mediate or modulate behavioral responses specifically elicited by noxious stimuli.

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149.22 ALTERATIONS IN NOCICEPTIVE THRESHOLD AND MORPHINE-INDUCED ANALGESIA FOLLOWING THE SELECTIVE DEPLETION OF SPINAL CORD MONOAMINES. H. K. Proudfit and T. L. Yaksh. Dept. Pharmacology, Univ. of Ill. Coll. Med., Chicago, IL 60680 and Mayo Fdn., Rochester, MN 55901.

These studies examined the proposal that serotonergic and noradrenergic neurons with axonal projections to the spinal cord are components of a neuronal system which modulates nociceptive threshold and mediates the antinociceptive actions of opiate drugs. This hypothesis was tested by examining the capacity of morphine to induce analgesia following the selective destruction of axon terminals in the caudal segments of the spinal cord which contain serotonin (5-HT) and norepinephrine (NE). Selective destruction of spinal monoamine terminals was achieved using specific neurotoxins injected directly into the spinal cord subarachnoid space near the lumbar enlargement. Thus, 5-HT, NE or both amines were depleted by injecting 5,6-dihydroxytryptamine (5,6-DHT), 6-hydroxydopamine (6-OHDA) and both neurotoxins, respectively. Both nociceptive threshold and morphine dose-response curves were determined between 7 and 21 days following intrathecal injection of neurotoxins. Spinal cord content of both 5-HT and NE determined 35 days after injections was found to be depressed to 10% of control values. The most prominent effect observed following depletion of spinal cord 5-HT was a pronounced, but transient, decrease in nociceptive threshold (hyperalgesia) which was evident on both the tail flick and hot plate tests. Depletion of spinal cord NE had identical effects. Although the spinal cord 5-HT and NE content remained depressed throughout the course of the study, the hyperalgesia exhibited recovery by 14 days. Despite alterations in nociceptive threshold following depletion of spinal cord 5-HT there was no alteration in the capacity of morphine to induce analgesia using either the tail flick or hot plate tests. The reduction of spinal cord NE produced a modest attenuation of morphine-induced analgesia. However, this effect was evident only when analgesia was assessed using the hot plate test, but not the tail flick test.

These studies support the following conclusions: (1) tonically active descending serotonergic and noradrenergic neurons regulate the transmission of nociceptive information at the level of the spinal cord; (2) descending serotonergic neurons do not appear to contribute measurably to the mediation of analgesia produced by the systemic administration of opiates; and (3) descending noradrenergic neurons appear to mediate some of the antinociceptive actions of opiates. (Supported by USPHS NS 12649 and NS 14629).

149.23 ANALGESIC TOLERANCE IS FACILITATED BY ANALGESIC TESTING. C. Advokat. Dept. Pharmacol., Univ. Ill. Med. Sch., Chicago, IL 60680.

Classical theories of opiate tolerance have emphasized the pharmacological mechanisms responsible for this phenomenon. Recent evidence, however, has shown that psychological, e.g. enrivonmental, variables play a significant role in tolerance development (Siegel, JCPP, 89:498-506, 1975). The present studies demonstrate a profound effect of the assessment environment on tolerance to opiate analgesia as measured by the spinal nociceptive tail flick reflex.

Naive, male albino rats were made tolerant by subcutaneous implantation of pellets containing 75 mg of morphine. In the first study, one group received morphine pellets while a second group received placebo implants. One half of the animals in each of these two groups was tested on the tail flick at 3, 24 and 48 hrs after the implant. At 3 hrs the Morphine group was significantly more analgesic than the Placebo group, however, by 48 hrs post implant these groups did not differ. At this time a 7.5 mg/kg subcutaneous injection of morphine produced significantly more analgesia in the Placebo group than in the Morphine group, further indicating that the Morphine group had become tolerant. In contrast, the remaining half of the Morphine group, which had not previously been tested, was still significantly more analgesic than its corresponding Placebo group at 48 hrs post implant. Furthermore, in response to the acute morphine injection these latter two groups were equally analgesic, indicating that the previously untested Morphine group was not tolerant.

In a second experiment, three groups of animals received morphine pellets. One group was tested 3, 6, 12, 24 and 48 hrs after the implant. A second group received only one test, at 48 hrs, in the same environment in which it had been housed and made tolerant. The third group also received only one test, at 48 hrs, but this occurred in a novel, unfamilar environment. These three groups differed significantly among themselves. The first group, which had received several successive tests, was significantly less analgesic than the other two groups, which received only a single test. Furthermore, these differences were maintained after an acute injection of 7.5 mg/kg of morphine.

These studies demonstrate profound modulation of analgesic tolerance to opiates by the context associated with the drug. The fact that such modulation can be obtained with a spinal reflex suggests that the mechanisms might be amenable to more detailed neuronal investigation. Such analyses could not only provide insight into processes underlying behavioral tolerance but to other forms of behavioral plasticity, e.g. learning, as well. (Supported by USPHS NS 12649).

149.24 EFFECTS OF INTRATHECALLY ADMINISTERED AMINE ANTAGONISTS ON NOCICEPTIVE THRESHOLD AND MORPHINE-INDUCED ANALGESIA. Donna L. Hammond and Herbert K. Proudfit, Mayo Fdn., Rochester, MN 55901 and Univ. of Ill. at the Med. Ctr., Chicago, IL 60612.

Indirect evidence has suggested that activation of serotonergic and/or noradrenergic bulbospinal pathways may mediate the production of analgesia by opiates. In addition, these projections may tonically inhibit responsiveness to noxious stimuli. Therefore, the present study examined the capacity of the amine antagonists, phentolamine (PTA) and methysergide (MSG) to decrease nociceptive threshold and attenuate morphine-induced analgesia when injected into the spinal cord subarachnoid space.

Male rats weighing 400-425 g were each implanted with an intrathecal catheter (PE-10) which extended 7.5 cm caudal from the atlanto-occipital junction. Seven days later, nociceptive threshold was measured using both the tail flick (TF) and hot plate (HP) tests. Each animal then received either 3.0 or 7.5 mg/kg morphine sulfate s.c.). Thirty minutes later the elevation of nociceptive threshold produced by morphine was assessed. Immediately following this assessment of analgesia, either 30 µg MSG maleate, 30 µg PTA HCl or saline was injected intrathecally in 15 µl of saline. Nociceptive threshold was then re-assessed 5, 15 and 30 min later. Comparison to the alterations in morphine-induced analgesia produced by intrathecally administered MSG or PTA with those produced by saline using a two-way ANOVA revealed that MSG failed to antagonize the analgesia induced by either 3.0 or 7.5 mg/kg morphine using either the TF or HP tests. In contrast, PTA produced a significant attenuation of the analgesia induced by 3.0 mg/kg morphine when assessed using the HP test. However, this antagonism was surmountable and was not evident following 7.5 mg/kg of morphine. No antagonism of the analgesia assessed using the TF test was produced by PTA. Both MSG and PTA decreased nociceptive threshold when injected intrathecally in separate groups of rats pretreated 30 min earlier with saline (s.c.).

The ability of either MSG or PTA to produce hyperalgesia when injected intrathecally supports the suggestion that bulbospinal aminergic neurons tonically inhibit the transmission of nociceptive information. However, these data do not support the participation of bulbospinal serotonergic projections in mediating morphine-induced analgesia. Some support is offered for the suggestion that noradrenergic projections to the spinal cord participate to some extent in mediating morphine-induced analgesia, although this may be dependent on the analgesiometric test used. Finally, these data agree with similar studies performed in rats with chronic depletion of spinal cord amine content.
(Supported by USPHS Grant NS 12649)

149.25 HYPOALGESIA INDUCED BY THE LOCAL INJECTION OF PHENTOLAMINE IN THE NUCLEUS RAPHE MAGNUS: REVERSAL BY THE INTRATHECAL INJECTION OF PHENTOLAMINE. J. Sagen* and H.K. Proudfit (SPON: E.G. Anderson). Dept. of Pharmacol., Univ. of Ill. Coll. Med., Chicago, IL 60680.

Numerous studies have demonstrated that activation of neurons in the n. raphe magnus (NRM) produces hypoalgesia. Noradrenergic (NA) projections to the NRM appear to be inhibitory since iontophoretically applied norepinephrine inhibits these neurons. In addition, blocking the NA input by the local injection of NA antagonists into the NRM produces hypoalgesia which may result from disinhibition of NRM neurons. These data suggest that brain stem NA systems function to enhance the sensitivity to noxious stimuli. However, bulbospinal NA systems appear to decrease pain sensitivity, since the injection of norepinephrine into the spinal cord subarachnoid space produces hypoalgesia (Yaksh et al., JPET, 208:466, 1979) and the NA antagonist phentolamine (PTA) produces hyperalgesia (Hammond and Proudfit, Neurosci. Abstr., 1980). Thus, NA systems in the brain stem appear to augment pain transmission while spinal NA system appear to have the opposite action.

The present studies provide evidence for an interaction between these two NA systems. More specifically, hypoalgesia induced by the local injection of PTA in the NRM can be antagonized by the injection of PTA into the spinal cord subarachnoid space. Male rats weighing 400-425 g were chronically implanted with both a guide sheath aimed at the NRM and an intrathecal catheter (PE-10 tubing) which extended to the lumbar spinal segments. Following recovery from surgery, nociceptive threshold was assessed using the tail flick test, and PTA (10 µg in 0.5 µl of saline, pH 6.9) was microinjected into the NRM through an injection cannula inserted through the guide sheath. Animals exhibiting significant hypoalgesia by 20 min received either an intrathecal injection of PTA (30 µg in 15 µl of saline and flushed with 10 µl of saline, pH 6.9) or saline (25 µl, pH 6.9) or a subcutaneous injection of naloxone (0.4 mg/kg). Nociceptive threshold was again determined at 5, 15 and 30 min after the intrathecal injection. Intrathecal injection of PTA, but not saline reversed the hypoalgesia induced by PTA injection into the NRM. Furthermore, this hypoalgesia was not altered by systemically-administered naloxone.

These data suggest that disinhibition of raphe-spinal NRM neurons by PTA induces hypoalgesia which is mediated, at least in part, by subsequent activation of spinally-projecting NA neurons. The failure of naloxone to alter the hypoalgesia produced by microinjection of PTA in the NRM does not support the participation of enkephalinergic neurons in this action. (Supported by USPHS Grant NS 12649).

149.26 A NEW QUANTITATIVE TEST FOR ANALGESIA IN RATS. P. Warren*. (SPON: C. Kellogg). Reflex Modulation Laboratory, University of Rochester, Rochester, New York 14627.

A new quantitative test for analgesia based on the startle response is described. Brief noxious shocks (approx. 1.0 mA, 20 msec.) and tones (110 dB, 10 KHZ, 20 msec.) reliably produce startle responses in rats. In the present experiment morphine (10 mg/kg, ip.) significantly reduced the amplitude of the startle response to shock, but had no significant effect upon the response to tone. This morphine induced inhibition of the response to shock was antagonized significantly by naloxone (10 mg/kg, ip.).

When the shock stimulus is presented 1 sec. before the tone, the response to the tone is reduced. This inhibition of the response to tone was not affected significantly by morphine or naloxone. When tone precedes shock by 1 sec., the amplitude of the response to shock is unaffected. Neither morphine nor naloxone affected this apparent lack of inhibition of tone upon the response to subsequent shock.

149.27 ELECTROCONVULSIVE SHOCK-INDUCED ANALGESIA: ANTAGONISM BY NALOXONE AND EFFECTS OF HYPOPHYSECTOMY. E. H. Chudler,* J. W. Lewis, J. T. Cannon, & J. C. Liebeskind. Dept. Psychol., UCLA, Los Angeles, CA 90024.

A variety of experimental manipulations have been shown to reduce reactivity to painful stimuli, presumably through activation of one or more endogenous pain-inhibitory mechanisms. Holaday and colleagues reported an array of opiate-like effects following electroconvulsive shock (ECS), including analgesia. Naloxone's antagonism of ECS-induced analgesia suggested the involvement of endorphins (Holaday et al., 1978). The source of those endorphins responsible for ECS-induced analgesia is unknown. That pituitary endorphins are involved in stress-induced analgesia is suggested by findings that hypophysectomy reduces this form of analgesia (Amir & Amit, 1979; Bodnar et al., 1978). Therefore, in this study we sought to determine whether ECS-induced analgesia was similarly affected by hypophysectomy.

Male albino rats subjected to hypophysectomy (n=12), sham surgery (n=12), or no surgery (n=12) were used. Each group was divided into saline or naloxone pretreated subgroups. Animals were tested on the hot-plate (51.5°C) for baseline paw-lick latencies, then injected with either naloxone (3 mg/kg) or saline. Rats were again tested 13.5 min later on the hot-plate to assess drug effects. Finally, all subjects received transauricular ECS delivered through stainless steel clips (140 volts, 60 Hz, 2 sec) and were retested on the hot-plate at 6 min intervals for up to 30 min.

ECS significantly increased paw-lick latencies in all groups. In sham and unoperated controls, this effect was markedly diminished by naloxone pretreatment ($p<.05$). In hypophysectomized rats, the analgesic response was considerably longer in duration. Naloxone, appeared to reduce the magnitude of this analgesic effect in some hypophysectomized rats, although the effect was not statistically reliable. Naloxone itself had no effect on paw-lick latencies.

Our findings serve to reinforce the importance of the opioid peptides in ECS-induced analgesia. That endorphins of CNS origin may be responsible for these effects was suggested by the potent analgesic response observed in the hypophysectomized rats. The failure of naloxone to alter reliably the analgesic response in these animals, however, makes it seem likely that another, non-opioid mechanism was called into play. These findings do not preclude the possibility that pituitary endorphins serve an important role in ECS-induced analgesia in intact rats. (Supported by NIH grant NS07628. JWL was supported by MHTP grant NH15345)

149.28 SPINAL ANALGESIA AND HYPERALGESIA IN THE MOUSE. Janice L. K. Hylden , Cathy J. Cleary and George L. Wilcox. Dept. of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

The spinal injection technique recently introduced by Hylden and Wilcox (Fed. Proc. 1980) was used to investigate the analgesic or hyperalgesic actions of various substances in the spinal cord of the mouse. A small incision is made in the skin of the back and a 30 ga. needle is inserted between two caudal lumbar vertebrae. The material is injected in a volume of 5 µl. Calibration experiments were performed by monitoring subdural distribution of methylene blue dye or [^{3}H]-morphine base. Not more than 5% of the injected material reached supraspinal structures. We observed tail flick and hot plate latencies, straub tail, and the occurrence of a characteristic behavioral response (after substance P and capsaicin). The following drugs were administered: morphine sulfate (MS), clonidine HCl (CLON), substance P (SP) and capsaicin (CAP).

MS was found to produce dose related, spinally mediated straub tail (ED50 0.5 µg, 0.25-1.0) and tail flick analgesia (ED50 0.85 µg, 0.35-2.0). Naloxone (2 mg/kg, s.c.) reversed these effects. CLON was 40 times as potent as MS in the tail flick assay (ED50 0.02 µg). SP elicited a characteristic biting and scratching response. The incidence, intensity and duration of this response were dose related (ED50 40 ng). Spinal MS did not inhibit this reaction (up to 20 µg). Thirty minutes following SP at 0.5 µg, mice showed tail flick analgesia. CAP evoked a biting and scratching response similar to that of substance P but of longer duration (2-3 min). These mice subsequently showed prolonged tail flick analgesia (>2 wks).

MS and CLON may exert an important part of their analgesic action at the spinal level. We believe our observations of SP's actions to be the first behavioral data consistent with the hypothesis that SP acts as a nociceptive neurotransmitter. That spinal MS does not inhibit the action of exogenous SP supports the contention that MS is not an antagonist for the SP receptor but exerts its antinociceptive action presynaptically. That analgesia follows SP administration by 30 minutes indicates that SP activates endogenous antinociceptive systems. The role of CAP as a SP releaser and depletor is supported by our observation of the biting and scratching response and subsequent analgesia. (Supported by NIDA Grant DA 01933 and U.S.P.H.S. Grant DA 00289.)

150.1 SOMATOTOPY OF PRESYNAPTIC CUTANEOUS NEUROPIL IN CAT DORSAL HORN. P. B. Brown and H. R. Koerber, Dept. of Physiology, West Virginia University Medical Center, Morgantown, WV 26506.

The projections of hindlimb cutaneous nerves to cat dorsal horn in segments L_2-Ca_1 were examined using transganglionic transport of horseradish peroxidase. Dorsal view maps were used to analyze the locations of terminal arbors in laminae III-IV. Nerves studied included three branches of the posterior femoral cutaneous nerve, medial and lateral branches of the sural nerve, the saphenous nerve, medial and lateral branches of the plantar nerve, and the lateral femoral cutaneous nerve. In order to compare the innervation and projection fields of pairs of nerves, two different nerves were loaded on opposite sides in the same cat.

Proximal preaxial nerves projected to segments rostral to L_6 and L_7, usually with a narrow band of terminals in lateral L_6 and L_7, skirting the representation of foot and toes which takes up the medial two thirds of laminae III-IV in these segments. Proximal postaxial nerves projected to segments caudal to L_6 and L_7, usually with a narrow band in lateral laminae III-IV in these segments. Nerves with both preaxial and postaxial components projected both rostral and caudal to L_6 and L_7. No somatotopically inappropriate projections were seen.

The degree of overlap or separation of projection fields of pairs of nerves was correlated with the degree of overlap or separation of their innervation fields on the leg, within 1-3 cm proximodistally and 2-4 cm circumferentially. The greater overlap circumferentially is consistent with the fact that within a given segment there are many dorsal horn cells with receptive fields overlapping both preaxial and postaxial skin. Thus, although there is a high degree of resolution in the organization of the terminal neuropil along the proximodistal skin axis at a given anteroposterior level, there is less resolution around the circumference of the leg. On the other hand, nerves with different circumferential locations of their innervation fields appear to project to different anteroposterior positions in the dorsal horn, as a consequence of the anteroposterior axis reflecting the dermatomal trajectory.

These results are consistent with the concept that the cells in laminae III-IV assemble their receptive fields largely by direct contact between their dendrites and cutaneous axon terminals, and that their receptive field locations, shapes and sizes are determined largely by the portions of the somatotopically organized presynaptic neuropil penetrated by their dendrites.

150.2 INHIBITORY AND WIDESPREAD CONVERGENCE OF SOMATIC AND VISCERAL INPUTS ONTO THORACIC SPINAL NEURONS IN THE CAT. Roger Thies and Robert D. Foreman, Dept. of Physiology and Biophysics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

We have studied with extracellular microelectrodes the discharges of single neurons in the T_2 and T_3 segments of the left spinal cord in chloralose-anesthetized cats. Of 33 neurons studied, 16 were identified as projecting to higher centers by antidromic activation from the ventral lateral quadrant at the spino-medullary junction, and eight were identified as spinoreticular by antidromic activation from the nucleus gigantocellularis. Of the nine remaining cells, four were excited and one was inhibited orthodromically by such stimuli. For visceral stimuli electrical shocks were applied to left or right sympathetic chains (at the stellate ganglion), to vagal branches from the heart, and to the central cut ends of cervical vagi. Twenty-four neurons had excitatory receptive fields in response to pinching skin or skin and muscle of the left upper foreleg and flank, similarly to spinothalamic neurons.

Eight of the nine neurons of interest here were inhibited by somatic stimuli; the ninth was excited by stimuli to all four limbs. Four of these were antidromically activated from the ipsilateral brain stem, and none were activated contralaterally. Their somatic fields showed inhibition in response to light touch or joint bending or firm pressure or pinching of one or more limbs, with contrasting excitatory fields from other limbs. Stimulation of left or right sympathetic chains inhibited two neurons, excited four, had no effect on four, and for one neuron excited from the left and inhibited from the right. These effects correlated with somatic stimulation overlying the visceral input. In addition, vagal stimuli had no effect on six neurons but inhibited three, a descending effect that we have reported previously (Thies & Foreman, Fed. Proc. 39:840, 1980).

These results show that some spinoreticular neurons in the upper thoracic cord have input from much of the body surface and viscera. This preprocessing of widespread information by some relay neurons may give higher centers an integrated view of sensory input, in comparison to the majority of neurons with restricted fields.

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150.3 ULTRASTRUCTURE OF DORSAL ROOT AFFERENTS TO THE VENTRAL HORN IN THE CAT. M.S. Beattie, J.C. Bresnahan and F. Liuzzi*. Depts. of Surg. and Anat., The Ohio State University, Coll. Med., Columbus, Ohio, 43210.

Details of the terminal patterns and synaptic organization of primary afferent input to laminae VII-IX have been difficult to obtain with degeneration methods. The ultrastructure of these terminations has only recently been described in the monkey using electron microscopic autoradiography (Ralston and Ralston, '79). We have employed the anterograde transport of horseradish peroxidase (HRP) after application to the central ends of cut dorsal rootlets to provide a more complete picture of the light and electron microscopic morphology of this system in the cat lumbosacral cord. The distribution of primary afferent axons to the ventral horn appears to be restricted to the region close to the entry zone. A medial projection travels through the intermediomedial zone and descends along the medial border of lamina VIII arborizing in the medial motor nuclei. In mid-lumbosacral enlargement where lateral motor nuclei are present, an additional but more diffuse bundle of axons descends through mid-lamina VII and arborizes among the cells in lateral lamina IX. Light microscopic observations suggest that synaptic terminations are most numerous around the cell bodies in lamina IX, but are also present in laminae VII and VIII.

Electron microscopic observations confirm the presence of labeled synaptic terminals on cell bodies in lamina IX. Some of these terminals are associated with subsurface cisternae. Contacts with dendrites and dendritic spines are also present. All terminals have round and oval vesicles. A prominent feature is the presence of axo-axonic contacts between primary afferents and axon terminals containing pleomorphic clear vesicles. (Supported by N.I.H. Grants NS-10165 and NS-14457).

150.4 THE SYNAPTIC ORGANIZATION OF THE DEEP DORSAL HORN OF THE MACAQUE MONKEY. H.J. Ralston III and D.D. Ralston*. Dept. of Anatomy, Univ. of California, School of Medicine, San Francisco, CA 94143.

A quantitative electron microscopic study of the deep dorsal horn (Rexed's laminae IV, V and VI) has been carried out in normal animals and in those having dorsal root afferents labeled by degeneration following dorsal rhizotomy, or by autoradiography following injection of H^3 leucine into dorsal root ganglia. In all three lamina, synaptic profiles with round vesicles (R) constituted from 30 to 40% of the total synaptic population; profiles with pleomorphic and flat vesicles (F) made up about 60% of all synapses; central profiles (C), which are large dilatations along the course of certain axons, and which contain round vesicles and make multiple contacts with postsynaptic structures, comprise from 2 to 6% of all synapses. Profiles with large granular vesicles (LGV's) are uncommon in all 3 laminae. The vast majority of synaptic contacts are axon-dendritic; axosomatic synapses are about 1 to 2% of the total (most being formed by F profiles); and 1 to 2% are axoaxonal, mostly being F to C contacts. Presynaptic dendrites appear to be uncommon in the dorsal horn.

18 hours after dorsal rhizotomy, early degenerative changes occur in many C terminals and a few of the R's. These early changes are principally the accumulation of filaments and a clumping of synaptic vesicles. Within the next few days post-rhizotomy, most of the C's and about 10% of the R's undergo degeneration. In addition ot the filamentous degeneration found at 18 hours, many C and R profiles exhibit lucent alterations, with a loss of vesicles and other organelles, and no filamentous changes. Such lucent alterations are particularly evident in V and VI. By 5 days post-rhizotomy, many of the degenerating C's have been cleared from IV and V, but are still common in VI. Degenerating R's remain in all laminae through the first week. The majority of axoaxonal contacts present are upon degenerating synapses. Autoradiography results in the labeling of most C's and some R profiles, as well as numerous axons.

It is concluded that the primary afferent axons make up most of the C's and 10-15% of the R's. The different types of synaptic degeneration suggests different classes of afferent fibers. Most of the axoaxonal contacts are upon dorsal root axons. (Supported by NS-11614 from NIH.)

150.5 STRUCTURAL DIVERSITY OF PRIMATE MARGINAL (LAMINA I) NEURONS IN THE LUMBOSACRAL SPINAL CORD: A GOLGI STUDY. J.A. Beal, Department of Anatomy, Louisiana State Univ. Med. Ctr., Shreveport, LA 71130

To facilitate their examination, marginal neurons from adult Macaque monkies were divided into four groups on the basis of major structural differences in conformation, distribution and specialization of dendritic arbors. Group I consists of neurons with large diameter dendritic trunks which taper gradually and have few spines. This is a heterogeneous group of neurons of varying shapes which can be further subdivided into several subgroups on the basis of cell size (large-small) and other structural features. Group II is composed of large to medium size neurons and characterized by spiny dendrites. The cells are primarily elongated along the longitudinal axis and have a fusiform or pyramidal shape. Variations in dendritic conformation and distribution of spines here generates several subgroupings. Group III is composed of small to medium size neurons which have slender aspiny dendrites. Group III is composed of 2 subgroups, one characterized by dendrites confined to lamina I and the other characterized by tortuous interstitial dendritic branches which ramify in Lissauer's fasciculus and the lateral funiculus. Group IV consists of small spiny neurons which are fusiform to pyramidal in shape. These cells give rise to delicate, longitudinal spiny dendrites and are prevalent in the deeper regions of lamina I.

In summary, the present study demonstrates that the marginal neurons in the lumbosacral cord of the primate exhibit greater morphological variation than has been previously reported. There is enough structural variation within the subgroups to predict several functionally distinct cell types. Studies involving intracellular staining and recording of marginal neurons will have to consider and account for these structural variations.

150.6 EFFECTS OF TEMPERATURE ON RESPONSES OF SPINOCERVICAL UNITS TO LOW-INTENSITY CUTANEOUS STIMULI. G. W. King, T. J. Ebner, J. R. Bloedel. Depts. of Neurosurg. and Physiol., Univ. of Minnesota, Minneapolis, MN 55455.

The effects of thermal stimuli on the encoding of cutaneous information by spinocervical cells were studied in cats anesthetized with alpha chloralose. Forty-three units in the lower lumbar enlargement were identified as spinocervical neurons by demonstrating that they were antidromically activated from the ipsilateral lower cervical cord but not from the cervical cord rostral to the C-1 roots. A probe was placed on the most sensitive part of the cutaneous receptive field to produce small sinusoidal deflections of fur and/or toes at frequencies of 0.5-50 Hz. and amplitudes up to 0.5 mm. Cycle histograms characterizing the responses were compared with and without the application of radiant heat (skin temperature 35 to 60°C.) on and off the receptive field. Units typically responded with short bursts near the peak of the sinusoidal stimulus and usually followed inputs up to 15 Hz. The responses were initiated at a precise phase of the input at each input cycle. Heating of the skin within the receptive field usually produced an increase in the background noise (band pass equal to 50 Hz.) and the occurrence of a first order harmonic or doublet at low input frequencies even in the absence of a change in the fundamental response. Heating of skin areas outside the receptive field usually reduced the amplitude of the response with a decrease in the background noise and an increase in the degree of phase locking. Transient rebound excitation lasting 2-5 min. appeared when the heat was discontinued. The effect of heating was dependent upon input frequency. At stimulus frequencies of 10 Hz. or higher, heating resulted in a greater modulation of unitary activity with an associated increase in the signal-to-noise ratio. At input frequencies below 7.5 Hz., heating generally increased the background noise and decreased the signal-to-noise ratio. These experiments show that high intensity thermal stimuli produce specific changes in the way spinocervical neurons encode low intensity peripheral stimuli. Selective effects on the first harmonic of the response were noted independent of changes in the amplitude of the fundamental. Furthermore, heating increased the upper range of frequencies at which the spinocervical neurons could be modulated while decreasing the signal-to-noise ratio at lower input frequencies. This suggests that the frequency response characteristics of the spinocervical system shifts when noxious thermal stimuli are applied to the extremity. Supported by NIH Grant No. 5 RO1-NS 13002.

150.7 LOCALIZATION OF THE LAST ORDER INTERNEURON IN THE PATHWAY PRODUCING PRESYNAPTIC DEPOLARIZATION OF Ia FIBER TERMINALS IN THE LUMBO-SACRAL SPINAL CORD OF THE CAT. P. Rudomin, S. Dueñas* I.Jiménez*and E. Jankowska* Department of Physiol. & Biophys., Centro de Investigación del IPN, México and Department of Physiol. Goteborg University, Sweden.

The relatively long latency (2.5 to 4 msec) of the primary afferent depolarization (PAD) has indicated that polysynaptic pathways are involved in its generation. In addition, studies on the activation patterns of spinal interneurons suggest that those mediating the PAD of group I fibers may be located in the intermediate nucleus (Eccles et al, J. Physiol. 1962, 161:237). To have more information on the presumed location of the PAD-mediating interneurons we studied the effects of intra-spinal (IS) stimulation on the excitability of individual Ia fibers ending either in the intermediate or in the motor nucleus. In the cat anesthetized with pentobarbital we observed that 1) The excitability of group Ia fiber terminals was increased by IS stimulation. The lowest threshold regions (<2μA) producing the shortest latency effects (0.6 - 0.8 msec) were located in the region where group I fibers terminate, that is, within the intermediate and motor nuclei. 2) The excitability increase produced by IS stimulation could be obtained with intensities below the threshold of the afferent fiber whose excitability was being measured. 3) IS stimulation failed to increase the excitability of rubro-spinal and vestibulo-spinal fibers ending close to group I fibers in the intermediate and motor nuclei, respectively. 4) Stimulation of cutaneous afferents reduced the PAD of group Ia fibers produced by group I flexor muscle afferents, but did not affect the excitability increase produced by IS stimulation. 5) The excitability increase of group I fiber terminals produced either by sensory nerve or by IS conditioning stimulation was depressed (but not abolished) by picrotoxin or bicuculline. These results suggest that: 1) IS stimulation activates neuronal elements, presumably interneurons, which are able to depolarize group I fiber terminals but not nearby descending fibers. 2) This rather specific action results, at least in part, from direct activation of the last interneuron in the PAD pathway, located close to the terminals of the target afferent fibers. 3) The inhibition that cutaneous volleys produce on the PAD of group Ia afferent fibers is probably not exerted on the last interneuron in the PAD pathway. This suggests that the shortest possible pathway for the PAD of Ia fibers is trisynaptic.

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150.8 ALPHA-CHLORALOSE SUPPRESSION OF SINGLE NEURON ACTIVITY IN THE DORSAL HORN OF THE SPINAL CORD. J.G. Collins, E. Homma* and L.M. Kitahata. Dept. of Anesthesiology, Yale University School of Medicine, New Haven, Ct. 06510.

It has recently been reported that the presence of an anesthetic agent can profoundly alter the functioning of neurons within the central nervous system (Collins and Roppolo 1980)[1]. Alpha-chloralose is widely used as an anesthetic agent in neurophysiological research, often because of the belief that it causes minimal suppression of neuronal activity. This study was undertaken in order to determine what effect an anesthetic dose of alpha-chloralose would have upon activity of single cells in the dorsal horn of the spinal cord in the absence of descending supraspinal influences.

We examined the effects of recrystallized alpha-chloralose on activity of neurons in the dorsal horn of decerebrate, spinal cord transected (L-1) cats which were paralyzed with gallamine triethiodide. The animals (2.2-4.8 kg) had been surgically prepared (tracheal, carotid artery and jugular vein cannulation and lumbar laminectomy, L-7, L-4) under halothane, nitrous oxide-oxygen anesthesia, but at least 3 hours passed between the end of anesthetic administration and the start of drug studies. Physiological parameters were monitored and maintained within normal limits. Tungsten microelectrodes were used to record extracellular single unit activity from low threshold, wide dynamic range, and proprioceptive neurons in the dorsal horn at the L-5, L-6 level. Light brushing was used to activate low threshold neurons. Noxious radiant heat (51°C) was used to activate wide dynamic range neurons. Spontaneous activity of proprioceptive neurons was recorded, in addition to the spontaneous activity of low threshold or wide dynamic range neurons. We report that a low anesthetic dose of alpha-chloralose (50 mg/kg I.V., dissolved in propylene glycol), caused a profound reduction, although not a complete elimination, of activity in neurons recorded from the dorsal horn of the spinal cord. Transection of the spinal cord eliminated the possibility that the effect was mediated by supraspinal mechanisms. Although extrapolation from this preparation to all parts of the central nervous system is not possible, the results of this study, in combination with recent reports (e.g. Haimann et al. 1978)[2] strongly suggest that the presence of alpha-chloralose in a neurophysiological preparation may cause significant changes in neuronal activity and thus influence the possible interpretation of the resultant data. [1] Collins, J.G. and Roppolo, J.R., J. Pharmacol. Exp. Ther. 213:337-345, 1980. [2] Haimann, C., Magnelli, M. and Sotgiu, M.L.: Exp. Neurol. 60:469-484, 1978. (Supported by NIH Grant NS-09871)

150.9 SEGMENTAL- AND BRAINSTEM-EVOKED DORSAL ROOT POTENTIALS (DRP) SELECTIVELY AFFECTS SPONTANEOUS ANTIDROMIC ACTIVITY. Barry D. Goldstein and Edmund G. Anderson. Dept. of Pharmacology, Univ. of Ill. Med. Ctr., Chicago, IL 60612.

Spontaneous antidromic action potentials observed in afferent nerves, called the dorsal root discharge (DRD), appears to be a normally occurring phenomenon associated with primary afferent depolarization (PAD), but is pharmacologically distinct from the process involved in generating evoked-DRPs (Repkin et al., Brain Res. 117:147, 1976; Kammerling and Anderson, Neurosci. Abst. 3: 502, 1977). Seventy percent of the afferent fibers exhibiting DRD have conduction velocities (CV) corresponding to group II fibers (40-72 m/sec), while the remaining 30% have group I conduction velocities (72-120 m/sec). Examination of multiple unit recordings have shown that synchrony of the DRD occurs during the segmentally-evoked DRPs (seg-DRP). Since seg-DRPs occur in group I fibers and brainstem-evoked DRPs (bs-DRP) occur in group II fibers (see Schmidt, Ergeb. de Physiol. 63:20, 1971), it was important to determine whether the DRD in afferents identified by conduction velocity was selectively influenced by stimulation at these two sites.

Dorsal laminectomies were performed on unanesthetized spinal and decerebrate cats. L7 and S1 dorsal roots were isolated and cut distally. Single unit DRD activity was obtained by carefully splitting the dorsal roots into filaments. The conduction velocities of fibers displaying DRD were determined from the latency between two pairs of recording electrodes. Once the unit was identified, group I threshold seg-DRPs were evoked via stimulation of the S1 dorsal root and bs-DRPs were evoked using an electrode stereotaxically placed in the brainstem.

In eight spinal cats, 30 single units were obtained with spontaneous DRD. Twenty-four units had CVs in the group II range and six had CVs in the group I range. Of the 24 group II fibers, only 17% discharged in synchrony with the seg-DRP as shown by post-stimulus time histograms. However, 67% of the group I fibers responded in synchrony with the seg-DRP. In five anemically decerebrate cats, 20 single units with spontaneous DRD were observed with 14 units having CVs in the group II range and six in the group I range. In these animals, both bs- and seg-DRPs were evoked. Seventy percent of the group II fibers responded in synchrony to the bs-DRP while none of the group I fibers responded. Conversely, 29% of the group II fibers and 100% of the group I fibers fired in synchrony to the seg-DRP.

These data show that PAD in group I fibers selectively affects group I DRD while PAD in group II fibers selectively affects group II DRD with modest cross-activation in the other fiber groups. (Supported by PHS NS-14985).

150.10 THE FUNCTIONAL ANATOMY OF THE COERULEO-SPINAL PATHWAY. R. T. Stevens†, C. J. Hodge, A. V. Apkarian†, G. D. Vogelsang†, H. J. Wisnicki* and O. Brown* (SPON: S. G. Nord) Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

Retrograde and antegrade transport studies have demonstrated projections, which are primarily ipsilateral, from the noradrenaline (NA) containing cells of the locus coeruleus (LC) to the spinal cord. Essentially all spinal catecholamines are contained in the terminals of the coeruleo-spinal and other bulbospinal pathways. Our previous studies have shown that the modulating effects of LC on spinal sensory units is functionally bilateral. This study was done to correlate anatomic and physiologic data demonstrating the location of the functional crossover of coeruleo-spinal and descending noradrenergic pathways.

Anatomic determination of the location and density of noradrenaline (NA) containing terminals in the dorsal horn was done using a glyoxylic acid histofluorescence method and confirmed by quantitative determinations using high pressure liquid chromatography. For these studies, various spinal cord lesions were made two weeks prior to the sacrifice. Physiologic studies entailed the determination of the effects of ipsilateral and contralateral stimulation of LC on lumbar dorsal horn unit responses to skin stimulation. For the physiologic experiments various acute spinal cord lesions were made. The cord lesions for the physiologic and anatomic studies were identical.

Midthoracic cord section resulted in complete loss of dorsal horn terminal fluorescence below the level of the lesion and abolished any effects that LC stimulation had on dorsal horn cell responses. After low thoracic hemisection significant amounts of equally distributed catecholamine (CA) fluorescence were found below the level of the lesion on both sides of the cord. Low thoracic hemisection had no effect on the inhibition of dorsal horn cell responses caused by either ipsilateral or contralateral LC stimulation. The addition of a midline sagittal section to the hemisection caused loss of CA fluorescent terminals only ipsilateral to the hemisection along the rostro-caudal extent of the sagittal section. Similarly only those cells in dorsal horn ipsilateral to the hemisection and within the rostro-caudal limits of the sagittal section were uneffected by LC stimulation.

These results suggest that the descending noradrenergic coeruleo-spinal pathway gains access to both sides of the spinal cord via both suprasegmental and local segmental crossover.

150.11 **NEURAL ORGANIZATION OF SACRAL SPINAL CORD IN CATS.** **B. Dubrovsky and P. Pacheco*. Neurophysiology Laboratory, Allan Memorial Institute, McGill University, Montreal, P.Q.**

The caudal end of the spinal cord; 2nd, 3rd sacral and caudal segments, is involved in reflex control of midline structures, notorious among which, in many quadrupedal mammals is the tail. Intactness of sensory and motor nerve fibers in the pudendal nerve (2nd and 3rd sacral divisions) is a necessary condition for maintenance of the tonic activity observed in the EMG of the external sphincter muscle of both anum and urethra. The caudal end of the spinal cord also mediates neural activity associated with sexual behavior. Further, tail and neck movements are integrated to various extents with the main girdes of the back of different species and have a balancing or cantilever function. In our studies the spinal cord was sectioned between the 1st and 2nd cervical level while the animals were under ether anesthesia. Monosynaptic connections described by Lloyd and Wilson (J. Gen. Physiol. 42, 1219) with direct dorsal root electrical stimulation in S3 were obtained only by stimulation of nerve trunks innervating tail musculature. Pudendal nerve stimulation as well as of the nerve of the levator ani muscle, only elicited polysynaptic reflexes recorded from proximal sections of S2 and S3 ventral roots. Neither with maximal stimulation, monitored by recording afferent volleys from corresponding dorsal roots, nor with tetanic priming stimulation was it possible to observe monosynaptic responses. The absence of monosynaptic responses to pudendal nerve stimulation suggests that spindle receptor organs in both external ani and urethral sphincter muscles subserve only the tonic component of the stretch reflex. Analogous phenomena, the existence of the tonic, but not phasic component of the stretch reflex was also noted by us in extraocular muscles of the cat (Barbas and Dubrovsky, Neurosc. Absts. 5, pp. 689).

The amplitude of polysynaptic potentials recorded in L7 and S1 ventral roots to maximal electrical sural nerve stimulation were significantly affected by cervix and perivaginal skin stimulation. The head piece of an otoscope was positioned in the vaginal cavity. Through the openings of the piece a glass rod could be directly applied to the cervix thus eliminating spurious sources (vaginal, cutaneous) of activation. Pressure on the cervix decreased ventral root potentials by 25-30% of control values. This effect could not be maintained, however, throughout the duration of the stimulation. After 4-5 sec. potentials returned to control levels. In contrast, perivaginal skin tapping decreased the amplitude of ventral root potentials by as much as 90% of the control amplitude. Moreover, this effect outlasted the effect of the tapping by as much as 5-10 sec.
P.P. is supported by the MRC, Ottawa.

150.12 THE STRUCTURAL ORGANIZATION OF SPLANCHNIC NEURONS IN POSTERIOR SPINAL SENSORY GANGLIA OF THE CAT STUDIED BY A MODIFIED HORSERADISH PEROXIDASE (HRP) REACTION USING THE CRYOSTAT. David C. Kuo*, George M. Krauthamer and Dwayne S. Yamasaki*. Anatomy Dept., Rutgers Medical School, Piscataway, N.J. 08854

The organization of visceral sensory neurons in the posterior spinal ganglion has not been investigated. This organization is elucidated in the present study by HRP technique. Retrograde transport of HRP from the central cut end of the major splanchnic nerve was used to label splanchnic sensory neurons in thoracic spinal ganglia. In addition, the conventional HRP reaction was modified using the cryostat to ensure proper orientation and sequence of tissue sections.

With the animal lying on its right side, a left laparotomy was performed. The left major splanchnic nerve was identified and dissected free from surrounding tissue. The nerve was cut. A small sheet of plastic was placed under the central nerve stump, which was exposed to HRP crystals for 60 min. Animals survived for two to five days.

In our initial experiments, left posterior spinal ganglia were cut into 50-75 μm sections using the freezing microtome. Sections were reacted free floating with tetramethyl benziding (TMB). Round to oval HRP labeled neurons of splanchnic origin were identified in spinal ganglia from T3 to T13 with the majority of cells concentrated between T5 and T11. In other experiments, in order to ensure proper orientation and sequence of tissue sections, the HRP reaction was modified using the cryostat. Serial cryostat sections (20-30 μm) cut at -20°C were plated immediately onto glass slides pretreated with chrome-alum. Sections were heat dried at 65°C for 45 to 60 min. and then incubated with TMB. Sensory ganglia were reconstructed by photomicroscopy and camera lucida drawings. Useful data for this study came from eight animals.

Our results showed that HRP labeled visceral neurons were distributed throughout the entire ganglion. Labeled cells measured between 20 and 80 μm. Clustering of visceral cell bodies was seen but there was no discernible pattern of localization. Visceral sensory organization is thus similar to somatic representations within posterior spinal ganglia. We speculate that cell clusters of visceral origin may represent functional segregation or, perhaps, specific innervation of individual viscera.
(Supported by NIH Grant NS 10922.)

150.13 ACCUMULATION OF TOTAL CALCIUM IN EXPERIMENTAL SPINAL CORD TRAUMA R. D. Happel*, K. P. Smith*, J. M. Powers*, J. D. Balentine*, N. L. Banik and E. L. Hogan, J. Byrd*. Depts. of Neurology and Div. of Neuropathology, Med. Univ. of South Carolina, Charleston, S.C. 29403.

An increase in tissue calcium may be involved in the pathogenesis of experimental spinal cord trauma. Recent EM studies demonstrated intracellular Ca^{2+} deposits in the lesion shortly after injury. (Balentine and Spector, Ann. Neurol:2,520, 1977). In order to examine further the cellular effects of trauma, we have quantitatively measured the time course of Ca^{2+} accumulation in the traumatic lesion. Spinal cord trauma was produced in rats by dropping a 10 gm weight from 30 cm on exposed dura-invested spinal cord. Lumbar sections of traumatized spinal cord and internal controls from non-traumatized cervical cord were excised and analyzed for Ca^{2+}. Spinal cord sections (9mm length) were lyophilized to dry weight and 50 ± 2mg dry weight tissue was digested in 0.5 ml HNO_3 (8 hrs. at 37°C). Following appropriate dilution with 1% $LaCl_3$ in 0.1 N HCl, the samples were centrifuged (15,000 xg, 10 min.) and total Ca^{2+} was determined in the supernatant using atomic absorption apectroscopy. Ca^{2+}-levels in the lesioned area were significantly elevated over control values within 45 minutes post-trauma ($p < .005$), with maximal increase at 8 hrs. (1147 ± 7 µg Ca^{2+}/gm dry wt. tissue). This value which represents a 4.1 fold increase in Ca^{2+} showed no evidence of decreasing after 72 hours post-trauma. Using Paired-t analysis the mean deviation between cervical control and lumbar lesion Ca^{2+} levels was 465.5± 120.7 (df=8) µg Ca^{2+}/gm dry weight tissue. In the same animal model, Banik et al. (abs. Soc. Neurosci. 9:396, 1979) found an increased proteinase activity which was inhibited by EDTA. The increase of tissue calcium in the lesion suggests that Ca^{2+} activation of neutral proteinase may be a mediator of the traumatic cell necrosis. (Supported by NIH-NINCDS Grant No. NS 11066).

150.14 CALIBRATION OF SPIKE AMPLITUDE PROFILES OF CELLS IN CAT SPINAL CORD LAMINAE I-VI: A PRELIMINARY TO EXTRACELLULAR CHARACTERIZATION OF CELL TYPES. H. R. Koerber and P. B. Brown (SPON: R. Millecchia), Dept. of Physiology, West Virginia University Medical Center, Morgantown, WV 26506.

The correlation of physiological properties of neurons with their anatomical properties, such as cell location, size, and dendritic geometry, is best accomplished by a combination of extracellular recording to characterize the cell's response properties and intracellular penetration to inject a dye to visualize the cell after histological processing. Unfortunately the yield is low using such a technique. For studies which require recording from large numbers of cells in the same animal, such as dorsal horn mapping experiments in which the somatotopy or right and left dorsal horn must be compared after unilateral partial deafferentation, extracellular recording is the method of choice, provided that some statements can be made about such anatomical attributes of the cells as their laminar, anteroposterior, and mediolateral locations, and cell size.

A beginning toward these objectives has been realized using a "smart electrode" program on the LM-SQUARE computer to control the stepping microdrive carrying the microelectrode, and an action potential wave form detector to follow the transformations of a single unit's wave form as a function of electrode depth. The smart electrode system can follow such transformations, even in the presence of other wave forms which are also changing with electrode position. A plot of the peak-to-peak amplitude of a unit's spike wave form, the amplitude profile, is used to determine the depth at which the recorded unit lies, the distance of the unit from the electrode track, and the relative size of the element generating the spike.

Calibration experiments reveal a close agreement of the depth of the recorded cell (indicated by a small electrolytic lesion at the depth of maximum amplitude) in a number of different tracks at different distances and in different directions from the unit. In addition, the distance of the cell from the electrode track has been demonstrated to be related to the slope of the amplitude profile, in triangulation experiments. Also, the relative cell size distributions, calculated from the peak amplitudes and the distances of units from electrode tracks, are correlated with the observed distributions of cell sizes in histological reconstructions.

The calibration functions thus obtained have been used to enhance the smart electrode system so that it can search for and select for cells lying within a specified recording radius of the electrode track and to select for relatively large or small cells.

150.15 TACTILE DISCRIMINATION FOLLOWING THORACIC CORD LESIONS IN CAT. G. P. Frommer. Dept. Psychol., Indiana U., Bloomington, IN 47405

The effect of lesions in thoracic cord of cats was tested on discriminations between touches on the left and right side, between rostrally and caudally directed strokes, or between large diam. (5 or 3.8 cm) and small diam. (.6 cm) discs. The cat had to turn its head toward food cups to its left or right, depending on which stimulus from a pair was manually delivered to its thigh. All but one cat preoperatively mastered the side of touch task to a criterion of > 90% correct on two of three consecutive days. All but three mastered one of the other tasks as well. Pain reactivity was tested with pin pricks and strong pinches. Orienting to hair movement was tested while the cat was eating. The lesions, made with the aid of a dissecting microscope, were aimed at (a) dorsal columns (DC) (N=2); (b) dorsal lateral columns (DLC) (N=2); (c) DC+DLC (N=2); or (d) ventral cord sparing DC (N=2) or DC+DLC (N=3). Animals are still being tested, so anatomical verification is not yet available.

DLC lesions permitted immediate recovery to preoperative levels of performance. After lesions in DC, cats took 46 and 122 days to recover mastery of the stroke direction and size discriminations, respectively. Lesions of DC+DLC prevented relearning within the allotted time. Performance on the simplest task (side of touch) remained at chance for 34 days. Above the level of the lesion this task was mastered within 25 days. When pin pricks below the level of the lesion replaced touch stimuli, one cat remained at or near chance for 43 days, while the other began performing consistently above chance after 33 days. Reactivity to painful stimuli was grossly normal postoperatively in all cats with lesions in the dorsal cord. Lesions in the ventral cord permitted learning or slow relearning of the side of touch task (2 cats) and the stroke direction task (2 cats). This lesion prevented recovery of the size discrimination (1 cat), which was the most difficult task to learn preoperatively. Cats with ventral lesions showed little if any reaction to painful stimuli below the level of the lesion, but they oriented reliably and consistently to hair movements.

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150.16 A METHOD FOR CONTINUOUS AND CONTROLLED DRUG DELIVERY TO THE TRANSECTED SPINAL CORD. J. C. de la Torre and N. Hayashi*. Dept. of Neurosurgery, Univ. Miami Sch. of Med., Miami, Fl 33101.

A technique has been developed to deliver drugs or fluids to experimentally transected spinal cord using an osmotic minipump connected to a catheter system.

Long-Evans hooded rats undergo laminectomy and transection of the spinal cord at T_{10} and after bleeding is controlled, a graft is inserted between the transected cord stumps.

The minipump is loaded with the drug or fluid and a 1.5 cm PE-60 tubing is loosely fitted to it while its distal end is connected to a "flared" PE-10 tubing. The latter tubing is shaped by heating to resemble the Greek letter "omega" or a "horseshoe" at its end so that the "arched" section of the tubing fits above, but does not touch, the transected cord tissue. The two ends or "legs" of this horseshoe-shaped tubing are anchored with 4-0 sutures to the muscles just lateral to the spinal cord. A syringe with a 30 gauge needle is connected to the end of the PE-10 tubing and the entire catheter is filled with the same solution contained in the minipump.

After the catheter system has been filled, the proximal end of the PE-60 tubing is connected to the flow moderator of the minipump. With a fine-tip ophthalmic cautery, a 1 mm puncture is made in the middle of the arched PE-10 tubing above the cord. A moist, pressed, gelfoam pledget is placed on the dorsal spinal cord to cover the transected ends while drugs or fluids from the punctured catheter above, drip on the gelfoam at a rate of 1 µl/hr. After surgery, the muscles and skin are closed in layers and the minipump is placed in a subcutaneous pouch. The minipump may be reloaded after 1 week.

Post-mortem studies on 9 rats show that minipump-catheter delivery of 10% Evans-Blue fluorescent dye to the transected cord area for 1-2 weeks, is taken up by the spinal cord tissue throughout its cephalo-caudal length as evidenced by fluorescent staining of blood vessels and tissue.

The method appears reliable for the continuous and rate controlled flow of drugs, fluids and dye markers to the spinal cord tissue and may be of value in CNS regeneration studies.

150.17 A RELIABLE E-M FIXATION PROCEDURE FOR THE ADULT RAT FUNICULI GRACILI.

P.K. Hill, J.C. de la Torre and S.B. Thompson*. Anatomy Dept. EVMS, Norfolk, VA. 23510 and Neurological Surgery Dept., U. Miami School of Med., Miami, Fla. 33101

To develop an adequate fixation of adult rat dorsal funiculi for ultrastructural study, experiments utilizing 5 female Sprague-Dawley rats (250-300 gm.) involved cannulation of the ascending aorta via the left ventricle. Five minutes before cannulation of the heart, the abdominal aorta immediately before its bifurcation is ligated and 8 cc. of 1% sodium nitrite-heparin is injected into the inferior vena cava (I.V.C.). The Harvard apparatus compact pump #975 delivers 600 cc. of 4% glutaraldehyde - 6% paraformaldehyde in 0.1M sodium cacodylate buffer containing 0.2% $CaCl_2$ (pH 7.4, r.t.) at 24 cc./min. for the first 3 min. and then 33 cc./min. for completion of prefixation. A small incision in the I.V.C. serves as a fluid release. The spinal cord is removed immediately and microscopic dissection of the right and left L-1 dorsal columns prepared (+4°C). The tissue is washed overnite in cold cacodylate buffer and post-fixed in a 1% osmium tetroxide (OsO_4) solution buffered in a 2% aqueous stock solution of OsO_4 that is diluted immediately before use. Dehydration involves 2 changes in cold 50% ethanol (EtOH) for 5 min. each, 1 change each in cold 70%, 80% and 95% EtOH, 2 changes for 10 min. each in cold 100% EtOH and 2 changes of propylene oxide (P.O.) for 15 min. each. The tissue is first infiltrated in a 1:1 mixture of P.O.: epon for 1 hr. at 37°C and then placed in pure epon for 1 hr. in a desiccator and embedded in flat molds with a fresh change of epon under vacuum at 5 lb./in.^{2} overnite at +60°C. One µm sections are prepared with glass knives and stained in aqueous 0.5% methylene blue-azure blue in 0.05% sodium borate. The fasciculi gracili are located medially and dorsally in the right and left L-1 dorsal columns and trimmed down for ultra-thin sectioning. Silver-toned sections prepared with the Dupont diamond knife are stained with a saturated ethanolic uranyl-acetate solution for 20 min. and 2.0% lead citrate for 3 min. Ultrastructural examination is done with the Philips 301 electron microscope. The results indicate consistently that unmyelinated, small-medium sized myelinated fibers, glia and blood vessels are well preserved. Some medium-sized myelinated fibers and large myelinated fibers show slight separation of lamellae. To remedy that problem, pressures that would potentially distort the entire tissue would have to be used. When comparing preservation of unmyelinated and myelinated fibers of the dorsal funiculus with those of the ventral gray matter, it is reassuring to know that the adequacy of fixation is similar inspite of a two-fold greater blood supply to the ventral gray matter.

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150.18 SPONTANEOUS ACTIVITY GENERATED IN SCIATIC NEUROMAS, A (^{14}C)2-DEOXYGLUCOSE UPTAKE STUDY IN THE RAT.

S. David* and A.J. Aguayo. The Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Canada.

Electrophysiological studies after sciatic nerve section in the rat have demonstrated that spontaneous activity is generated at the site of nerve section; the peak of this activity occurring between 5-15 days after injury (Brain Res. 159:406-410, 1978). In the present study we have examined the effects of such electrical discharges on spinal cord activity utilizing the (^{14}C)2-deoxyglucose (2DG) technique.

Both sciatic nerves were transected above the knee in 200-300 gm. Sprague-Dawley rats. The cut end of the nerves was ligated and capped with a polyethylene tube to prevent regeneration. Thirteen days after nerve section animals were anesthetized with sodium pentobarbital and the neuroma on one side was exposed and bathed in 2% Xylocaine. Animals were then injected with 2DG (15µCi/100 gm.), sacrificed 45 minutes later and segments of the lumbar spinal cord prepared for 2DG autoradiography and histology. The autoradiographs were scanned with a Zeiss Axiomat microscope and T.V. densitometer along with a PDP 11 computer. Results of the microdensitometric analysis indicate an increase in 2DG uptake at the L_4-L_5 levels of the spinal cord particularly in laminae I to V and IX on the side of the neuroma not blocked with Xylocaine.

Since there was a reduction in 2DG uptake on the side of the spinal cord ipsilateral to the nerves treated with local anaesthetic, the increase in autoradiographic density seen on the untreated side is considered to be largely due to the spontaneous ongoing activity generated at the site of the neuroma. These findings support the view that similar activity from injured nerves might be responsible for the sensory symptoms observed in patients with post-traumatic neuromas (Nature 248:740-743, 1974).

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150.19 DISTRIBUTION, DENSITY, AND SIZE OF MUSCLE RECEPTORS IN CAT TAIL.

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In nembutal anesthetized adult cats, dorsolateral tail muscles on both sides were exposed to 1 or 2.5% OsO4 in 0.1M cacodylate buffer for 20-40 minutes, washed in Ringer, divided into segments, and stored in glycerol. Under a dissecting microscope, darkly stained myelinated nerve fibers were easily recognized and traced to their termination (or terminal branches) on extrafusal muscle fibers as well as in muscle spindles and tendon organs (GTO). The size and distribution of muscle receptor capsules (ie. visible capsule space) along the tendon and entire muscle length were determined for each side of each caudal segment (starting with #4 or #5). Data were obtained for three tails.

For an entire tail side, the number of receptors and muscle mass per segment decreased caudally. The average numbers of receptors per segment were: 7 spindles (5-8) and 6 GTOs (5-6). The average total numbers of receptors per side were: 63 (56-71) spindles and 53 (43-58) GTOs. An average of 25% of ipsilateral spindle capsules were 'tandem'; there were 7 tandems (avg.) per side. The average ipsilateral receptor densities were: 142 spindles/g-muscle (92-188); 121 GTO/g-muscle (79-166). Opposing side total receptor numbers were similar: the range of departure from right vs. left average was 3-12% for spindles and GTOs; for a given segment, the dissymmetry could be larger. Correcting for multi-GTO innervation by single axons, the average ipsilateral Ia/Ib afferent axon ratio was 1.3 (1.0-1.7).

Averaging the means of receptor capsule space size distributions for the three whole tail samples gave:

Spindles - Length: 900µm(360-1510), Center Width: 140µm(40-240);
GTO - Length: 870µm(350-1120), Center Width: 75µm(30-100).

Scatter plot regression analysis revealed significant correlation between spindle capsule space length and center width; for GTO capsules, this correlation obtained in 2 of 3 tails.

The average spindle position-expressed as percent extrafusal muscle length from tendon to capsule center-was 45% (avg. range: 2-99%). While spindle capsules were distributed uniformly over 75% of the muscle length from the tendon, there was a lower (avg. 53%) occurrence over that 25% muscle length closest to the origin. There was no correlation between spindle capsule space length or center width and capsule center position on the extrafusal muscle longitudinal axis.

GTO capsules received an average of 10 input muscle fibers (avg. range: 3-22). In 2 of 3 tails, the number of input muscle fibers was significantly correlated with capsule width.

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150.20 MUSCLE SPINDLE DENSITY IN REGIONS WITH VARYING OXIDATIVE INDICES OF PIGEON FOREARM MUSCLES.

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Preferential location of muscle spindles in regions with high oxidative index (OI; sum of number of slow-twitch oxidative and fast-twitch oxidative-glycolytic fibers/total number of fibers x 100) has been noted first in rat hindlimb muscles (Yellin, Am. J. Anat., 125:31-46, '69), and since then has been reported for other species. From these findings the view arose that muscles with high OI have greater spindle densities than muscles with low OI. In the past, OI calculated from single histochemical series have been routinely used to characterize whole muscles and their associated spindle densities, but with few exceptions (e.g. cat soleus), muscles are not histochemically homogeneous. The present work took regional histochemical variation into account when considering relations of OI to spindle density in 13 muscles of the pigeon forearm. The muscles were grouped into wrist flexors, wrist extensors, flexors of digits, extensors of digits, pronators, supinator and anconeus. Three specimens of each kind of muscle were serially cross-sectioned. Each fifth section was mounted and stained by the van Gieson technique. At appropriate intervals, sections incubated for myofibrillar adenosine triphosphatase or succinic dehydrogenase were interposed to evaluate the histochemical profile at different points along the length of muscles. This allowed the partitioning of individual muscles into from 7 to 27 segments of different histochemical composition. Spindle density was calculated per mm^3 for each separate segment. The level of OI below which no spindles were found in a fascicle ranged from 50% (pronators) to 67% (supinator). Plots of spindle density against OI showed a positive correlation for each muscle. Means of populations of slopes representing concomitant increases in spindle density with increasingly greater units of OI were not significantly different ($p \leq 0.01$) among the seven muscle groups. However, correlation coefficients (r) varied within muscle groups, or even among specimens of the same kind of muscle. The lowest values of r were seen in flexors and extensors of the wrist, anconeus and supinator. The correlation held best for extensors of the digits. In these, r was significantly greater ($p \leq 0.01$) than in flexors and extensors of the wrist, anconeus and supinator. Nevertheless, in any muscle group there was a range of spindle density values for the same value of OI. Low densities for OI greater than 90% were as frequent as high densities for OI below 70%. It has been suggested (Botterman et al., Am. Zool., 18:135-152, '78) that the distribution of spindles is preferentially linked to readily recruited "oxidative" motor units. The scatter in density indicates that this presumed functional association can proceed without strict incremental allotments of spindles for increasingly greater values of OI.

150.21 MECHANORECEPTOR FATIGUE IN CAT TRIGEMINAL NEURONS.

Paul D. Shepard*, Kathy L. Howard* and David J. Barker. Dept. of Physiology, North Texas State University/Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

In the course of investigating the effects of sympathetic efferent stimulation on facial mechanoreceptors in the cat, we observed that fatigue was prominent in most receptors. Fatigue is defined as a reduction in number of impulses to repeated presentations of the same mechanical stimulus. Fatigue differs from adaptation which is a reduction in number of impulses during a constant stimulus. Fatigue is reversible with rest periods of sufficient length. We also observed that phentolamine, a sympathetic noradrenergic antagonist produced a temporary reversal of the fatigue process. The present study was undertaken to (a) Examine the relationship between fatigue and various stimulus parameters (amplitude, duration, interstimulus interval); and (b) Further examine the reversal of fatigue by phentolamine.

Single units were recorded from the trigeminal ganglion of anesthetized (pentobarbital) cats. Facial cutaneous mechanoreceptors were stimulated with trapezoidal waveforms delivered by an axial displacement generator. The following stimulus parameters were included: (a) amplitude: 250, 500 and 1000 microns; (b) duration: 1, 5 and 10 sec; (c) interstimulus interval: 10, 20 and 40 sec. The following results were obtained: (1) Fatigue occurs to some extent in all mechanoreceptors, depending on the particular combination of stimulus parameters; (2) The magnitude of fatigue is greater among slowly adapting receptors (average of 50% decline in number of spikes over 10 trials of the same stimulus) than among rapidly adapting receptors (20% decline in spikes over 10 trials); (3) A 3 minute rest period is sufficient to reverse fatigue; that is, fatigue does not occur when the interval between stimuli is this long; (4) Generally, among slowly adapting receptors, the magnitude of fatigue varies directly with stimulus amplitude and duration, and varies inversely with interstimulus interval. However, the effects of interstimulus interval are different at different amplitudes and durations; (5) In about 50% of the slowly adapting receptors, IV injection of phentolamine (5 mg) resulted in periodic reversal of fatigue and/or an increase in spontaneous firing (during the interstimulus interval). The peak magnitude of this effect and duration coincided with the time to maximal response and duration of action of this drug. Our results suggest that fatigue can be a significant component of mechanoreceptor response profiles and may be a significant source of variability in studies of mechanoreceptor coding. The phentolamine data suggests that sympathetic efferents may alter viscoelastic properties of skin which may be important in mechanoreceptor fatigue.

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151.1 HYPOPHYSECTOMY PREVENTS THE STRIATAL DOPAMINE RECEPTOR SUPERSENSITIVITY PRODUCED BY CHRONIC HALOPERIDOL TREATMENT. Lynn M. Ludmer* and Robert E. Hruska (SPON: Paul D. Thut). Neurotoxicology Section, NINCDS, NIH, Bethesda, MD 20205.

Chronic Haloperidol (HAL) treatment of adult male rats is well known to produce supersensitive dopamine (DA) receptors in the striatum. This phenomena has been proposed to result from a compensatory increase in DA receptor number. This supersensitivity is thought to be important in the clinical development of tardive dyskinesia, which results from chronic neuroleptic treatment. In the pituitary DA acts to inhibit prolactin secretion. Blockade of the DA receptors by HAL produces an increase in prolactin release, which is maintained during chronic HAL treatment. We have tested the effects of chronic HAL treatment on striatal DA receptors in rats after hypophysectomy (Hypox). Male rats were obtained from Taconic Farms on the day after Hypox. These rats were maintained on a drinking solution containing 5% dextrose and a balanced salt solution, and kept in the warmest part of the animal room (25°C). At the same time a group of weight matched non-operated control rats were obtained and housed in the same area. The experiment was initiated on the second day after Hypox. One-half of the control rats and one-half of the Hypox rats were injected daily, i.p., with 1 mg/kg HAL for 3 weeks. The remaining rats were injected with saline. Then treatment was discontinued for one week before sacrifice by decapitation. DA receptors were measured in the striata using [^{3}H]spiroperidol in our routine assay procedure (Eur. J. Pharmacol. 61: 397). In the control rats, HAL treatment produced the expected increase in the number of striatal DA receptors (28%), but did not change their affinity. Hypox, by itself, did not alter either the number or affinity of the DA receptors. However, Hypox did prevent the development of striatal DA receptor supersensitivity normally produced by chronic HAL treatment. It should be noted that during the 4 weeks of this experiment, the Hypox rats gained no weight or lost some, while the control rats gained an average of 130 g. Chronic HAL treatment did not appear to affect weight gain in either treatment group. This experiment suggests that the striatal DA receptor supersensitivity produced by chronic HAL administration is mediated through the pituitary or requires a pituitary cofactor. Further it is possible that the production of this supersensitivity depends on prolactin, since HAL increases prolactin levels in control rats, while there is no prolactin available in the Hypox rats. This may be of relevance in the mechanism of the development of tardive dyskinesia after chronic neuroleptic treatment, and may explain the reported higher incidence of tardive dyskinesia in females, who have higher circulating levels of prolactin.

151.2 THE STRIATAL DOPAMINE RECEPTOR SUPERSENSITIVITY PRODUCED BY ESTROGEN MAY BE MEDIATED THROUGH PROLACTIN. Robert E. Hruska, Lynn M. Ludmer* and Ellen K. Silbergeld. Neurotoxicology Section, NINCDS, NIH, Bethesda, MD 20205.

We have previously reported that estrogen treatment of rats results in an increase in the number of dopamine (DA) receptors in the striatum without a change in their affinity (Eur. J. Pharm. 61: 397). Behavioral experiments, such as stereotypy, also showed an increase in DA receptor sensitivity after estrogen treatment. The effect of estrogen on DA receptors has been extensively characterized. Only the β-diastereomer of estradiol is active. Other striatal receptors are not affected. DA uptake, a presynaptic function, remains unaffected by estrogen treatment. Estrogen in vitro does not affect DA receptors. In order to determine a possible mechanism of action of estrogen, we treated male rats, after hypophysectomy (Hypox) with 125 μg 17β-estradiol valerate in sesame oil, s.c., 6 days before measurements were made. Striatal DA receptors were measured using [^{3}H]spiroperidol in our routine assay procedure, and stereotypy was measured using apomorphine (4 mg/kg) (see above ref.). Hypox, by itself, did not alter the number or the affinity of the DA receptors; however, it did prevent the increase in DA receptor number normally produced by estrogen. Hypox also prevented the increase in apomorphine-induced stereotypy which occurs after estrogen treatment. This suggests that a pituitary secretion is involved in the increase in DA receptor sensitivity. Since estrogen directly increases prolactin secretion by the pituitary, prolactin may mediate the increase in DA receptor sensitivity. To test this hypothesis we implanted male rats, s.c., with Alzet mini-pumps which delivered 120 ng of ovine prolactin per hr for 7 days. The rats were then evaluated behaviorally and biochemically for striatal DA receptor sensitivity. Prolactin treatment significantly increased the number of striatal DA receptors (27%). The DA receptor affinity did not change. Similarly, prolactin administration enhanced stereotypy produced by either apomorphine (4 mg/kg) or by amphetamine (2.5 mg/kg). These results indicate that prolactin may influence striatal DA receptors, and that the effect of estrogen may be mediated through prolactin. Chorea of pregnancy or chorea associated with the use of oral contraceptives is thought to be related to a relative increase in striatal DA sensitivity. Estrogen has been suggested to be the agent which produces this form of chorea; however, our data suggests that further investigation of prolactin may be indicated.

151.3 6-HYDROXYDOPAMINE DIFFERENTIALLY AFFECTS PNMT ACTIVITY AND EPINEPHRINE CONTENT IN RAT HYPOTHALAMUS: EVIDENCE FOR COMPENSATORY ENHANCEMENT IN NEURONAL FUNCTION. Susan K. Burgess*, Lee E. Eiden, Richard E. Tessel* (SPON: Ronald T. Borchardt); U. of Kansas, Lawrence, KS, 66045, and Lab. Clin. Sci. NIMH, Bethesda, MD, 20205.

The observation that phenylethanolamine N-methyltransferase (PNMT) activity in the hypothalamus (HT) is unchanged 10 days after i.vent. 6-hydroxydopamine (6HD) has been interpreted as evidence that epinephrine (EPI) neurons are intact after 6HD treatment (Jonsson et al., Med. Biol. 54:421, 1976). However EPI content in HT is substantially depleted by 6HD (Tessel et al., Brain Res. 153:615, 1978) indicating that these neurons are subject to 6HD neurotoxicity. The mechanism for the discrepancy between enzyme activity and neurotransmitter content in EPI neurons has been explored. The EPI/PNMT disparity is not a general consequence of lesioning: surgical isolation of the HT resulted in equivalent decreases in EPI and PNMT in the medial basal HT. Whereas dopamine (DA) content was not significantly changed by either chemical or surgical lesions, norepinephrine (NE) and EPI contents were markedly depleted by both lesions. The apparent 6HD-resistance of DA and PNMT neurons is not due to a lack of 6HD affinity for their neuronal uptake systems. 6HD in vitro released endogenous EPI, DA, and NE from chopped HT with approximately equal potencies, indicating that 6HD was taken up by all three types of neurons.

PNMT activity was significantly lowered one day after 6HD injection (compared to sham or day 10), suggesting that a compensatory increase in enzyme activity has occurred by day 10. In addition, MAO inhibition with pargyline (50 mg/kg, 2½ h), which did not increase HT catecholamines in control rats, more than doubled the EPI and NE content of 6HD-treated rats (day 10). This suggests that, in EPI and NE neurons of the HT, catecholamine storage capacity is impaired and/or turnover rate is increased after 6HD. The percentage of endogenous EPI, NE, or DA released in the presence of pargyline, either by depolarization or by amphetamine, was the same in HT tissue obtained from 6HD-treated or control rats. This argues against either the selective destruction of intraneuronal storage vesicles, or the long-term impairment of the uptake system in undamaged neurons after 6HD.

These data suggest that EPI neurons more closely resemble NE than DA neurons in the HT, and are consistent with the hypothesis that a compensatory increase in EPI synthetic capacity of the residual EPI neurons accounts for the EPI/PNMT disparity after 6HD lesioning.

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151.4 **AN EVALUATION OF THE BIOACTIVITY OF AN ENDOGENOUS INHIBITOR OF ^{3}H-DIAZEPAM BINDING.** **Leonard G. Davis, Helen McIntosh*, Dean Reker*, Luci Fikes* and Ron Cohen*. Missouri Institute of Psychiatry, Univ. of Missouri, 5400 Arsenal St., St. Louis, MO 63139.**

The isolation from bovine brain of a factor that is a competitive inhibitor of ^{3}H-diazepam binding (BBRC 92, 141, 1980) has supported the possibility that an endogenous diazepam-like compound exists. The present preliminary studies are concerned with the determination of the bio-active actions that this isolated factor might have in comparison to diazepam and placebo. The intraventricular injection, through chronically implanted cannula, of 5μl of either solution preceded the behavioral testing by thirty to sixty minutes. The test situations included motility, ataxia, EEG, suppression of hyperthermia, behavioral disinhibition (conflict) and retardation of pentylenetetrazole induced convulsions. In each situation, the material isolated from the brain has affects similar to those observed for diazepam. In particular, the endogenous material was able to relieve the shock-induced inhibition associated with the bar press in the conflict test. It was also possible to reduce the onset of convulsions with this brain extract. These observations support the concept of an endogenous diazepam-like substance. Studies are presently aimed at the purification and structural characterization of this putative-natural ligand for the benzodiazepine receptor.

151.5 CATECHOLAMINE AND CYCLIC NUCLEOTIDE CONCENTRATIONS IN THE CEREBELLUM OF PCD MUTANT MICE. B. D. Sawyer,* S. Hemrick-Luecke,* M. J. Schmidt, R. W. Fuller and B. Ghetti. The Lilly Research Laboratories, Indianapolis, IN 46285 and Indiana University Medical Center, Indianapolis, IN 46202

Purkinje cells in the cerebellum receive noradrenergic input from the locus coeruleus, and this input inhibits Purkinje cell firing possibly through an elevation of cyclic AMP. Unexpectedly, we observed that in vitro in the presence of norepinephrine, levels of cyclic AMP were dramatically increased in cerebellar slices prepared from mutant mice (pcd or nervous), animals in which Purkinje cells degenerate. The present experiments were done to determine if elevated levels of cyclic AMP were present and concentrations of norepinephrine were altered in the cerebellum of pcd mice in vivo. Determinations were made in animals ranging in age from 23-290 days. Mice were sacrificed by exposure to high level microwave radiation, and cyclic nucleotides were determined in the cerebellum by radioimmunoassay. Catecholamine concentrations were determined in the cerebella of mice which had been decapitated and the brains frozen shortly after death. HPLC and electrochemical detection were used to assay amines.

Cyclic AMP levels were similar in control and affected mice prior to 44 days of age, at which time cyclic AMP was 45 percent higher in pcd mice. Cyclic AMP remained elevated in affected animals at all times thereafter, with a peak elevation at 60 days of age. The concentration of cyclic GMP, on the other hand, was lower in pcd mice at all ages.

Norepinephrine levels were not significantly different in control and pcd mice at 23 days of age, but at all later ages concentrations of norepinephrine were markedly elevated in the cerebella of mutant mice. The greatest difference was observed in the oldest mice in which norepinephrine levels were 150 percent higher in affected animals. In contrast, the total content of cerebellar norepinephrine was not different in pcd and control mice. Apparently, the increased norepinephrine concentration is due to the reduction of cerebellar volume. These results indicate that despite the loss of a major post-synaptic target (Purkinje cells), the cerebellar noradrenergic input remains stable. The present studies illustrate that increases in cyclic AMP and norepinephrine in vivo are coincident with marked norepinephrine stimulated accumulations of cyclic AMP in vitro in the cerebella of pcd mutant mice.

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151.6 AN ACID HYDROLYZABLE CONJUGATE OF DOPAMINE IN HUMAN VENTRICULAR FLUID. N.S. Sharpless, G.M. Tyce*, L.J. Thal*, J.M. Waltz*, K. Tabaddor*, and L.I. Wolfson*. Albert Einstein College of Medicine, Bronx, NY 10461, Mayo Clinic and Mayo Foundation, Rochester, MN 55901, and St. Barnabas Hospital, Bronx, NY 10457.

In earlier work (J. Neurochem. 34:210, 1980) a substantial amount of a conjugate of dopamine (DA) was found in lumbar CSF of 2 Parkinson patients being treated with Sinemet (a 10:1 mixture of L-DOPA and a peripheral decarboxylase inhibitor). We now confirm the presence of an acid hydrolyzable conjugate of DA in CSF from patients taking Sinemet and also present evidence for the presence of conjugated DA in ventricular CSF from patients never treated with L-DOPA. Free DA was removed from an aliquot (2 ml) of ventricular CSF by adsorption onto alumina. The alumina-treated CSF was acidified with $HClO_4$ and heated at 100°C for 45 min to hydrolyze the DA conjugate. Liberated DA was adsorbed onto alumina which was then washed and eluted with 0.5 M acetic acid. The eluates were freeze-dried, reconstituted in 0.1 ml, and aliquots (25 µl) were analyzed for DA by high-performance liquid chromatography with electrochemical detection (Column: µBondapak C_{18}, 4 mm x 30 cm; Solvent (1.0 ml/min): 0.07 M NaH_2PO_4(pH 4.8), 0.2 mM Na_2EDTA, 0.5 mM heptanesulfonate, 8% methanol). Recovery of DA added to CSF was 75%; recovery of DA liberated from DA-sulfate during hydrolysis was 71% from DA-3-sulfate and 79% from DA-4-sulfate. Although only trace amounts (58 pg/ml or less) of free DA were detected in ventricular fluids from 7 patients with movement disorders, all of the hydrolyzed fluids had a clearly defined peak with the same elution time (11 min) as authentic DA. With 6% methanol and 1 mM heptanesulfonate authentic DA and the CSF peak both eluted at 17 min; with 2 mM heptanesulfonate the peaks appeared at 23.5 min. Levels of conjugated DA in ventricular CSF from 3 Parkinson patients who had taken Sinemet ranged from 210-1508 pg/ml and correlated inversely with the time between the last dose of Sinemet and withdrawal of the fluid (23-65 h). The estimated half-life for disappearance of conjugated DA from ventricular CSF after cessation of L-DOPA was 15 h. Values for conjugated DA in ventricular CSF from 4 patients with movement disorders (cerebral palsy; multiple sclerosis) who had never taken L-DOPA ranged from 105-218 pg/ml. Ventricular fluid from one patient who had recently sustained a severe head injury had increased concentrations of both free (483 pg/ml) and conjugated (435 pg/ml) DA. The physiological significance of conjugated DA in human ventricular CSF is not yet known. Sulfation of the meta hydroxyl group of DA may compete with O-methylation for inactivation of released DA. Conjugated DA may also represent a convenient transport form for removal of DA from brain and CSF.

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151.7 EFFECTS OF DRUGS ON DOPAMINE RELEASE AS ESTIMATED BY SIMULTANEOUS RADIOENZYMATIC ASSAY OF DOPAMINE AND 3,4-DIHYDROXYPHENYL ACETIC ACID. C.H. Cheng and G.F. Wooten. Depts. of Neurology & Pharmacology; Washington University School of Medicine; St. Louis, Missouri 63110.

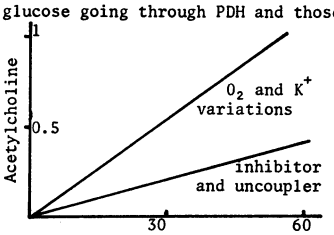
The formation of 3,4-dihydroxyphenyl acetic acid (DOPAC) in the striatum (CS) appears to occur primarily in terminals of dopaminergic neurons and may reflect metabolism of released dopamine (DA) which has been recaptured. Thus short-term changes in relative concentrations of DA and DOPAC may be a useful index of the rate of DA release. We have developed a highly sensitive and specific radioenzymatic assay for simultaneous estimation of DA and DOPAC. These compounds were enzymatically transmethylated on the meta-hydroxy groups in the presence of partially purified catechol-O-methyltransferase with S-[methyl-^{3}H] adenosyl-L-methionine serving as the methyl donor. The reaction products were isolated by organic solvent extraction, resin treatment, and thin layer chromatographic separation on silica gel plates. The method provides a sensitivity of 10 pg for DA and 100 pg for DOPAC. Cross-contamination between DA and DOPAC was less than 1%. Control rat brain levels of DA were 9.6 ± 0.3 µg/g wet weight in CS and 5.0 ± 0.3 µg/g in olfactory cortex-nucleus accumbens (OA); while control DOPAC levels were 1.2 ± 0.1 µg/g and 1.0 ± 0.2 µg/g in CS and OA respectively.

Treatment with haloperidol 1 mg/kg SC, a DA receptor antagonist, resulted in a 2.7-fold increase at 1 hour and a 3.1-fold increase at 2 hours of DOPAC in CS. DA levels were not affected. In contrast, administration of apomorphine 0.5 mg/kg SC, a dopamine agonist, resulted in a 50% reduction in striatal DOPAC 1 hour after drug administration while no changes were noted in striatal DA. However, neither DA nor DOPAC levels were changed in OA after apomorphine administration suggesting that mesolimbic dopaminergic neurons may not be subject to feedback control as is the case for nigrostriatal dopaminergic neurons. Injection of amphetamine 2.5 mg/kg SC, a drug which increases turnover of DA by enhancing release and blocking re-uptake, produced a small (20%) but significant increase in striatal DA 1 hour after administration; however, DOPAC levels were reduced by 61% and 49% at 30 minutes and 1 hour respectively after injection.

Thus simultaneous estimation of DA and DOPAC provides a useful index of DA release in most instances. But in the special case of drugs such as amphetamine which block DA re-uptake, estimation of DA and DOPAC levels may falsely suggest a reduction in the rate of release.

151.8 ACETYLCHOLINE SYNTHESIS AND $^{14}CO_2$ PRODUCTION FROM VARIOUSLY LABELLED GLUCOSES. H.J. Ksiezak* and G.E. Gibson, Dept. Neurology, Cornell Univ. Medical College, Burke Rehab. Center, White Plains, NY 10605

A variety of data suggest that there is a close link between oxidative metabolism and acetylcholine (ACh) synthesis. This is shown by a parallel inhibition of ACh synthesis and of $^{14}CO_2$ production from pyruvate or glucose by a variety of compounds (J.Neurochemistry 26, 1073). However, the molecular basis of this link is unknown. Furthermore, some compounds stimulate CO_2 production but inhibit ACh synthesis (e.g. dinitrophenol, DNP). We studied this relation further by comparing ACh synthesis from [U-^{14}C]glucose to $^{14}CO_2$ production from [3,4-^{14}C]glucose (a measure of pyruvate dehydrogenase reaction, PDH), [2-^{14}C]glucose (a measure of the tricarboxylic acid cycle, TCAC) and from [U-^{14}C]glucose (a measure of overall oxidative metabolism). We studied these variables in synaptosomes and in brain slices under several conditions which stimulate or inhibit oxidative metabolism: low O_2 content (<0.01%), low K^+ (5 mM) and high K^+ (35 mM) concentrations, with an inhibitor of electron transport (antimycin) and with an uncoupler of oxidative phosphorylation (DNP). Under all of these conditions we analyzed the relation of ACh synthesis to the decarboxylation of [3,4-^{14}C]glucose, [2-^{14}C]glucose, [U-^{14}C]-glucose and to the efflux of metabolites from the TCAC. The latter was calculated as the difference between the nanomoles of glucose going through PDH and those decarboxylated by the TCAC.



Efflux of metabolites from the tricarboxylic acid cycle

The highest correlation was between ACh synthesis and the efflux of metabolites from the TCAC (r=0.860) as compared to any of the other relations (r<0.741). When O_2 and K^+ variations data were grouped separately (see Figure), the correlation was much higher (r=0.986). For the rest of the data with the inhibitor or uncoupler the correlation coefficient was r=0.946. The lower slope with the inhibitor and uncoupler suggests that in these conditions the efflux is directed more into metabolites other than ACh. In summary, ACh synthesis is linearly related to the efflux of metabolites from the TCAC under a variety of conditions. The physiological control of this efflux and its implications to the control of ACh synthesis has not been determined.

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151.9 PURIFICATION OF CHOLINE ACETYLTRANSFERASE FROM DROSOPHILA MELANOGASTER. J.R. Slemmon*, P.M. Salvaterra, and E. Roberts. Division of Neurosciences, City of Hope Research Inst., Duarte, CA 91010

The heads of Drosophila melanogaster are one of the richest known sources of choline acetyltransferase (ChAT) activity, with a specific activity approximately twice that of Torpedo electric organ and ten times that of Rat or Rabbit brain. We are continuing the work begun in this laboratory (Driskell et al., J. Neurochem. 30:1135, 1978) to purify and characterize this important neurotransmitter synthetic enzyme. The results of our current purification procedure are summarized below. Homogenization was carried out in low pH (5.1) sodium citrate buffer. The supernatant was precipitated with polyethylene glycol. The precipitate from 5 to 15% polyethylene glycol was resuspended in citrate phosphate buffer, pH 5.9, and chromatographed on DEAE-Bio Gel A. ChAT activity was eluted with a 0 to 0.3 M sodium chloride gradient. The pH of the pooled ChAT activity was adjusted to 5.1, and the sample was chromatographed on an Octyl Sepharose column. The column was washed with one volume of buffer (sodium citrate, pH 5.1) and the activity eluted with 10 mM citrate phosphate, pH 5.9. The pooled concentrated sample was added to a Green A-Agarose column so that the sample volume was entirely included in the column. The column was left to equilibrate for 30 min followed by a wash with 10 mM citrate phosphate, pH 5.9. ChAT activity was eluted with 20 mM sodium phosphate, pH 7.0, containing 0.5 mM Coenzyme A. Polyacrylamide gel electrophoresis in SDS showed two protein bands at 67,000 and 54,000 daltons.

ChAT Purification from 100 g Fly Heads

	Volume (ml)	Units (μmole/min)	Protein (mg)	Sp/Ac (units/mg prot)
1. Homogenate	1040	540	11700	0.04
2. Supernatant	870	341	2436	0.15
3. Polyethylene glycol precipitate	93	300	403	0.74
4. DEAE Chromatography	40	266	42	6.30
5. Octyl Sepharose Chromatography	7	147	1.9	80
6. Green A-Agarose Chromatography	2	100	0.2‡	300

‡ Estimated from staining intensity of Coomassie Blue stained SDS gel (BSA standard)

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151.10 SPECIFIC ENZYMATIC SYNTHESIS OF γ-GLUTAMYLHISTAMINE IN APLYSIA CNS IN VITRO. C. Stein* and D. Weinreich. Dept. of Pharm. & Exptl. Therap., Univ. MD, Sch. Med., Baltimore, MD 21201.

Much evidence supports a neurotransmitter role for histamine (HA) at identified synapses in Aplysia cerebral ganglia. A mechanism for inactivation of synaptically released HA would provide further evidence for such a role. To this end, we have examined the possibility that metabolism participates in terminating the action of neuronal HA. Previous studies of intact Aplysia CNS incubated with radiolabeled HA revealed formation of a single labeled product, γ-glutamylhistamine (GHA; Weinreich, D., J. Neurochem. 32:363, 1979).

In the present study, we have developed an in vitro enzyme assay to characterize the properties of the dipeptide-forming enzyme(s). Homogenates of ganglia were incubated in the presence of radiolabeled HA. The unique formation of GHA was detected by high voltage electrophoresis, and column and thin layer chromatography by comparison with standards. Formation of the peptidoamine was linear with tissue and time. In addition, neither anisomycin (5–500 μM) nor ribonuclease T_1 (10 mg/ml) had any effect on GHA synthesis. It seemed probable, therefore, that metabolism of HA was under enzymatic control.

The enzyme(s) mediating GHA synthesis demonstrated an absolute requirement for ATP which could not be replaced with AMP, ADP, GTP or c-AMP. Dithiothreitol and Mg^{++} ions were also essential for activity. The enzyme had a K_m and V_{max} for HA of 240 μM and 17 nmol/mg protein/hr, respectively, and a pH 8 optimum. A variety of imidazole compounds and HA analogs had no effect on synthesis of GHA. Glutamate was essential for product formation. Glutathione and glutamine, two major γ-glutamyl compounds in tissue, were not appreciably incorporated into peptide linkage with HA. Methionine sulfoximine, an inhibitor of glutathione and glutamine biosynthesis, had minimal effect on GHA formation, supporting the view that glutamate is a substrate rather than a precursor. Glutamine and methionine, active substrates for γ-glutamyl transfer by γ-glutamyl transpeptidase, did not affect GHA synthesis. Serine and borate, known inhibitors of γ-glutamyl transpeptidase, did not decrease formation of product.

Since GHA (up to 10^{-3} M) has no discernible neuronal effects, this enzyme(s) could participate in the inactivation of synaptically released HA.

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151.11 BRAIN REGIONAL DISTRIBUTION OF GLUTAMATE-INSENSITIVE CYSTEINE SULFINATE DECARBOXYLASE ACTIVITY. Ronald M. Spears* and David L. Martin (SPON: A. T. Campagnoni), Department of Chemistry, University of Maryland, College Park, MD 20742.

Cysteine sulfinate decarboxylase (CSAD) catalyzes one step in the biosynthesis of taurine. During purification of the enzyme from hog brain, CSAD activity was resolved into two distinct components by chromatography on hydroxylapatite (referred to as Peaks I and II). When the hydroxylapatite column eluate was assayed for glutamate decarboxylase (GAD) activity, a single peak was observed which coincided with Peak II of CSAD activity. There was no detectable GAD activity in Peak I of CSAD. The value of K_m for cysteine sulfinate for Peak I of CSAD was 0.4 mM. The values of K_m for Peak II were 3.8 mM for cysteine sulfinate and 1.2 mM for glutamate. Kinetic studies with Peak II showed cysteine sulfinate and glutamate to be mutually competitive but glutamate did not inhibit CSAD activity in Peak I. These results suggested that CSAD activity in Peak II was due to GAD. Studies in other laboratories have shown that highly purified GAD also has CSAD activity. We have developed a procedure to measure the glutamate-insensitive CSAD activity (Peak I) in the presence of Peak II (GAD) activity. The decarboxylation of cysteine sulfinate was determined using 3.0 mM [^{35}S]cysteine sulfinate as substrate. The product, [^{35}S]hypotaurine, was separated from substrate on small Dowex 1-X8 columns (0.5 x 3 cm). To measure the glutamate-insensitive CSAD activity (Peak I) in the presence of GAD activity (Peak II), CSAD assays were carried out in the presence of 100 mM glutamate. Under assay conditions, 100 mM glutamate inhibited the decarboxylation of cysteine sulfinate by Peak II by more than 90%, but inhibited Peak I by less than 5%. To measure total cysteine sulfinate decarboxylation by the tissue, no glutamate was added. Glutamate-insensitive and total CSAD activity was measured in various regions of hog brains. The glutamate-insensitive CSAD activity was 35-45% of the total activity in all regions examined except the superior colliculus and the pons in which it was 20% and 60%, respectively. Highest levels of glutamate-insensitive CSAD were found in the caudate/putamen/globus pallidus and lowest values were found in the thalamus. Other brain regions examined were hippocampus, amygdala, inferior colliculus, lateral and medial geniculate bodies, and medulla.

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151.12 A COMPARISON OF THE ION EXCHANGE RESIN AND THE SOLVENT EXTRACTION RADIOCHEMICAL METHODS FOR THE ASSAY OF MONOAMINE OXIDASE ACTIVITY. S.M. Anderson* (SPON: P. Lerner). NINCDS, NIH, Bethesda, MD 20205.

The commonly used radiochemical methods for the assay of monoamine oxidase activity (MAO) involve the incubation of the enzyme with the radioactive substrate for a fixed time period. After the reaction is stopped the aldehyde, acid and/or alcohol products are separated from the unchanged amine and quantitated by liquid scintillation counting. Two separation methods are in wide use. The products and the substrate may be separated by the use of negatively charged ion exchange resin columns or by the extraction of the product into an organic solvent. The present study compares the two assay methods in the measurement of MAO activity in crude tissue homogenates of mouse brain and liver. MAO activity was measured by the oxidation of three substrates: phenylethylamine (PEA), tyramine (TYR) and 5-hydroxytryptamine (5-HT). Activity measured by a variation of the solvent extraction method of Wurtman and Axelrod (Biochem. Pharmacol., 12:1439, 1963) was compared with activity measured by a variation of the ion exchange resin procedure of Donnelly and Murphy (Biochem. Pharmacol., 26:853, 1977). Toluene:ethyl acetate was found to be an efficient solvent for the extraction of all three substrates. Both Dowex-50 and Amberlite CG-50 were found suitable for the retention of the unchanged amines in the ion exchange method. Recovery of the metabolites from these two methods is equivalent. Inbred strain mean rank ordering of MAO activity in crude brain homogenates suggest that these two methods yield similar results (Pearson Product Moment correlation coefficients describing the relationship between these two procedures are 0.92, 093 and 0.83 for PEA, TYR AND 5-HT, respectively). Sample to blank ratios from the ion exchange method, however, are low. The ion exchange resins failed to retain 3-5% of the unchanged substrates when the columns are washed. Crude liver homogenates from mice contain 20% MAO-A and 80% MAO-B (determined by chlorgyline and deprenyl inhibition curves). When low levels of MAO activity are measured in mouse liver homogenates using 5-HT as the substrate, sample to blank ratios are unacceptable (<2). The solvent extraction procedure yields sample to blank ratios >5 even for low levels of activity as found in mouse liver with 5-HT as the substrate. In general, results of studies presenting data on MAO activity measured by the oxidation of PEA, TYR or 5-HT by either of these methods should be comparable. On the other hand, the solvent extraction method is superior if 5-HT is the substrate and low levels of activity are expected.

151.13 O-METHYL-CATECHOLAMINE METABOLITE ANALYSIS USING ^{3}H•DANSYL CHLORIDE. C. F. Saller* and I. J. Kopin. Laboratory of Clinical Science, NIMH, Bethesda, MD 20205

Dansyl chloride was introduced as a means of producing fluorescent derivatives of phenolic and amino compounds, but radiolabelled dansyl chloride has been used to extend the level of sensitivity in assaying such compounds. We have optimized the production and isolation of ^{3}H•dansyl chloride derivatives of O-methylated catecholamines in perchloric acid extracts of tissue to develop a sensitive and specific assay for these catecholamine metabolites. Samples of brain or other tissues are homogenized in 4 volumes 0.2 N $HClO_4$. After centrifugation, 36 µl of the supernatant is removed and added to 4 µl 0.2 N $HClO_4$ or 4 µl 0.2N $HClO_4$ containing 10 picomoles each of 3-methoxytyramine, normetanephrine, and metanephrine as internal standards. Next, 10 µl 1N NaOH and 20 µl 1 M Na_2CO_3 are added and the samples are incubated at 37°C for 30 min. to destroy catecholamines. Then, the samples are washed with 1 ml toluene to remove non-phenolic amines. The aqueous phase is frozen and the toluene decanted. After thawing, 10 µl 0.8 N HCl are added and the samples are divided into three 20 µl aliquots. Twenty µl 2.5 mM (1 µCi) dansyl chloride in acetone are added to each aliquot and this mixture is incubated at 37°C for 15 min. Five µl proline (150 mg/ml H_2O) are added and the incubation is continued for another 10 min. Benzene (80 µl) containing 25 µM of each dansyl derivative is added and the ^{3}H•dansyl derivatives are extracted by vortexing. Sixty µl of the benzene extract are applied to each channel of 19-channel silica TLC plates and the derivatives are separated using benzene : triethylamine (1:1). The fluorescent derivatives are located under uv light, the appropriate areas scraped into scintillation vials, and the tritium assayed by liquid scintillation spectrometry. Less than 2-4 picomoles of each metabolite can be assayed. Their levels in several tissues and brain areas and changes induced by drug administration will be presented.

151.14 GLUCOCORTICOID INDUCTION OF TRYPTOPHAN OXYGENASE: ATTENUATION BY DIETARY CARBOHYDRATES AND CARBOHYDRATE METABOLITES. A. Yuwiler and A. Altar*. Neurobiochemistry Lab., VA Med. Ctr Brentwood, Los Angeles, CA 90073.

Tryptophan oxygenase (TPO) is a glucocorticoid-inducible liver enzyme which appears to regulate the amount of dietary tryptophan catabolism and nicotinic acid anabolism in the rat. The glucocorticoid-induced increase in TPO synthesis has been associated with an increase in both *in vitro* and *in vivo* tryptophan degradation within the kynurenine pathway. TPO induction can be blocked by pretreatment with glucose, but the attenuation may involve a metabolite of glucose such as NADPH and/or NADH. The present experiments determined the extent to which glucose or other intermediates of carbohydrate metabolism which regenerate liver HADH and NADPH attenuate the induction of TPO by short- or long-acting glucocorticoids.

Continuous *in vivo* TPO activity in male rats was estimated from the rate of production of 14-CO_2 after the intragastric administration of 14-C-2-tryptophan. Hydrocortisone-21-sodium succinate (Solucortef) or Triamcinolone acetonide were injected to respectively elevate hepatic TPO activity on an acute (4 hr) or chronic (24 hr) basis. Glucose, fructose, glycerol, or pyruvic acid were intragastrically intubated in doses ranging from 2 to 16 mMoles in attempts to reverse either the acute or chronic TPO inductions.

Solucortef produced a dose-related elevation of TPO over the range of 0, 25, and 50 mg/kg while the 50 mg/kg dose produced an inverted U-shaped induction of TPO over time which was maximal 3-4 hours post-injection. 16 mMoles of any carbohydrate intermediate completely abolished the TPO induction by Solucortef when given one-half hour before 14-C-tryptophan. Lower doses of these compounds were capable of somewhat less attenuation of TPO induction. Both fructose and glycerol, the only intermediates tested following Triamcinolone pretreatment, were capable of blocking chronic TPO induction. The present findings are consistent with the notion that dietary constituents that are capable of regenerating hepatic NADH and/or NADPH are capable of blocking glucocorticoid-induced elevations of TPO.

151.15 ABNORMALITIES IN ENDOGENOUS PHOSPHORYLATION IN A HEREDITARY MODEL OF EPILEPSY. J.M. Tuchek,* D.D. Johnson, S.L. Polvi*and R.D. Crawford.* Dept. of Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0

The relationship between high seizure susceptibility and endogenous protein phosphorylation was investigated by comparing the *in vitro* phosphorylation of brain proteins from carrier and epileptic fowl. The high seizure susceptibility in epileptic fowl is due to an autosomal recessive mutation. Homozygotes (epilpetics) undergo spontaneous seizures and also convulse in response to stroboscopic stimulation or hyperthermia whereas heterozygotes (carriers) do not develop seizures.

The incorporation of ^{32}P from radiolabelled ATP into brain proteins from epileptics and nonepileptic fowl was studied by incubating 100 µg protein in 100 µl of 50 mM Tris-HCl buffer pH 7.4 containing 10 mM $MgCl_2$, 10 mM $CaCl_2$ and approximately 5 µM [α^{32}P] ATP at 35°C for 20 seconds. The reactions were terminated by the addition of 50 µl of a stop solution consisting of 5% SDS, 5% sucrose, 3 mM EDTA, 30% mercaptoethanol and 30 mM Tris-HCl pH 7.5. The proteins were then subjected to SDS polyacrylamide gel electrophoresis, stained with coomassie blue, dried and placed in contact with x-ray film for a period of time ranging from 24-72 hours.

No differences were observed in the SDS-polyacrylamide brain protein profiles of carriers and epileptics. However, three major electrophoretic bands in the epileptics showed significantly less net incorporation of ^{32}P than did the corresponding bands from non-epileptic fowl. These proteins had approximate molecular weights of 72, 60 and 16 x 10^3. Although these proteins were present in all the brain areas studied, the abnormal phosphorylation was evident only in the proteins from the cerebral hemispheres and the optic lobes. Subfractionation of the homogenates revealed that the low molecular weight protein was associated with the myelin fraction whereas the two higher molecular weight bands were present in the 100,000 x g supernatant fraction.

To our knowledge, this is the first reported abnormality in endogenous brain protein phosphorylation that is associated with an observable neurological syndrome. The finding of abnormalities in brain protein phosphorylation associated with high seizure susceptibility may provide a valuable model for studies on the cause of epilepsy as well as providing some insight into the role that phosphorylation of neuronal proteins has in normal brain function.

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151.16 ACUTE EFFECTS OF KAINIC ACID ON ENERGY STATE IN THE STRIATUM OF THE MOUSE. Konrad C. Retz and Joseph T. Coyle, Dept. of Pharmacol. and Expt. Therapeut., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

The proximate mechanism of neuronal degeneration caused by intra-striatal injection of kainic acid (KA) may be an irreparable alteration in the energy state of affected neurons due to continuous depolarization. To test this hypothesis, striatal energy metabolism was measured in the mouse. KA (1.64 nmoles in 0.3 1 saline) was stereotaxically injected into the left striatum of ether-anesthesized adult male mice using the coordinates 0.6 mm A, 2.0 mm L and 3.4 mm V to the bregma. At 48 hr after treatment, the specific activities of choline acetyltransferase and glutamic acid decarboxylase in the striatum were reduced by 51 ± 7% and 50 ± 7% respectively, whereas specific activity of tyrosine hydroxylase was increased by 145 ± 22% of contralateral (n = 7-8). Histologic examination of Nissl-stained sections through the forebrain revealed a sphere of neuronal degeneration of 2.7 mm in diameter in the head of the striatum. Except for neuronal degeneration in the deep layers of the overlying frontal cortex, structures outside the striatum including the pyriform cortex and dentate gyrus of the hippocampus were unaffected. Thus, KA causes a pattern of selective neuronal degeneration in the mouse striatum similar to that described in the rat.

Striatal parameters of energy metabolism were measured in mice sacrificed by focused microwave irradiation (1300 V; 2450 MHz; 0.95 sec) at 0.5, 1 and 2 hr after the 1.64 nM KA injection. In control striatum, the values in umoles/g were: phosphocreatine 3.4 ± 0.2,ATP - 2.2 ± 0.1, ADP 0.42 ± 0.04, AMP 0.12 ± 0.01, total adenosine nucleoside phosphates (Ad-P) - 2.8 ± 0.1, glucose 1.3 ± 0.1 and lactate 1.8 ± 0.2 with an energy charge of 0.89 ± 0.01. The content of ATP and phosphocreatine was significantly decreased at all time points but was lowest at 2 hr with a 23 ± 4% decrement in ATP and a 30 ± 5% fall in phosphocreatine in the lesioned striata relative to the contralateral (n = 8). ADP content was not significantly altered whereas AMP was increased by 70 ± 28% and the Ad-P pool was decreased by 20 ± 4% resulting in an energy charge depression from 0.87 ± 0.01 in the contralateral to 0.79 ± 0.02 in the lesioned striatum ($p < 0.005$; n = 8). An increase in glycolytic activity was evidenced by a 44 ± 8% increase in lactate levels in the lesioned striatum accompanied by a 58 ± 7% decrease in glucose content as compared to the contralateral striatum (n = 7). These findings demonstrate that the acute neurotoxic affects of kainic acid are associated with a significant alteration in the energy state of the lesioned brain area.

151.17 EPILEPTOGENIC EFFECT OF KAINIC ACID ON HIPPOCAMPAL SLICES MAINTAINED IN VITRO. E. Lothman and J. Ferrendelli. Div. of Clin. Neuropharm. and Dept. Neurology, Wash. Univ. Med. Sch., St. Louis, MO 63110.

We have previously shown that intravenous kainic acid (KA), a rigid analogue of glutamate, is a potent and selective activator of seizures in limbic structures (Neurology 30:386, 1980). To investigate possible mechanisms for this epileptogenic action of KA, we have studied the effects of this drug in vitro.

Slices of guinea pig hippocampus (400 µm) were prepared and maintained according to standard techniques and constantly perfused with an oxygenated, physiological buffer with an ionic composition similar to brain ECF (127 mM NaCl, 2 mM KCl, 1.5 mM $MgSO_4$, 1.5 mM $CaCl_2$, 26 mM $NaHCO_3$, 1.1 mM KH_2PO_4, 10 mM glucose, pH 7.4). Extracellular recordings were led from CA_3 and CA_1, and stimulation of mossy fibers and Schaffer collaterals provided a measure of the efficiency of orthodromic excitatory connections.

KA produced several changes in the spontaneous activity of hippocampal slices. From 0.01 to 0.1 µM KA the discharges of single units increased. Between 0.1 to 1 µM this increased activity became synchronized into discrete epileptiform bursts (interictal spikes-IIS), similar to those produced by penicillin. Up to 1 µM, IIS had a regular rate proportional to the concentration of KA, but they disappeared with higher concentrations. IIS were synchronized in CA_3 and CA_1 with intact slices, and transections between these two areas blocked IIS in CA_1 but not in CA_3. Both potentials evoked in CA_3 by mossy fiber stimulation and potentials evoked in CA_1 by Schaffer collateral stimulation were augmented and prolonged with afterdischarges over certain concentration ranges of KA, beginning at a level subthreshold for IIS (0.01 µM) and ranging to 1 µM (CA_3) and 10 µM (CA_1). Above these levels the potentials were suppressed or reversed in polarity.

We conclude that, since KA produces epileptiform activity in hippocampal slices in the presence of physiological ion concentrations and selectively induces seizures in limbic centers in vivo, this system is a useful model to study mechanisms of epilepsy, especially temporal lobe seizures. Based on the changes in evoked potentials and results obtained in other systems, we suggest that epileptogenic amounts of KA enhance excitatory glutamatergic connections while higher concentrations tonically and excessively depolarize neurons. Our data also imply that within the hippocampus epileptic discharges arise at discrete loci with secondary spread to other areas.

Supported by USPHS Grant NS-14834 and a grant from the Pharmaceutical Manufacturers Assoication Foundation.

151.18 TOPICAL PENICILLIN REDUCES GABA-MEDIATED INHIBITION IN FELINE CEREBELLAR CORTEX IN VIVO. J. Davenport. VA Medical Center, Departments of Neurology and Physiology, University of Missouri, Columbia 65201

A current working hypothesis for the epileptogenic effects of penicillin (PCN) ascribes induced cellular hyperactivity to the reduction of synaptic inhibition mediated by GABA. Evidence from many simple synaptic systems supports the hypothesis, but such an effect has been at times difficult to demonstrate in more complex mammalian tissues.

Experiments were conducted with intact, anesthetized cats, using standard stimulation and recording techniques in the cerebellar vermal cortex. Glass micropipette electrodes recorded extracellular field potentials at several levels in the molecular and Purkinje layers, evoked by stimulation of nearby cortical surface and of Purkinje axons in subcortical white matter.

Topical application of 133 mM Na-PCN to the cortical surface reduced both the slow positive field potential following surface stimulation attributed to GABA-mediated basket cell IPSP currents, and the surface-evoked inhibition of antidromic Purkinje activation. Considerable variability of these effects was seen among several experiments.

In the absence of intracellular recordings from Purkinje and basket cells, these data are presumptive evidence of a local blockade by PCN of GABA-mediated postsynaptic inhibition in feline cerebellar cortex.

Supported by VA Medical Research and the Epilepsy Foundation of America

151.19 **SUBSTANCE P DEGRADATION AND THE DEVELOPMENT OF STABLE ANALOGS.** **C.M. Lee*, M.R. Hanley, B.E.B. Sandberg* and L.L. Iversen*. MRC Neurochemical Pharmacology Unit, University of Cambridge, England.**

Substance P (SP) is degraded rapidly by neural tissues. In view of its possible role as a neurotransmitter, attempts were made to identify the enzymatic cleavage sites involved in its inactivation. A SP-degrading enzyme preparation was highly purified from a membrane fraction of human diencephalon. Enzyme activity was monitored by measuring the rate of disappearance of added SP with radioimmunoassay.

By a combination of high performance liquid chromatography and amino acid analysis, the break-down products were identified and the enzyme preparation was shown to cleave SP between Gln^6-Phe^7, Phe^7-Phe^8 and Phe^8-Gly^9 with an endopeptidase action. A similar cleavage pattern was also demonstrated in membrane fractions prepared from rat brain. This information was used in the design of stable analogs of SP. Stabilization against enzymatic digestion was tested by incubation with the purified enzyme preparation and with washed rat brain slices. Biological activities were routinely tested on guinea pig ileum, isolated rat parotid gland fragments, rat vas deferens and rat salivation in vivo. Several analogs have been synthesized which are stable towards enzymatic digestion and have prolonged biological activities both in vitro and in vivo.

151.20 STIMULATION DEPLETES SEROTONIN AND NORADRENALINE FROM VESICLES OF PINEAL SYMPATHETIC NERVES. G. Jaim-Etcheverry and L. M. Zieher*. Instituto de Biología Celular, Fac. de Medicina, Buenos Aires, Argentina.

The sympathetic fibers innervating the pineal gland of the rat contain serotonin in addition to the neurotransmitter noradrenaline. Previous cytochemical studies at the ultrastructural level have shown that both amines are stored in the cores of the vesicles that are characteristic of those nerves. Indirect biochemical and pharmacological evidence suggests the possible coexistence of both amines in these nerve vesicles. Thus, it is conceivable that serotonin might be released together with noradrenaline from pineal nerves by stimulation.

To study this possibility, the preganglionic nerves to the superior cervical ganglia of rats were bilaterally stimulated with square wave pulses (25 Hz, 1 msec, 20 min) at supramaximal voltage. After stimulation or sham-stimulation, the pineals were processed with conventional techniques for electron microscopic observation and with cytochemical procedures for the ultrastructural demonstration of serotonin and of noradrenaline plus serotonin (Jaim-Etcheverry, G. and Zieher, L.M., J. Histochem. Cytochem. 16:162, 1968). Electrical stimulation markedly reduced the number of dense cores of nerve vesicles in conventionally processed pineals. Vesicular size and shape were modified by nerve stimulation. The cores reacting for serotonin that are normally present in these nerves, were almost absent after nerve stimulation. A similar observation was made in tissues processed with the technique showing noradrenaline and serotonin. These results indicate that both amines are depleted from their vesicular stores by nerve stimulation. The content of noradrenaline was reduced in stimulated pineals when compared with that of controls. Stimulation of the nerves for longer periods (60 min) at a frequency of 10 Hz, resulted in a similar reduction of cytochemically reactive cores. When the glands were incubated "in vitro" in the presence of 5-hydroxydopamine after stimulation, the vesicles incorporated the exogenous amine, showing that even after depletion by stimulation, they retain the capacity to store monoamines.

These findings support the assumption that several substances with presumed transmitter or modulatory functions might be released simultaneously by nerve stimulation from a given terminal. (Supported by grants from CONICET, SECYT and Secretaría de Salud Pública, Argentina).

151.21 SMALL GRANULAR VESICLES IN THE LOCUS COERULEUS MAY INDICATE DENDRITIC RELEASE OF NOREPINEPHRINE. L.Y. Koda, G. Aston-Jones, and F.E. Bloom. The Arthur V. Davis Center for Behav. Neurobiol., The Salk Institute, La Jolla, CA 92037.

Small granular vesicles (SGV's) appear to be a distinguishing characteristic of monoamine-containing structures. SGV's have been tentatively localized within dendrites of norepinephrine (NE)-containing neurons in the rat locus coeruleus (LC). This result may indicate that LC neurons release NE from their dendrites, and follows from previous evidence suggesting dendritic release of transmitter from DA-containing neurons in substantia nigra.

Male rats (150-250 g) were perfused with 150-200 ml of a cold 2% glyoxylic acid-0.5% paraformaldehyde solution. Razor blade sections from the rostral pons were trimmed to include the LC. Tissue blocks were fixed (30-45 minutes at 4°C) in potassium permanganate, then stained (in 1% uranyl acetate), dehydrated, embedded and sectioned. SGV's were observed in LC perikarya and neuropil. SGV's within perikarya were often clustered near the periphery of the soma, but were also seen individually within a group of agranular vesicles. In the neuropil, SGV's were found in large (1-2 μm), medium (0.5-1 μm) and fine (200-300 nm) processes. The large processes (tentatively identified as dendrites) contained mitochondria and few vesicles. The medium-sized profiles contained mitochondria and many vesicles, and accounted for about 2.5% of the total synaptic bouton population. Approximately 9% of these medium-sized SGV-containing profiles were in synaptic-like apposition to dendritic profiles. The fine processes, although rare, have not been observed to make synaptic-like appositions and presumably represent intervaricose segments of NE-containing axons. All SGV's within the LC were absent 4-6 hrs after a 10 mg/kg i.p. dose of reserpine, indicating that endogenous SGV's may reflect storage sites for NE within LC.

The presence of SGV's in LC dendrites does not in itself indicate dendritic release of NE; additional support for this notion is found in certain electrophysiological properties of LC neurons. The waveform typical of LC neuron spikes suggests that substantial dendritic depolarization follows antidromic or orthodromic soma impulse activity. In addition, there is pharmacological as well as electrophysiological evidence for recurrent collateral interaction among the NE-containing neurons within LC.

Further anatomical and electrophysiological studies are underway to test more definitively the possibility that SGV's in LC neuron dendrites reflect release sites of NE. (Supported by the B.W. Foundation of L.A. and AA 07273).

151.22 RELEASE OF NOREPINEPHRINE FROM HYPOTHALAMUS AND BRAINSTEM OF GENETICALLY OBESE MICE(obob). M.F. Callahan*and G.A. Oltmans. Dept. Pharmacol., Chicago Medical School, Chicago IL 60612.

Recent work suggests an abnormality in noradrenergic function in genetically obese(obob) mice. Mice with the obob mutation have significantly elevated levels of hypothalamic(HT) and brainstem norepinephrine(NE)(Lorden et al., 1976, Brain Res.; Feldman et al., 1979, Hormone Res.). Other studies indicate that the obob mouse is less sensitive than its lean littermates to depletion of HT NE by 6OHDA or reserpine(Lorden, 1979, J.Comp.Physiol.Psych.; Oltmans, 1980, Neuropharmacol.). In the current study, adrenergic function was further explored by examining the effects of K^+ and amphetamine(AMPH) upon NE release from chopped suspensions of HT and brainstem tissue of obob and lean mice.

Baseline NE release(15 min at 37°C) in unstimulated HT tissue was significantly higher in obob mice(3.22 +/- 0.99 ng/mg protein N=11) than in lean mice(2.22 +/- 0.60, N=12). When stimulated by 15 mM K^+, NE release increased more in lean than in obob tissue, producing nonsignificantly different amounts of total NE release for the two groups(lean=2.82 +/- 0.97; obob=3.27 +/- 0.81). At high K^+ concentrations(85 mM) obob HT tissue suspensions again released greater amounts than suspensions from lean tissue(obob= 6.18 +/- 0.86; lean=4.32 +/- 0.85). However, when the stimulated release was expressed as a change from baseline release, no significant differences between obob and lean mice were found in the amount of NE released at the higher K^+ concentrations.

AMPH stimulated release was similar to K^+ release. At low AMPH concentrations(10^{-8} to 10^{-7}M) HT NE release did not differ between lean and obob mice. At higher AMPH concentrations (10^{-4} M) HT tissue from obob mice released more NE than HT tissue from lean mice(4.32 +/- 0.72 vs. 2.60 +/- 0.69, $p < .05$). Again, when the stimulated release was expressed as a change from baseline, there were no differences between lean and obob mice.

Both K^+ and AMPH stimulated release from brainstem preparations paralleled the findings with HT tissue.

In summary, nonstimulated baseline release of NE is higher in obob than in lean mice. At low concentrations of either K^+ or AMPH these differences disappear. They then reappear when the concentrations of K^+ and AMPH are increased. Although NE release in obob mice is not different than in lean mice in all cases, the data at high K^+ and high AMPH concentrations are consistent with the possibility of abnormal NE activity in obob mice. (Supported in part by grant # 1 RO 1 NS15600 from NINCDS)

151.23 ADRENAL INFLUENCE ON NORADRENALINE RECEPTORS IN THE HIPPOCAMPUS. D.C.S. Roberts and F.E. Bloom. A.V. Davis Ctr. for Behav. Neurobiology, Salk Inst., La Jolla, CA 92037.

Bilateral 6-hydroxydopamine (6-OHDA) lesions of the dorsal noradrenergic bundle (DB) have been shown to increase ^{3}H-dihydroalprenolol (DHA) binding in the hippocampus. The effect of adrenalectomy on this increase in receptor binding was examined and a significant interaction between adrenalectomy and the 6-OHDA treatment was observed. Male 300g rats received bilateral stereotaxically placed infusions of 6-OHDA (4 μg/1μl) into the DB (A:+2.6, L:±1.1, D:+3.7 from stereotaxic zero; incisor bar, -4.2) followed 3 weeks later by either bilateral adrenalectomies or sham operations, and were sacrificed 1 week later. Binding of ^{3}H-DHA was determined in the presence and absence of 1 μM alprenolol. Scatchard analysis revealed the following values (from 4-7 replications):

	K_D (nM)	max binding ± S.E.M. (fmole/mg protein)
control	1.67	56.0 ± 4.3
DB-6OHDA	1.72	*79.4 ± 3.9
DB-6OHDA-adrenalectomy	1.51	†*94.4 ± 4.8
Adrenalectomy	1.38	61.7 ± 7.1

*$p < .05$ significantly different from control
†$p < .05$ significantly different from DB-6OHDA

No significant increase in receptor binding was observed after adrenalectomy. The 6OHDA treatment produced a 41% increase in maximum binding and the combined treatment produced a significantly greater increase (68%). Daily treatment with corticosterone (s.c., 1.0 mg/kg/12 hours) for one week following the adrenalectomy reduced the effect of the adrenalectomy. These data argue for a steroid involvement in the regulation of central noradrenergic receptors in the hippocampus.

Previous behavioral experiments have shown an apparent interaction between lesions to the dorsal noradrenergic bundle and adrenalectomy. Either treatment alone did not significantly affect the acquisition of shock avoidance behavior, whereas rats which received both treatments were markedly impaired in their ability to learn the appropriate response (Ogren and Fuxe, Neurosci. Letters, 5 (1977) 291). These observations together with the present data suggest that steroids may interact with central NA receptors to be an intregral part of the learning process in stress related situations. (Supported by Medical Research Council of Canada.)

152.1 IONTOPHORETIC APPLICATION OF ADRENERGIC AGONISTS POTENTIATES ANGULAR BUNDLE EVOKED FIELD POTENTIALS IN THE DENTATE GYRUS. C. Harley and R. Neuman. Dept. of Psychology and Basic Sciences Division, Memorial Univ. of Nfld., St. John's, Nfld. A1B 3X9.

An interest in norepinephrine (NE)'s putative involvement in forebrain plasticity led us to explore the effects of iontophoretically applied NE on evoked field potentials in the hilar region of the dentate gyrus.

Adult rats were first anesthetized with halothane in oxygen and then maintained on urethane 1.1 mg/kg supplemented as required. Seven barrel micropipettes were used for recording and drug applications. Solutions used included 4M NaCl for recording and current balance barrels; 0.1M NE; 0.5M tyramine; and 0.5% HRP with 0.05% adenosine in phosphate buffer, pH 6,8. Other putative neurotransmitters and a variety of adrenergic agonists and antagonists were also included in particular experiments.

The micropipette and a concentric stimulating electrode were located relative to bregma with the rat's skull adjusted to the horizontal plane. Co-ordinates for the micropipette were AP - 3.5 mm and ML ± 2 mm. Depth was determined by the shape and amplitude of the dentate field potential. Placements in the granular cell layer of the upper blade and in the infragranular layer of the dentate hilus were confirmed by HRP iontophoresis. Field potentials were evoked by constant current biphasic pulses delivered through a stimulating electrode angled at 20° in the AP plane (AP - 8.5 mm, ML ± 4.1 mm, DV - 3.75 mm). Stimulus frequency was varied between 0.2 and 0.1 Hz. Stimulus intensity was adjusted to produce a submaximal population spike. Evoked potentials were recorded via the center barrel of the micropipette, processed by computer and displayed on a chart recorder.

Application of NE and tyramine (80-100nA) for 2-4 min. resulted in increases in the population spike amplitude ranging from 20% to 400%. These increases outlasted the drug application by at least several minutes and often continued for as long as observations were made (20-40 min.). With this complex potential it was not always possible to evaluate EPSP changes but in some cases a clear enhancement was observed. Preliminary analyses with adrenergic agonists and antagonists suggest that beta receptors rather than alpha receptors are mediating the potentiation obtained.

These results imply that NE plays a modulatory role in gating throughput in the hippocampus. Further, the long time course of these effects suggests a possible role for NE in long term plasticity.

Supported by grants from MRC and NSERC of Canada.

152.2 Frequency specific effects of dopamine in the nucleus accumbens. J.F. DeFrance, R.W. Sikes, G. Palmer, and R.B. Chronister. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX and Department of Anatomy, University of South Alabama, Mobile, Ala.

Significant controversy has arisen in the past several years concerning the function of dopamine (DA) in the central nervous system. The purpose of the present is to show some attributes of DA function in the nucleus accumbens that add additional complexities to any model of DA function.

Electrophysiological observations were taken from rabbits, anesthetized with urethane. Stimulation micropippettes (1-5 meg ohm) were placed in the lateral margin of the ipsilateral fimbra (IFIM). Multi-barrel micropipettes were used for recording field potentials in the nucleus accumbens following IFIM stimulation, and for the iontophoresis of DA (10 mM-50 mM, pH 6.5). To test for the specificity of the DA effect, haloperidol (Haldol, 0.3-0.7 mg/kg) was injected intravenously.

To determine the potential for DA to stimulate adenyl cyclase activity, the nucleus accumbens was removed and homogenized in cold glycylglycine buffer (2 nM + 1 nM Mg SO_4) + 0.1 mM EGTA, pH 7.4). All assays were in duplicate.

DA had a differential effect which depended upon the frequency of IFIM stimulation. At 0.5 Hz, DA had a suppressive effect which was relatively slow to develop and slow to dissipate. In contrast to the suppressive effects at 0.5 Hz, DA has essentially no effect on responses at a stimulation frequency of 6.0 Hz. Haloperidol was able to reverse the suppressive effects of DA at 0.5 Hz. The data indicated that the suppressive effects are independent of modifying intrinsic inhibitory mechanisms.

Concentrations of 0.1 - 100μM DA were tested for the ability to elicit adenylate cyclase activation. Maximal enzyme activation (47%) was observed at a 100 μM concentration; the ED (50) value was 4.5 μM. Fluphenazine effectively blocked the DA activation of adenylate cyclase.

These results suggest that DA is capable of enhancing cyclic AMP activity in the nucleus accumbens and the resultant inhibition is expressed largely when incoming signals are arriving at relatively low frequencies.

152.3 EFFECT OF IONTOPHORETICALLY APPLIED DOPAMINE ON THE RESPONSE OF NUCLEUS ACCUMBENS NEURONS TO ELECTRICAL STIMULATION OF THE AMYGDALA. C. Y. Yim* and G. J. Mogenson. Dept. of Physiology, University of Western Ontario, London, Canada N6A 5C1.

The nucleus accumbens (NA) receives inputs from various limbic structures and in turn projects to the globus pallidus. It also receives strong dopaminergic (DA) projections from the ventral tegmental area (VTA). The NA has received considerable attention as a possible interface between the limbic and motor systems of the brain. The present study investigates the effect of DA on the response of NA neurons to amygdala (AMG) stimulation to test the possible role of DA in gating the transfer of signals from the limbic system to the motor system.

Extracellular single unit recordings were obtained from neurons in NA of urethane-anesthetized rats using glass micropipettes filled with 0.5 M sodium acetate. Recordings were obtained from the medial part of NA (1.1-1.5 mm lateral to the midline) shown to receive the heaviest DA projection from the VTA. Effects of electrical stimulation of the AMG (500 μA, 0.15 ms) was investigated. In some experiments, 7-barrel glass micropipettes were used for recording and iontophoretic application of DA or glutamate.

NA neurons were observed to have a slow discharge rate with bursts of 3-4 spikes. Electrical stimulation of AMG elicited in 64% (89/139) of the units tested a relatively short latency (mean = 9.5 ms) excitation followed by a prolonged period of inhibition. 17% (24/139) were inhibited only and 19% (26/139) did not respond. Iontophoretically applied DA at > 15 nA decreased the baseline firing rate (BFR) of all units tested. When DA was applied at 5-10 nA the excitatory response to stimulation of the AMG was either markedly attenuated or abolished in 28 of 32 units tested, even in the absence of a significant change in BFR. The prolonged inhibition that followed the original excitation remained unchanged. For neurons inhibited only by AMG stimulation, 4 out of 14 showed an enhanced inhibition when DA was applied.

These observations indicate that the AMG sends a strong excitatory projection to a large proportion of NA neurons. Dopamine appears to modulate this excitatory response. The mechanism of this modulatory effect is not known but it is of interest to note that there was no significant change in BFR and the inhibitory response that followed was not modified. It is also not known if the recorded units are output neurons from NA and if DA released from the VTA-NA projection would have the same effect. These possibilities are being investigated.

(Supported by MRC of Canada)

152.4 EVIDENCE THAT BENZODIAZEPINES AND NOREPINEPHRINE ACT THROUGH A COMMON MECHANISM TO FACILITATE PURKINJE CELL RESPONSES TO GABA. W. Smith*, B. D. Waterhouse, H. C. Moises, H. H. Yeh, H. M. Geller, and D. J. Woodward. (SPON: L. Grandison). Dept. of Cell Bio., Univ. of TX Health Sci. Ctr., Dallas, TX 95235; and CMDNJ-Rutgers Med. Sch., Dept. Pharmacology, Piscataway, NJ 08854

We have previously shown that microiontophoretically released flura zapam (FLU) and norepinephrine (NE) can potentiate Purkinje cell (PC) inhibition induced by iontophoresis of GABA. The hypothesis tested here was that both FLU and NE share a common mechanism to alter cerebellar neuronal responsiveness to GABA.

Extracellular single unit PC activity was recorded from halothane-anesthetized, naive FLU-tolerant and FLU-withdrawn albino rats using a multibarreled pipette. Responses to microiontophoretic pulses of GABA were examined before, during, and after NE (0.5 M) or FLU (0.1 M) iontophoresis. Peri-event histograms were used to quantitate effect of NE and FLU on spontaneous activity and GABA-induced inhibition. Doses of GABA sufficient to produce depression of PC activity in naive rats (5-40 nA) depressed firing rate in all 19 PC cells tested in tolerant animals. In 14 tolerant cells, responses to GABA were either unchanged (10 cells) or reduced (2 cells) during FLU (2-25 nA) iontophoresis, despite suppression of spontaneous firing. Similarly, in 8 of 12 cells, NE was ineffective in potentiating GABA actions despite causing depression of background discharge. Augmentation of GABA was observed with FLU (2 cells) or NE (4 cells); however, such facilitating effects were substantially weaker than similar actions demonstrated in naive rats. Tests in tolerant animals, 48 hr after withdrawl of FLU injections, showed potentiation of GABA inhibitory action by FLU and NE in 4 of 5 and 3 of 5 cells, respectively. In naive animals, interactions between FLU and GABA were examined during concurrent iontophoresis of sotalol. Gaba potentiation by NE was reversibly antagonized by sotalol in 2 of 3 cells; no antagonism of FLU potentiation by sotalol was observed in 3 other cells. In summary, the results presented here suggest that NE and FLU potentiate PC response to GABA via a common mechanism beyond the point of noradrenergic receptor activity. Moreover, results presented here represent the initial electrophysiological demonstration of tolerance to benzodiazepine actions in the CNS. (Supported by NIDA DA-02338 to D.J.W.; NIH 2F 32N5-056-99-03 to B.D.W.; NIH NS 15468 and NSF BNS 79-14003 to H.M.G., and the Biological Humanics Foundation.)

152.5 ONTOGENY OF NORADRENERGIC INPUT TO RAT CEREBELLUM: INTERACTIONS OF NOREPINEPHRINE WITH IMMATURE CEREBELLAR CIRCUITRY. Hermes H. Yeh, Hylan C. Moises, Barry D. Waterhouse and Donald J. Woodward. Dept. Cell Biology, Univ. Tx. Hlth. Sci. Ctr., Dallas, Tx. 75235

We have begun an initial series of experiments to characterize the course of functional development of the norepinephrine (NE)-containing fiber afferents to the cerebellum. Earlier work from our laboratory has demonstrated that chemosensitivity exists in Purkinje cells (PC) at birth, and that PC responses to parallel and climbing fiber synaptic inputs can be elicited by day 3. The present study was conducted to evaluate the interaction between iontophoretically applied NE and GABA, and between NE and climbing fiber synaptic input in the immature cerebellar circuitry.

Multibarrel micropipettes were used to apply drugs in halothane-anesthetized rat pups. Single-barrel recording electrodes attached to the multibarrel assembly were critical for obtaining stable recordings of PC unit responses while applying drugs. Three shocks (0.2 msec duration at 0.1/sec) were applied to the sensorimotor cortex through an array of 3 bipolar concentric electrodes to evoke climbing fiber responses. Poststimulus time or drug response histograms were computed before, during and after NE iontophoresis.

In 27 cells tested on selected days over the first 2 weeks of postnatal development, PC spontaneous discharge was consistently inhibited by NE and GABA iontophoresis. In 13 cells tested for NE interaction with evoked climbing fiber complex spikes, there occurred a preferential inhibition of spontaneous activity over complex spikes in all cases. Sweep-by-sweep analysis of oscilloscope tracings revealed that NE induced a bias toward complex spikes by converting inactivation spike and waves to groups of full-size action potentials. In many cases, single spikes with no evidence of inactivation waves were converted to distinct climbing fiber bursts during NE. These results were observed as early as day 6. In 7 of 9 cells, inhibitory responses to GABA were augmented during application of NE at levels subthreshold for inducing significant depressant effects on background discharge. This NE enhancement of GABA inhibition exists by day 5 when neither basket-stellate cell contacts nor glial cell investment of neuronal processes have fully matured.

In summary, evidence is provided here that NE can exert differential effects early in development by inhibiting spontaneous activity while promoting activity evoked by afferent climbing fiber inputs. Furthermore, the ability of NE to enhance GABA-induced inhibitions at day 5 suggests that PC postsynaptic receptivity as revealed by interactions between putative transmitters is programmed early into development and is not dependent on the emergence of adult synaptic function and circuitry. (Supported by NSF BNS77-01174 and the Biological Humanics Foundation)

152.6 MODIFICATION OF VISUAL RESPONSE PROPERTIES OF CEREBELLAR NEURONS BY NOREPINEPHRINE. Hylan C. Moises, Richard A. Burne and Donald J. Woodward. Dept. of Cell Biology, The University of Texas Health Science Center, Dallas, Texas 75235.

Previous studies from our laboratory have demonstrated in the rat that norepinephrine (NE), released iontophoretically or by electrical stimulation of the noradrenergic pathway from the locus coeruleus, can enhance the responses of cerebellar Purkinje (P) cells to both excitatory and inhibitory afferent input. To determine the functional significance of this noradrenergic action in cerebellar information processing, we characterized here the effects of local NE iontophoresis on the response properties of visually sensitive cells in the rat paraflocculus, a newly identified cerebellar visual cortical projection zone (Burne and Woodward, 1978, Neurosci. Abst. 4:64).

Extracellular recordings of single unit activity were obtained in unanesthetized, immobilized Long-Evans hooded rats, and perievent raster and histogram analyses were employed to determine neuronal response properties following the presentation of computer controlled visual stimuli. Noradrenergic effects on the response pattern of a cell to visual presentation were assessed by construction of raster and histogram records before, during and after iontophoresis of NE.

Presentation of visual stimuli elicited pure excitatory, inhibitory or mixed excitatory-inhibitory responses in 13 of 19 total neurons and in 9 of 14 identified paraflocular P cells. Responses of visually sensitive cells were elicited by flashed on-off stationary or moving bars of light (at 80-240°/sec velocities) and showed evidence for directional, velocity and positional sensitivities. NE was applied at levels which depressed background neuronal activity in 15 of 19 cells. In 11 of 13 visually responsive units tested with NE, stimulus-related excitation was increased above control levels (5 cells) or enhanced relative to the suppression in background firing (6 cells) such that signal to noise ratios were markedly increased. Moreover, during NE application marked excitatory or inhibitory responses were elicited in 4 of 5 P cells previously found unresponsive to presentations of visual stimuli during control periods.

In summary, the overall effect observed with NE application on visual units in the parafloculus was a sharpening of their response properties to stimulus presentation above the ongoing level of background activity. Our hypothesis is that one physiological expression of endogenous noradrenergic activity in cerebellar visual integration may be the refinement of the spatial-temporal response properties (i.e., receptive fields) of visually sensitive cells.

(Supported by NIDA Grant DA-02238 to DJW and a grant from the Biological Humanics Foundation)

152.7 LOCUS COERULEUS STIMULATION POTENTIATES SOMATOSENSORY CORTICAL NEURONAL RESPONSES TO AFFERENT SYNAPTIC INPUTS. Barry D. Waterhouse, Hylan C. Moises and Donald J. Woodward. Dept. Cell Biology, Univ. Tx. Health Sci. Ctr., Dallas, Tx. 75235

Previously we reported that iontophoretically released norepinephrine (NE) facilitated somatosensory cortical neuronal responses to both excitatory and inhibitory afferent synaptic inputs and to direct application of the putative cerebrocortical transmitter agents, acetylcholine and GABA. In this study we examined whether endogenous NE released via electrical stimulation of the locus coeruleus (LC) could enhance somatosensory cortical neuronal responsiveness to afferent synaptic input.

Single glass micropipettes were used to record extracellular activity of neurons in the somatosensory cortex of halothane-anesthetized albino rats. Somatosensory afferent pathways were activated by suprathreshold mechanical stimulation (500 micron punctate deformation of glabrous skin at 0.5 Hz) of the contralateral forepaw. Post stimulus time histograms were used to quantitate the neuronal response before and at various time intervals after preconditioning stimulation of the LC.

All cortical neurons studied were spontaneously active and had one of the following responses to forepaw stimulation: 1) short latency excitation, 2) inhibition or 3) excitation followed by a period of inhibition. In 9 of 13 (69%) cells tested, synaptically evoked excitatory responses were potentiated (from 1.0 to 1.26 spikes/stimulus) when preceded by LC conditioning stimulation (3-6 shocks of 0.2-0.5 msec duration, 100-300 microamp at 100 Hz) which alone produced no change in cortical neuron spontaneous discharge. In a similar fashion, subthreshold LC stimulation increased by 46% the magnitude of stimulus bound inhibition or post-excitatory suppression of activity in 9 of 13 (69%) additional cells. This enhancement of cortical neuronal responsiveness resulting from LC activation was typically observed when conditioning preceded forepaw stimulation by 100-600 msec. Stimulation in areas outside of LC antagonized or had no effect on stimulus bound excitation and inhibition in 6 of 8 and 4 of 8 cases, respectively. These results suggest that increased activity in the coeruleus-cortical pathway may enhance the efficacy of conventional afferent input to somatosensory cortical neurons. Moreover, these data are consistent with the notion that a primary physiological function of the noradrenergic projection system may be to modulate the transmission of sensory information within the CNS.

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152.8 SEROTONERGIC SUPPRESSION OF SOMATOSENSORY CORTICAL NEURONAL RESPONSES TO PUTATIVE NEUROTRANSMITTERS AND AFFERENT SYNAPTIC INPUTS. Donald J. Woodward, Barry D. Waterhouse and Hylan C. Moises. Dept. Cell Biology, Univ. Tx. Hlth. Sci. Ctr., Dallas, Tx. 75235.

The present study was conducted to investigate the action of serotonin (5HT) on transmission of information through somatosensory cortical neuronal circuits. Extracellular activity of cortical neurons was recorded from halothane-anesthetized albino rats using multibarrel micropipettes. Responses to microiontophoretic pulses (10 sec duration at 40 sec. intervals) of the putative cerebrocortical transmitters, acetylcholine (ACH), glutamate (GLUT) and gamma-aminobutyric acid (GABA), were examined before, during and after 5HT iontophoresis (3-20 nanoamps). Excitatory and inhibitory responses of single units to mechanical stimulation (500 micron punctate deformation of glabrous skin at 0.5 Hz.) of the contralateral forepaw were also examined before, during and after 5HT administration. Post-drug and post-stimulus time histograms were used to quantitate 5HT effects on spontaneous and drug or stimulus induced activity.

In 10 of 16 neurons, 5HT suppressed stimulus bound excitation more than background discharge such that signal to noise ratio was decreased approximately one third. Evoked spiking in 6 cells was depressed below control levels during 5HT application despite little change in background activity. Similarly, excitatory responses to ACH and GLUT were antagonized by iontophoretic 5HT in 6 of 10 and 4 of 5 neurons, respectively. In 7 of 7 cases, 5HT blocked stimulus bound inhibition while producing a concomitant, slight depression of background discharge. In a similar fashion, the magnitude of GABA-induced inhibition was effectively reduced relative to baseline firing rate during 5HT iontophoresis in 6 of 9 neurons. The response to GABA was completely blocked in 2 cells.

In summary, these results suggest that low levels of 5HT reduce the responsiveness of somatosensory cortical neurons to afferent synaptic inputs and putative transmitter agents. Parallel studies on norepinephrine (NE) in somatosensory cortex have demonstrated largely opposite actions, i.e. amplification of neuronal responses to synaptic inputs and to application of putative transmitter agents, despite a common effect of both 5HT and NE to suppress background firing. The data suggest that 5HT, along with other monoamines may modulate activity of other conventional transmitter systems as a means of regulating information transfer in the cerebral cortex.

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152.9 EFFECTS OF 5-HT DENERVATION AND DEPLETION ON NEURONAL RESPONSIVENESS TO BIOGENIC AMINES IN CEREBRAL CORTEX: A MICROIONTOPHORETIC STUDY IN THE RAT. A. Ferron*, T.A. Reader and L. Descarries. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada.

In adult rats under urethane anesthesia (1.25 g/kg, i.p.), a vast majority of spontaneously active neurons in the fronto-parietal cortex respond by prolonged depression of their firing rate to microiontophoretic applications (50-100 nA during 30 s.) of serotonin (5-HT), noradrenaline (NA) or dopamine (DA). To further investigate the role of 5-HT in cerebral cortex, the responses to these amines were examined after destruction of 5-HT afferents with 5,7-dihydroxytryptamine (5,7-DHT, 200 µg F.B., i-vt, 2 weeks earlier), or pharmacological depletion of 5-HT stores by pretreatment with p-chlorophenylalanine (PCPA, 400 mg/kg, i.p., q.d. for 2 or 14 d.). In 5,7-DHT deafferented cortex, depression of spontaneous firing rate by 5-HT was of longer duration (mean 14 min.) than in controls (mean 5 min.). Moreover, small ejection currents of 5-HT (2 nA) were sufficient to induce a prolonged depression. In contrast, responsiveness to NA and DA was markedly reduced in units which were supersensitive to 5-HT. In PCPA-pretreated rats, there was no change in the responsiveness to 5-HT despite a mean 90% depletion of cortical 5-HT content. However, as in denervated cortex, responsiveness to NA and DA was significantly decreased. In a third series of experiments, a selective 5-HT uptake blocker, fluoxetine (20 mg/Kg, i.p.), was administered in control and PCPA-pretreated rats to determine to which extent the suppression of 5-HT reuptake contributes in the denervation supersensitivity to 5-HT. Fluoxetine induced prolongation of the response to 5-HT but failed to enhance the sensitivity of cortical neurons to small currents of 5-HT.

These data indicate that, in cerebral cortex, 5-HT supersensitivity results primarily from the removal of 5-HT terminals and not from depletion of 5-HT content. Two components may be involved in the denervation supersensitivity to 5-HT: the suppression of reuptake (presynaptic component) could account for the prolonged duration of responses to 5-HT, whereas a modification of the 5-HT receptor on target cells (postsynaptic component) could be mainly responsible for the greater sensitivity to 5-HT. The decreased sensitivity to NA and DA following 5-HT deafferentation or depletion suggests that the responsiveness to NA and DA may be regulated by 5-HT release.

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152.10 QUIPAZINE FACILITATION OF SPINAL MOTONEURON EXCITABILITY IN 5-7-DIHYDROXYTRYPTAMINE PRETREATED RATS. S.R. White and R.S. Neuman. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3V6.

The purported 5-HT agonist, quipazine (QPZ), has serotonin mimetic actions in the central nervous system which may result from activation of postsynaptic 5-HT receptors, blockade of presynaptic 5-HT reuptake, release of 5-HT or some combination of these mechanisms. To test these alternatives, the ability of iontophoretically applied QPZ to mimic 5-HT enhancement of motoneuron excitability was examined in the spinal cords of 5-7-dihydroxytryptamine (DHT) pretreated rats and control rats which received no pretreatment. Rats were anesthetized with urethane and prepared for spinal cord motoneuron unit recording combined with multibarrel microiontophoretic drug application as described previously (White & Neuman, Brain Res. 188, 1980, 119). Motoneuron activity was evoked by short uniform applications of glutamate cycled automatically. Following the establishment of stable responses to glutamate application, the effects of other drugs on the glutamate elicited activity were examined.

In control rats both QPZ (10-40nA, 30-120 sec) and another purported 5-HT agonist, MK-212 (10-40nA, 60-180 sec), facilitated glutamate elicited motoneuron activity, as did 5-HT (10-30nA, 30-60 sec). The facilitation produced by all three drugs was often, but not always, preceded by short periods of depression during current application. While the period of facilitation often lasted for several minutes for all three drugs, the delay to peak facilitation was greater for QPZ and MK-212 than for 5-HT. Metergoline, 0.1 mg/kg i.v., antagonized the facilitatory effects of 5-HT, QPZ, and MK-212, but not norepinephrine.

DHT pretreated rats received intracisternal injections of DHT (100-200 µg in 20 µL of a saline-ascorbic acid solution) 45 to 90 min. following injections of desmethylimipramine (25 mg/kg, i.p.). The effectiveness of this treatment for destroying spinal cord 5-HT terminals was assessed 2 to 5 days later by the application of the 5-HT releasing agent, p-chloramphetamine (PCA). In control rats, PCA (50-100nA, 30-60 sec) increased glutamate elicited motoneuron activity, while in DHT pretreated rats, PCA (60-100nA, 60-120 sec) produced only initial depression of motoneuron activity with no subsequent facilitation.

Although the PCA results indicated that 5-HT was not being released from nerve terminals in the vicinity of the motoneurons in the DHT-treated rats, QPZ (10-50nA, 60-120 sec) was still capable of facilitating glutamate elicited motoneuron activity. These results suggest that at least some of the serotonin mimetic actions of QPZ can be attributed to direct effects on postsynaptic 5-HT receptors.

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152.11 MODIFICATIONS IN THE EXCITABILITY OF LOCUS COERULEUS SYNAPTIC TERMINALS BY ADRENERGIC AGENTS. S. Nakamura*, J.M. Tepper*, S.J. Young*, and P.M. Groves. (SPON: M. Dubin). Dept. Psychiatry, Univ. Calif., San Diego, La Jolla, CA 92093.

Many experimental studies have suggested the existence of presynaptic receptors on the terminals of catecholaminergic neurons which may participate in feedback regulation of transmitter release, synthesis and other processes. It is possible that presynaptic receptor activation alters the polarization of the terminal membrane in a way similar to the self-inhibitory effects proposed for catecholamines released within such cell groups (Groves et al., Prog. Neuropsychopharm., 3:315, 1979). To test this possibility, the excitability of locus coeruleus neurons was determined using direct electrical stimulation of their terminal fields following local administration of adrenergic agents. Experiments were performed on urethane anesthetized, curarized and artificially respired subjects. Antidromic responses of locus coeruleus neurons were recorded extracellularly following bipolar stimulation of frontal cortical terminals. The tip of a 32 gauge cannula was placed approximately 100 microns from the site of stimulation. Prior to drug infusion, stimulus current was varied in order to determine a current just sufficient to produce 100% antidromic invasion. Adrenergic agents were then infused for a duration of 5 minutes at a rate of 0.0625 microliters/minute and the threshold value redetermined at frequent intervals. Infusion of between 10^{-6} to 10^{-5} molar amphetamine produced a decrease in excitability in 20 experiments, an increase in 7 and no effect in 5 cases. Phentolamine over the same range of concentrations produced an increase in terminal excitability in 5 experiments and no effect in 5 others. Propranolol at these concentrations decreased terminal excitability in 8 experiments with no effect in 2 others. Control infusions of saline in 5 experiments did not alter terminal excitability. Spontaneous activity and action potential waveforms were unaffected by drug infusion. Antidromic latency appeared to covary with changes in terminal excitability. These results indicated that the excitability of noradrenergic terminals can be modified by adrenergic agonists and antagonists and suggest that activation of presynaptic receptors alters the electrical properties of the presynaptic membrane. (Supported in part by grant DA 01467 and Research Scientist Development Award DA 00056 from the National Institute on Drug Abuse to PMG.)

152.12 LOCUS COERULEUS MODULATES LATERAL GENICULATE NEURON EXCITABILITY VIA AN α-ADRENOCEPTOR. Michael A. Rogawski and George K. Aghajanian. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Microiontophoretic application of norepinephrine (NE) in the vicinity of dorsal lateral geniculate (LGN) neurons produces an increase in their spontaneous firing rate and facilitates their responsiveness to optic pathway stimulation. These effects appear to be mediated by an α-adrenoceptor (Brain Res. 182: 345-359; Exp. Neurol., in press). The LGN receives a dense noradrenergic innervation originating exclusively in the locus coeruleus (LC). In the present study, we compared the effects of LC stimulation and iontophoretically applied NE to provide evidence for a transmitter role of NE in the coeruleogeniculate pathway.

Electrical stimulation (1 msec pulses at 10 Hz, 0.3-0.8 mA, 10-60 sec trains) was delivered with coaxial electrodes in the vicinity of the ipsilateral LC of chloral hydrate anesthetised rats. Such stimulation invariably produced an increase in the spontaneous firing rate of LGN neurons. Iontophoresis of NE (5-10 nA) near the same neurons mimicked this effect. LGN neurons were not driven by single shocks to the LC nor was a field potential ever observed. As with iontophoretically applied NE, the effect of LC stimulation was delayed in onset (requiring 1-30 sec to attain a peak response) and sustained beyond the duration of the stimulus train (up to 20 sec). Optimal effects were obtained when the electrode tip was either within the LC or just anterior to the LC, presumably in the dorsal NE bundle. The activating effect of LC stimulation was transiently suppressed by systemic (100 µg/kg, i.v.) or iontophoretic (15-30 nA) administration of the α-adrenoceptor antagonist WB-4101.

When afferent excitation from the retina is eliminated by enucleation of the eyes, many LGN neurons cease firing spontaneously. Silent neurons in enucleated animals generally did not respond to iontophoretically applied NE although they were easily activated by the excitatory amino acid glutamate. However, low currents of NE (5-10 nA) markedly enhanced the responsiveness of these cells to glutamate, suggesting that NE acts in a neuromodulatory fashion to increase their excitability. Similarly, LC stimulation generally failed to excite silent cells in enucleated animals but was able to facilitate the response to glutamate. Both the facilitatory effects of NE iontophoresis and LC stimulation were antagonized by WB-4101. We conclude that activation of the coeruleogeniculate pathway and local application of NE are physiologically and pharmacologically similar in their modulation of LGN neurons excitability.

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152.13 LOCUS COERULEUS NEURONS IN BEHAVIORALLY-INTACT RATS ALTER THEIR SENSORY-RESPONSE AND SPONTANEOUS DISCHARGE CHARACTERISTICS DURING THE SLEEP-WAKING CYCLE. G. Aston-Jones[1] and F.E. Bloom. A.V. Davis Ctr. for Behav. Neurobiol., Salk Inst., La Jolla, CA 92037 and [1]Div. of Biology, Calif. Inst. of Tech., Pasadena, CA.

We previously reported that norepinephrine-containing locus coeruleus (NE-LC) neurons in freely moving rats discharge as a function of sensory stimulation and stages of the sleep-waking cycle (S-WC) . These neurons characteristically responded to non-noxious environmental stimuli with a 15 to 20 msec latency excitation, usually followed by a more variable and longer duration inhibition. During the S-WC, spontaneous rates were highest during active waking (2.03 Hz), lower during slow-sleep (0.65 Hz) and very low in paradoxical sleep (PS) (0.02 Hz).

In our continuing studies on this system, we have found additional characteristic properties of NE-LC neuron discharge. 1) The magnitude of sensory response in NE-LC neurons varies during the S-WC: The greatest response is elicited by stimuli which awaken the animal from slow-sleep (SWS), a smaller response follows stimuli presented during waking (W), and an even smaller response is elicited by stimuli within SWS; NE-LC neurons appear to be unresponsive to such stimuli during uninterrupted PS. 2) Spontaneous rates during the S-WC are higher for neurons located on the edge of the nucleus than for non-edge cells; in addition, neurons in the ventrocaudal pole of LC tend to fire at lower rates than other NE-LC neurons. 3) NE-LC neuron discharge rates anticipate the EEG signs of all S-WC transitions (except PS-to-W, in which discharge activity preceeded only the EMG signs of W); for example, the mean discharge rate during the last sec of SWS before W is higher than the mean rate during SWS or during W. 4) Even during SWS, when NE-LC neurons discharge very slowly, systemic fluctuations in rate accompany EEG spindle activity: The mean discharge rate one sec before spindles (0.43 Hz) is less than the mean rate for SWS (0.53 Hz), while the highest mean rate occurs during spindles (1.11 Hz); the mean rate is then lower for the sec following spindles (0.87 Hz) but remains higher than the mean rate during SWS (all rates differ significantly at $p < .005$ by repeated measures t-tests). These LC impulse rate fluctuations appear to anticipate phasic EEG events within SWS, analogous to the rate fluctuations which anticipate the longer duration EEG signs of SW-C transitions. (Supported by AA 07273 and NS 16209.)

152.14 CATECHOLAMINERGIC MODULATION OF THE CORTICAL SENSORIMOTOR RHYTHM AND THE LIMBIC 40 HZ RHYTHM J. M. Simard*, C. C. Turbes and G. T. Schneider* (SPON: F. J. Clark). Dept. of Anatomy, Creighton Univ., Omaha NE 68178.

In awake, freely-behaving cats with chronically implanted cortical and subcortical electrodes and instrumented for telemetric recording, the differential roles of dopamine and norepinephrine in the management of the state of cortical synchrony (low voltage fast activity vs. the sensorimotor rhythm) and the limbic 40 Hz rhythm were assessed by: 1) comparing the effects of the dextro- and levo-stereoisomers of amphetamine (2.5 mg/kg) on the brain rhythms; 2) assessing the development of tolerance of the brain rhythms to repeated administration of amphetamine. The data were quantified using spectral techniques.

The results obtained indicated that 1) the two isomers of amphetamine were approximately equipotent ($p > .05$) in effecting cortical desynchrony and 2) that within three days of successive daily administration, marked tolerance to the drugs' cortical desynchronizing effect was evident as a return of the sensorimotor rhythm ($p < .05$). Conversely, it was found that 3) the dextro isomer was significantly ($p < .05$) more potent than the levo isomer in activating the limbic 40 Hz rhythm and 4) that the effect of d-amphetamine on the 40 Hz rhythm exhibited no tolerance with successive daily administration.

It was concluded that norepinephrine is a critical modulator of the frontal cortical EEG whereas dopamine is a critical modulator of the limbic 40 Hz rhythm.

152.15 STIMULATION OF LOCUS COERULEUS ALTERS REGIONAL CEREBRAL BLOOD FLOW IN THE RAT. S. M. Feldman[1], S. Baez*[2], R. E. Breger*[1] and G. P. Lu*[2]. Department of Psychology, New York University, New York, NY 10003[1] and Department of Anesthesiology, Albert Einstein College of Medicine, Bronx, NY 10461[2].

To investigate the contribution of locus coeruleus (LC) to the regulation of cerebral circulation, we have examined the effect of LC stimulation on transit time of an intracerebral optical marker. Under pentobarbital anesthesia, a unilateral craniotomy exposed a 1 cm area of parietotemporal cortex in rats. A sealed saline-filled transparent plastic chamber, affixed to the skull, provided a neutral environment for the exposed cortical tissue. Artificial ventilation allowed adjustment to maintain arterial pH, $PaCO_2$ and PaO_2 in normal ranges. The pial microvasculature was viewed microscopically, displayed on a TV monitor, and permanently recorded on tape. A small bolus of colloidal carbon (0.06 to 0.15 ml) was rapidly injected intrajugularly; carbon transit time (TTc) was the interval between first appearance of the visual marker in a selected penetrating arteriole (18 to 25 µm o.d.) and its emergence from underlying brain via selected collecting venules (Caceres, Chung, Baez and Orkin, Fed. Proc., 1975, 34: 749). TTc thus measured circulation time through the cerebrum, which is affected by diameter changes in resistance vessels. Trains of rectangular electrical pulses were delivered to ipsilateral brainstem through permanently indwelling bipolar electrodes for 15 to 20 sec prior to injection of the carbon marker. Stimulation in or near LC (as verified histologically) was accompanied by substantial increases in TTc, with a single exception. The changes in TTc (Mean = +39 ± 9.6%) varied from -1.3% to +77.0%; accompanying changes in mean arterial blood pressure (MAP) varied from -10.2% to +14.5% (Mean = +1.7 ± 3.5%). TTc and MAP are both expressed as per cent changes from control or sham stimulation trials. Effective frequencies of stimulation ranged from 5 to 50 pps. Well placed electrodes required minimal stimulating current to effect large increases in TTc: e.g. 50µa at 25 pps caused a 77.0% increase in TTc, with an accompanying 2.0% increase in MAP. Because changes in MAP and TTc were not inversely related, the small changes in systemic arterial blood pressure can not account for the observed increases in TTc; both increases and decreases in MAP were seen equally. Because regional changes in cerebral vascular resistance would vary directly with TTc, we conclude that the LC stimulation affected regional cerebral blood flow by causing vasoconstriction of intraparenchymal resistance vessels.
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152.16 Role of forebrain noradrenaline in self-administration of ethanol. Michael E. Corcoran, Stephen T. Mason, and Hans C. Fibiger. Dept. of Psychology, University of Victoria, Victoria, B.C., Canada; and Division of Neurological Sciences, University of British Columbia, Vancouver, B.C., Canada.

Bilateral injections of 6-hydroxydopamine (6-OHDA) were made into the dorsal tegmental noradrenergic bundle, and the effects of the resulting depletion of forebrain noradrenaline (NA) on oral self-administration of ethanol were examined. When given a choice between water and a 15% (v/v) solution of ethanol on alternate days, NA-depleted rats drank significantly less ethanol than controls. There was no difference in forced intake of quinine-adulterated water by NA-depleted and control rats, however, suggesting that NA depletion does not produce hyperreactivity to aversive tastes. In a separate experiment ethanol rejection thresholds were determined by offering rats a choice between water and an ascending series of concentrations of ethanol; the lowest concentration totally avoided was arbitrarily designated as the rejection threshold. NA-depleted rats showed significantly lower rejection thresholds than control rats and persisted in drinking less ethanol when subsequently offered a choice between water and ethanol at rejection concentration on alternate days for 40 days. These effects of NA depletion on initiation of self-administration of ethanol were presumably not due to a potentiation of the aversive pharmacological effects of ethanol, as NA-depleted and control rats did not differ in acquisition of a conditioned aversion to a novel flavor paired with intraperitoneal injections of ethanol.

To determine whether depletion of forebrain NA would also disrupt an already established pattern of ethanol self-administration, additional rats were allowed to develop a home-cage preference for ethanol solutions over water before injections of 6-OHDA were performed. Although depletions of forebrain NA comparable to those described above were produced, there was no significant effect on intake of ethanol. Similarly, other rats were induced to consume large quantities of 5% (v/v) solutions of ethanol in the schedule-induced polydipsia paradigm, and injections of 6-OHDA were subsequently performed. Once again depletion of forebrain NA had no significant effect on an established pattern of ethanol self-administration.

These results suggest that forebrain NA is somehow involved in the initiation of oral intake of ethanol but that it is not required for the maintenance of established patterns of ethanol self-administration.

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152.17 ESTROGEN-INDUCED SUBSENSITIVITY OF DOPAMINE AUTORECEPTORS. L.A. Chiodo, A.R. Caggiula and R.R. Lucik*. Psychobiology Program, Department of Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260

Oral administration of conjugated estrogens can significantly ameleorate the clinical symptoms of unipolar depression and also increase the efficacy of antidepressant drugs in women. We recently demonstrated that antidepressants induce a subsensitivity of DA autoreceptors in the substantia nigra of rats, and suggested that the resulting increase in DA function might account for their therapeutic effects (Antelman & Chiodo, this volume). In the present study, we asked whether estrogen produces a similar alteration in autoreceptor sensitivity.

We first tested the effects of estradiol benzoate on the decrease in open field activity produced by doses of apomorphine that are throught to preferentially stimulate DA autoreceptors. All doses of apomorphine tested (25, 200, 400, 800 ug/kg, s.c.) reduced square crossings recorded every 5 min. for 20 min., relative to a pre-drug baseline. Moreover estradiol benzoate (EB; 10 ug/kg s.c.), given 48h before the test, significantly attenuated the decrease in locomotion produced by the 3 highest doses of apomorphine, when compared to oil injected controls.

We also measured the sensitivity of autoreceptors electrophysiologically. Females treated as above were anesthetized with chloral hydrate (400 mg/kg, i.p.) and DA neural activity was sampled through glass micropipetts (2M NaCl, 4-12 megohms). We used a dose of apomorphine (4 ug/kg, i.v.) that has been shown to preferentially stimulate these receptors without affecting postsynaptic DA receptors (Skirboll et al., Science, 206, 80, 1979). Estrogen significantly attenuated the ability of apomorphine to inhibit the activity of type B DA neurons (a class of cells that are inhibited by activating environmental stimuli), but did not alter apomorphine's effect on type A cells (cells stimulated by such stimuli) (mean percent inhibition of firing $\pm$ S.E.: Type A; oil = 51.5 $\pm$ 12.9, EB = 72.3 $\pm$ 12.8. $p > .05$; type B; oil = 65.3 $\pm$ 11.7, EB = -2.6 $\pm$ 9.7, $p < .001$).

In summary, we have presented both behavioral and electrophysiological evidence that a single injection of estrogen induces a significant subsensitivity of DA autoreceptors. This provides one of the first demonstrations of a direct effect of estrogen on mesencephalic DA neuronal activity. It might also account, at least in part, for our finding that estrogen potentiates the stereotypy produced by DA agonists (Chiodo and Caggiula, 1978). Finally, the fact that estrogen, at least in the dose and time course used, induced subsensitivity only in the type B neuron provides further evidence that A and B represent two functionally different classes of DA cells.

152.18 AGE RELATED CHANGES IN DOPAMINE AUTORECEPTOR SENSITIVITY. R.R. Lucik*, L.A. Chiodo and A.R. Caggiula. Psychobiology Program, Dept. of Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Recent evidence suggests that aging is associated with a decline in the functioning of central dopamine (DA)-containing systems. For example, as rats grow older, there is a decrease in DA levels and turnover in the striatum which is accompanied by an impairment in the animals' response to activating stimuli. In the present study we asked if the sensitivity of DA autoreceptors in the substantia nigra also changes with age.

Single unit activity of DA neurons was sampled extracellularly through glass micropipetts (2M NaCl, 4-12 megohms) in 1, 6 and 24 month old male albino rats. As expected, a dose of apomorphine (4 ug/kg, i.v.) that has been shown to preferentially stimulate DA autoreceptors (Skirboll et al., Science, 206, 80, 1979) inhibited the discharge rate in the 6 month old males by 67.7 $\pm$ 6.3% (mean percent inhibition of firing rate $\pm$ S.E.). In contrast, all cells in the 24 month group, and 50% of the cells in the 1 month group increased their activity in response to this dose of apomorphine (group means: -82.4 $\pm$ 31.1% and -28.7 $\pm$ 31.5% respectively). Subsequent administration of a postsynaptic dose of apomorphine (50 ug/kg, i.v.) suppressed discharge rate in all cells of the 1 and 24 month old animals. All neurons sampled increased their activity in response to haloperidol (.2 mg/kg, i.v.), confirming the dopaminergic nature of these cells.

These data suggest that both old (24 month) and young (1 month) animals display a significant degree of DA autoreceptor subsensitivity relative to 6 month old animals. It is possible that in the older animal, the subsensitivity is a compensatory response to an overall decline in DA function, whereas in the young animal, it represents incomplete development of DA regulatory mechanisms. Regarding the fact that a majority of subsensitive cells actually increased their activity in response to a presynaptic dose of apomorphine, two hypotheses might be considered to account for this response reversal. First, the confirmation of the autoreceptor may be altered to such a degree that apomorphine binds to the receptor without stimulating it, and thus exerts an antagonist-like action. Alternately, since both agonist and antagonist receptors appear to exist with respect to apomorphine, perhaps only the agonist receptor becomes subsensitive (or is absent) and thus only the actions of the antagonist receptor are observed.

152.19 CORTICAL MODULATION OF STRIATAL FUNCTION IN THE RAT. B. Scatton, P. Worms*, K.G. Lloyd and G. Bartholini . Synthélabo-L.E.R.S., Research Department, 58 rue de la Glacière, 75013 Paris, France.

The effect of complete surgical destruction of the cortico-striatal projections or selective ablation of the frontal cortex on behavioral and biochemical parameters related to striatal dopaminergic function were investigated in the rat. Bilateral complete surgical destruction of the cortico-striatal projections as well as frontal cortex ablation virtually abolished the cataleptogenic action of haloperidol in the rat. This effect was observed as soon as 3 days after surgery ; the time course of behavioral modifications paralleled changes in glutamate uptake. Thus, glutamate uptake was reduced 2 hours to 16 days following frontal cortical ablation, the maximal effect (- 55 %) being observed after 2 days. Rats bearing such lesions were also found to be more susceptible than sham-operated animals to the stereotypy-inducing effects of apomorphine. In this respect, the duration of stereotyped behavior but not the maximal efficacy of apomorphine was enhanced.

The behavioral changes induced by cortical lesions do not appear to result from changes in striatal dopaminergic and/or cholinergic neuron activity. Thus : 1) acute (2 hours to 7 days) lesions of the cortico-striatal projections or frontal cortex ablations fail to alter baseline levels of, and neuroleptic or apomorphine-induced changes in, striatal homovanillic acid and dihydroxyphenylacetic acid levels in the rat ; 2) striatal acetylcholine (Ach) concentrations were transiently increased 2 hours after frontal cortex ablation, but were unaffected 2-7 days following surgery ; 3) haloperidol decreased and apomorphine increased Ach levels in the striatum to a similar extent in lesioned and sham-operated rats ; 4) finally, lesions of the cortico-striatal pathways failed to affect the ability of apomorphine to reduce the potassium (20 mM)-evoked ^{3}H-Ach release in rat striatal slices preloaded with ^{3}H-choline.

The above findings suggest that the excitatory cortico-striatal (possibly glutamatergic) pathways play an important role in dopamine-dependent behavioral events. As indicated by the biochemical data, the site of this action is likely distal to striatal dopaminergic and cholinergic neurons, and independent of the nigro-striatal feed back activation of dopaminergic neurons.

Other data showing a greater degree of tolerance of cortico-striatal lesioned rats of the increases in striatal dopamine metabolite levels after repeated treatment (11 days) with haloperidol or sulpiride suggest that the cerebral cortex also plays a role in the development of dopamine target cell supersensitivity.

152.20 EFFECTS OF PIMOZIDE ON SIMULTANEOUS DISCRIMINATION PERFORMANCE IN THE RAT. T.N. Tombaugh.* (SPON: W. Webster). Dept. of Psychology, Carleton University, Ottawa, Ontario, Canada K1S 5B6.

The effects of pimozide, a dopamine receptor blocker, were investigated on the ability of a rat to maintain a previously established simultaneous discrimination. Sixteen rats were trained to bar-press for food reward in a two-bar operant chamber. Responding on the bar associated with the appropriate light cue was reinforced on a variable interval 15 sec. schedule of reinforcement. Responses on the other bar were not reinforced. Every 30 sec. the cue randomly alternated between the bars. The status of the cue was counterbalanced across subjects so that an illuminated lamp above the bar signalled reinforced periods (S+) for half of the animals and nonreinforced periods (S-) for the other half. After 20 days of training, 95% or more of all responses occurred during the S+ periods. During subsequent test sessions each animal received 5 doses of pimozide (vehicle, 0.05, 0.10, 0.30 and 0.60 mg/kg) delivered in a Latin Square sequence. Five non-drug days separated each injection. Results showed that pimozide did not disrupt the well established discrimination behavior at any of the doses even though a substantial decrease in rate of responding was observed at the two higher doses. These response decrements were not accompanied by any change in the distribution of responding between the two bars. The relationship between responding and accuracy of responding is consistent with earlier results obtained with a successive discrimination paradigm with pigeons (Tombaugh, Psychopharmacology, 1980, in press). The congruence of these results is important because it demonstrates that pimozide produces similar results regardless of whether the initiation and inhibition of response tendencies occurs sequentially or simultaneously, suggesting that dopaminergic neurons are not essential in the mediation of previously learned associations.

152.21 APOMORPHINE AND S3608 INCREASE LOCOMOTOR ACTIVITY VIA DIFFERENT DOPAMINERGIC SYSTEMS IN THE RAT BRAIN. J. Offermeier* and J.M. Van Rooyen* (SPON: T.N. Chase). Res. Unit for the Design of Catecholaminergic Drugs, Potchefstroom Univ., Potchefstroom 2520, South Africa.

Apomorphine (APO) and S3608 increase locomotor activity (LA) via dopaminergic mechanisms in the rat brain as is evidenced by antagonism of these LA increases by dopamine (DA) antagonists. LA increases produced by these 2 drugs appear to be mediated via different dopaminergic systems, since (a) combination of these 2 drugs at doses producing maximal increases of LA of the drugs given individually results in an additive effect, (b) sulpiride antagonism of LA produced by APO is surmountable but sulpiride antagonism of S3608 is insurmountable, (c) bilateral kainic acid lesions of the nucleus accumbens suppress the activity of DA-sensitive adenylate cyclases in this area as well as S3608-induced LA but not APO-induced LA, (d) S3608 antagonises DA-induced cAMP formation whereas APO is a low-affinity dualist on this system, (e) APO-induced LA is not significantly altered by pretreatment with atropine, whereas S3608-induced LA is clearly potentiated by such pretreatment, (f) lithium carbonate pretreatment suppresses APO-induced LA but potentiates S3608-induced LA and, (g) fenfluramine pretreatment slightly suppresses APO-induced LA but clearly potentiates S3608-induced LA.
It is concluded that APO is an agonist on non-adenylate cyclase dependent DA receptors (D-2 receptors) and S3608 is an antagonist on adenylate cyclase dependent DA receptors (D-1 receptors). These 2 systems are influenced differently by other transmitter systems.

152.22 CHRONIC ADMINISTRATION OF DOPAMINERGIC AGONISTS AND RAT BEHAVIOR: DIFFERENTIATION OF APOMORPHINE FROM ET 495, BROMOCRIPTINE AND TRH. F. Porreca*, A. Cowan and R.J. Tallarida*, Dept. Pharmacol., Temple Univ. Sch. Med., Philadelphia, PA 19140.

A large dose of apomorphine (APO, 20 mg/kg, s.c.) induces aggressive attitudes in male rats (McKenzie, Brain Res. 34: 323, '71). We have found that this dose of APO causes 'sham-boxing' in only 20% of male S.D. rats tested. In contrast, we observed that chronic APO (1 mg/kg, s.c.) at 0900 h and 1800 h elicits a bizarre behavioral syndrome (BBS) in all male and female rats tested. The syndrome is characterized by tail vibration (within 5 d), 'sham-boxing' (after 9-11 d), and an increasing hyperexcitment which includes defecation, mounting (in males only), jumping, cliff- jumping, and climbing. Tail vibration was not observed in rats which were isolated, indicating a social behavior which requires the presence of another rat (mice presented either before or after the APO injection were ignored). In addition, no noise or other sensory stimulus was required to produce the BBS; latency to 'sham-boxing' decreased with repeated injections, being seen within 2-3 min by the 15th d. 'Sham boxing' was always accompanied by intense vocalization. Rats chronically treated with APO and injected with APO circled continuously, almost exclusively in one direction, when placed in a circular container or open field, and did not rear. However, when these animals were injected with saline no circling occurred, but rearing was present.

Chronic s.c. administration of ET 495 (5 mg/kg), bromocriptine (1 mg/kg) or thyrotropin releasing hormone (TRH, 10 mg/kg) for at least 20 d did not give the BBS.

The APO-induced BBS was blocked by d-butaclamol (0.64 mg/kg, s.c. at -20 min) but not by l-butaclamol (0.64 mg/kg) nor by naloxone (5 and 10 mg/kg, s.c. at -20 min). If d-amphetamine (1 and 5 mg/kg, s.c.) was substituted for APO, tail vibration and excessive mounting (but no 'sham-boxing') were seen in males. ET 495 (1,5, and 10 mg/kg, s.c.), when substituted for APO, had effects similar to amphetamine with the exception that 'sham-boxing' occurred at 10 mg/kg if noise was introduced. When APO was substituted for ET 495, bromocriptine or TRH, no signs of a BBS were seen. There was no tolerance to the BBS over 90 d. The BBS was still present 5,10,15,25, and 31 d after the last dose of APO, in animals treated chronically with APO.

These results suggest that a) a long term pharmacological change occurs after chronic treatment with APO which does not occur with ET 495, bromocriptine or TRH; b) the BBS produced by chronic APO may be an example of agonist-induced supersensitivity; and c) the model may provide a means of classifying dopamine receptors in vivo. (Supported by Grant DA 02322 from NIDA.)

152.23 THE COMPARATIVE EFFECT OF CHRONIC ADMINISTRATION OF d-AMPHETAMINE AND APOMORPHINE TO SELECTED MEMBERS OF PRIMATE SOCIAL COLONIES. R.F. Schlemmer, Jr., N. Narasimhachari, and J.M. Davis. Illinois State Psychiatric Institute, Chicago IL 60612.

Street use or supervised administration of large quantities of amphetamines over a short period of time often precipitates a paranoid psychosis. It has been suggested that many of the symptoms of amphetamine psychosis are mediated through central dopamine systems. However, administration of the more selective dopamine agonist apomorphine (APO) to normal volunteers does not induce psychotic behavior. But, only relatively low doses of APO have been used in these studies. Large doses of APO have rarely been given to humans because of the emetic effect. However, APO does not induce emesis in monkeys. Also, chronic administration of d-amphetamine (d-AMPH) to selected members of primate social colonies induces several behavioral changes which resemble those seen in humans during amphetamine psychosis. Therefore, a comparative study of the behavioral effects of chronic d-AMPH and APO in the same monkeys was conducted. In the first experiment, d-AMPH, 1.6 mg (base)/kg, in time-release form was given n.g. every 12 hours for 12 consecutive days to 4 adult Stumptail macaques (Macaca arctoides) from a colony of 5 monkeys. Only 2 monkeys received drug treatment at one time. 1 year later, APO, 0.5 mg (base)/kg, was given i.m. 2 x daily for 12 consecutive days to the same monkeys using a cross-over design as before. Each drug treatment period was preceded by a 2 week baseline observation period including sham drug treatment. Behavioral observation was conducted by a "blind observer" for 1 hour each day during baseline and drug treatment. During treatment, d-AMPH and APO both induced stereotyped behavior and significantly increased activity, checking (vigilance) submissive gestures, and distancing scores of all treated monkeys. Both drugs significantly reduced social grooming initiated by treated monkeys. Some differences were noted, however. APO increased locomotion more than d-AMPH. Only d-AMPH increased self grooming. The results of this study demonstrate many similarities between the behavioral changes seen in monkeys with chronic d-AMPH and chronic APO treatment. Of particular interest is that both drugs induced social withdrawal, increased submissiveness, stereotyped behavior, hypervigilance, and hyperactivity, all of which have been reported during amphetamine psychosis. This study demonstrates the important role of dopamine systems in the mediation of primate behavior and provides additional support for the role of dopamine in the mediation of amphetamine psychosis in humans.

152.24 CENTRAL MONOAMINE CHANGES IN CHRONICALLY STRESSED RATS. Kevin A. Roth*, Ivan N. Mefford* and Jack D. Barchas. Nancy Pritzker Laboratory of Behavioral Neurochemistry, Dept. Psychiatry and Behavioral Sciences, Stanford Univ. Sch. Med., Stanford, CA 94305 and Richard J. Katz*, Mental Health Research Institute, Univ. Michigan, Ann Arbor, MI 48109..

Rats were exposed to a chronic stress procedure designed to maximize the unpredictable nature of various stressors and time of stressor delivery. Stressors, such as water deprivation, individual housing, switching of cage mates, one hour of unpredictable electrical footshock (avg. 1 mA, 1-10 sec. duration; avg. 1 shock/min), and light/dark cycle reversal, were delivered in a semi-random fashion every 1-3 days throughout the day/night cycle for 21 days. Following the chronic stress procedure unstressed controls and experimental animals were sacrificed and the brains dissected. Tissue samples were stored on Dry Ice until assayed. The concentrations of tissue epinephrine, norepinephrine, MHPG, dopamine, DOPAC, HVA, serotonin and 5-HIAA were measured using HPLC with electrochemical detection.

Chronic stress failed to effect the levels of dopamine, its metabolites HVA and DOPAC, norepinephrine, and its metabolite MHPG. Hypothalamic epinephrine levels were decreased over 20% by chronic stress and caudate serotonin and 5-HIAA were both decreased about 30%. These changes may have implications to the underlying neurochemistry of human depression. The chronic stress procedure produces changes in rat behavior that may be similar to human depressed behavior. Preliminary results indicate that the chronic administration of antidepressants prevents the decrease in hypothalamic epinephrine produced by exposure to chronic stress.

152.25 EFFECTS OF CHRONIC NEUROLEPTIC TREATMENT OF CHRONICALLY STRESSED RATS. Ivan N. Mefford*, Kevin A. Roth*, Jack D. Barchas (SPON: James Dewson). Nancy Pritzker Laboratory of Behavioral Neurochemistry, Dept. Psychiatry and Behavioral Sciences, Stanford Univ. Sch. Med., Stanford, CA 94305.

Male Sprague Dawley rats, 250-300 g were injected with haloperidol (1 mg/kg), thioridazine (5 mg/kg), clozapine (10 mg/kg), chlorpromazine (5 mg/kg) or saline once daily for 21 days. A second group was chronically stressed with the drug treatment superimposed. Both chronically stressed and unstressed control groups were maintained (Katz et al., 1980).

Animals were sacrificed and brains removed and dissected for neurochemical analysis. Concurrent determination of biogenic amines was accomplished using HPLC with electrochemical detection (Mefford et al., 1980). Serotonin, 5-HIAA, norepinephrine, epinephrine, dopamine, MHPG, HVA and DOPAC were analyzed in each tissue sample.

We have previously shown that chronic stress leads to decreases in hypothalamic epinephrine and striatal 5-HT and 5-HIAA. Chronic administration of haloperidol to unstressed rats produced a marked depletion of both 5-HT and 5-HIAA in striatum. When superimposed on the chronic stress regimen, chronic haloperidol treatment led to an increase in both striatal 5-HT and 5-HIAA (about 50% in each case, $p < .05$). The same general trends were observed in both hypothalamus and frontal cortex. We are presently screening the neuroleptics to determine if the responses found after haloperidol treatment are a common characteristic of this class of drugs.

152.26

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152.27 ELECTROCONVULSIVE SHOCK TREATMENT INCREASES RESPONSIVENESS OF FOREBRAIN NEURONS TO SEROTONIN: A MICROIONTOPHORETIC STUDY IN THE RAT. C. de Montigny, Centre de recherche en sciences neurologiques and Département de psychiatrie, Université de Montréal, Québec, Canada.

Even though electroconvulsive therapy is the most effective treatment of major depressive disorders, its mode of action is still unclear.

Electroconvulsive shocks (ECS) (150 V, 10 ms pulses at 50 Hz for 1 s) were administered thrice weekly in the late afternoon for 2 weeks to male Sprague-Dawley rats (200-250 g) under light halothane anesthesia. Paired control rats were similarly anesthetized and given a foot shock. Iontophoretic experiments were carried out 36-40 hrs after the last shock. Five-barrelled glass micropipettes were prepared in a conventional manner. The central and one side barrel, filled with 2 M NaCl solutions, were used for recording and current balancing. The other barrels were filled with three of the following solutions: serotonin (5HT) creatinine sulfate (0.002 M in 0.2 M NaCl, pH: 3.8); 5-methoxydimethyltryptamine (5MeODMT) (0.05 M, pH: 4), (-) norepinephrine (NE) bitartrate (0.1 M; pH: 4), γ-aminobutyric acid (GABA) (0.05 M in 0.05 M NaCl; pH: 4) and acetylcholine (Ach) chloride (0.02 M in 0.2 M NaCl; pH: 4). Recordings were obtained from CA_3 hippocampal pyramidal neurons. Small currents of Ach were used to activated silent or slowly discharging units. Responsiveness to iontophoretic applications of drugs was estimated from the charge $I \cdot T_{50}$ required to reduce the firing rate by 50% {charge $I \cdot T_{50}$ = current (nA) x time (s)}. The same micropipette was used to assess neuronal responsiveness in both the control and the ECS-treated rats of each pair.

A first series of double-blind experiments, carried out under urethane anesthesia (1.25 g/kg, i.p.), revealed a 2-3 fold increase in responsiveness to 5HT in ECS-pretreated rats. In contrast, the effect of NE remained unchanged. In a second series, GABA and 5MeODMT, a 5HT agonist which is not a substrate for the high affinity 5HT reuptake process, were studied. Whereas the effectiveness of GABA was unaltered, the responsiveness to 5MeODMT was enhanced 2-3 times in ECS-pretreated rats. In a third series, a low *cerveau isolé* preparation was used to rule out any possible effect of general anesthesia. The sensitivity to both 5HT and 5MeODMT was found to be increased 3-4 times by ECS pretreatment.

These data indicate that ECS induce a selective increase in the sensitivity of 5HT postsynaptic receptors of hippocampal pyramidal cells in the rat. Tricyclic antidepressants also have been shown to increase responsiveness of forebrain neurons to 5HT (de Montigny and Aghajanian, 1978; Gallager and Bunney, 1979). It is therefore possible that sensitization of forebrain neurons to 5HT contributes to the therapeutic effect of both types of treatment. (Supported by M.R.C. (MA-6444) and C.R.S.Q. grants.)

152.28 ACUTE VERSUS CHRONIC EFFECTS OF SEROTONIN UPTAKE BLOCKERS ON SEROTONIN METABOLISM. Melvin H. Van Woert* and Eunyong Chung Hwang (SPON: T. Elizan). Depts. of Neurology and Pharmacology, Mt. Sinai Sch. Med., New York, N.Y. 10029.

A single injection of a serotonin uptake blocker increases the concentration of serotonin reaching post-synaptic sites, an action which has been presumed to enhance serotonergic neurotransmission. The functional effects of chronic administration of serotonin uptake inhibitors had not been examined. Therefore, we compared the effect of a single intraperitoneal injection with 14 days of treatment with 10 mg/kg of the serotonin uptake blockers fluoxetine, paroxetine, zimelidine, trazodone and amitryptyline on potentiation of the "serotonin syndrome" (lateral head weaving, Straub tail, hindlimb abduction, tremor, hyperactivity, reciprocal fore-paw treading, salivation and piloerection), produced by the monoamine oxidase inhibitor, tranylcypromine (20 mg/kg) alone and tranylcypromine (20 mg/kg) plus L-tryptophan (50 mg/kg) in mice. A single injection of fluoxetine, paroxetine and zimelidine significantly potentiated the "serotonin syndrome" produced by tranylcypromine with L-tryptophan and tranylcypromine alone. This potentiating effect on the "serotonin syndrome" of fluoxetine paroxetine and zimelidine was no longer evident after 14 daily injections of these serotonin uptake blockers. Neither acute nor chronic pretreatment with trazodone and amitryptyline had any effect on the "serotonin syndrome".

Although acute and chronic fluoxetine treatment had similar effects on mouse brain synaptosomal (^{3}H)-serotonin uptake, only chronic fluoxetine administration decreased serotonin synthesis (33%, $p < 0.01$) and (^{3}H)-tryptophan uptake into synaptosomes (26%, $p < 0.01$). Synaptosomal (^{3}H)-tryptophan uptake also was significantly reduced by chronic but not acute treatment with zimelidine, trazodone and amitryptyline. Brain 5-hydroxyindoleacetic acid was decreased by 46% and 52% ($p < 0.001$) after acute and chronic fluoxetine treatment, respectively. Neither acute nor chronic fluoxetine administration had any effect on brain serotonin levels, tryptophan hydroxylase, (^{3}H)-5-hydroxytryptophan uptake or (^{3}H)-serotonin receptor binding.

Our behavioral and biochemical findings suggest that there may be no significant enhancement of brain serotonergic neurotransmission during chronic therapy with serotonin uptake blockers because a reduction in tryptophan uptake and serotonin synthesis counteracts the effects of serotonin uptake inhibition. (Supported by USPHS grants NS 12341, NS 05802 and NS 71631).

152.29 Electroconvulsive Shock and Reserpine: Effects on Adrenergic and Serotonergic Receptors in Rat Brain. Judith A. Butler*, Caren S. Cascio*, Debra A. Bergstrom*, and Kenneth J. Kellar (Spon: F.G. Standaert). Dept. of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, D.C. 20007.

Chronic electroconvulsive shock (ECS) decreases beta-adrenergic receptors in rat cortex. It does not alter alpha adrenergic or 5-HT_1 receptors in the cortex or dopamine receptors in the striatum. The present experiments further examined the specificity of the effects of ECS on neurotransmitter receptors in the cortex and hippocampus and in particular examined the effects of ECS on receptors previously altered by chronic reserpine administration.

Rats were treated with vehicle or reserpine (0.25 mg/kg i.p.) for 30 days and then subjected to either ECS or sham shock (handled and placed to electrodes) for 10 days. One day after the last ECS or sham treatment, the rats were sacrificed and the brains dissected. Beta-, $alpha_1$-, and $alpha_2$-adrenergic receptors were measured using ^{3}H-dihydroalprenolol, ^{3}H-WB-4101, and ^{3}H-clonidine, respectively. Serotonin$_1$ and serotonin$_2$ receptors were measured using ^{3}H-5-HT and ^{3}H-spiperone, respectively.

Chronic ECS reduced beta-adrenergic receptors in rat cortex as we have previously reported. In addition, beta-adrenergic receptors in the hippocampus were reduced to the same exent. Further, 10 days of ECS completely reversed reserpine-induced increases in beta-adrenergic receptors. Eleven days after the last reserpine injection beta-adrenergic receptor binding in the sham-shocked group was increased by 22% compared to vehicle-injected, sham-shocked controls. In contrast, the binding in rats which received reserpine followed by 10 days of ECS was the same as vehicle controls. Chronic ECS had no apparent effect on $alpha_1$ or $alpha_2$ receptors in the cortex, nor on ^{3}H-5-HT binding sites in the cortex or hippocampus. Serotonin$_2$ receptors, measured by ^{3}H-spiperone binding in the cortex were not reduced by chronic ECS. This is in contrast to the effects of chronic administration of tricyclic antidepressants and MAO inhibitors (Peroutka and Snyder Science, in press, 1980).

Data on the specificity of the effects of ECS on reserpine-induced increased neurotransmitter receptors will be presented.

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152.30 OPPOSITE AND ANTAGONISTIC EFFECTS OF INTRAHIPPOCAMPAL INJECTIONS OF SEROTONIN AND NOREPINEPHRINE ON BEHAVIOR Fred H. Gage and Joe E. Springer* Chemistry of Behavior Program, Texas Christian University, Fort Worth, TX 76129 On the basis of converging histofluorescent, autoradiographic, microiontophoretic and biochemical assay techniques, NE and 5-HT have been implicated as putative neurotransmitters within the hippocampal formation (HPF).

Convincing evidence for an antagonistic role of 5-HT and NE has been obtained in experiments aimed at elucidating the neurochemical substrate of behavioral arousal and emotional behavior. The HPF has been associated with related behaviors, more specifically the HPF has been suggested to be involved in the integration of internal and external stimuli and in particular appears to function in the recognition of novel stimuli. The present study was designed to answer the following questions: 1)Do intrahippocampal injections of NE and/or 5-HT effect behavioral responsiveness to novel stimuli? 2)Do 5-HT and NE effect behavior in the same direction? 3)Do NE and 5-HT act antagonistically? 4)Do NE and 5-HT respectively have the same effect when injected along the dorsal and ventral axis of the HPF?

Groups of rats were designated by dorsal or ventral HPF cannula placements and then further divided depending upon whether the subject was to receive NE or 5-HT. The animals received an injection and were tested once a day with a two day interval. Five concentrations of the amines were used: .005, .05, .5, 2.5 and 5.0μg/μl in 2μl injected at a rate of 1μl/minutes.

The behavioral tests included two with noxious stimuli, pawlick latency in response to electric shock; one with non-noxious stimulus, ambulatory behavior in response to an open field.

Our results demonstrate a clear dose-dependent change in behavior following both NE and 5-HT injections. NE increased responsiveness of rats in all three behavioral tests. 5-HT decreased responsiveness to noxious stimuli and increased open field behavior. Injections of NE and 5-HT simultaneously antagonized the effects of either transmitter when injected individually. Clear differences are observed for NE and 5-HT depending on whether the neurotransmitters were injected into the dorsal or ventral HPF. These results demonstrated an interaction or balance between 5-HT and NE in the HPF is important in integration of external stimuli into behavioral responses.

152.31 VAGINAL STIMULATION-PRODUCED ANALGESIA IS MEDIATED BY SPINAL NOREPINEPHRINE AND SEROTONIN IN RATS. J.L. Steinman*, B.R. Komisaruk, T.L. Yaksh and G.M. Tyce. Inst. Animal Behavior, Rutgers Univ., Newark, NJ 07102 and Mayo Clinic, Neurosurgical Research and Physiology, Rochester, MN. 55901.

The present findings demonstrate that 1) administration of α-adrenergic (NE) and serotoninergic (5-HT) receptor blocking agents directly to the spinal cord significantly antagonized the analgesic effect of vaginal stimulation (VS), and 2) NE and 5-HT measured in spinal cord superfusate increased significantly in response to VS. The drugs were injected via catheters (PE-10 tubing) passed to the lumbar level of the spinal cord through an incision in the cisterna magna according to the method of Yaksh and Rudy (Physiol. Behav., 17: 1031, 1976). VS (200g force) was applied via a calibrated glass probe assembly (modified 1 cc syringe plunger). Two indices of analgesia were used: latency of tail flick to radiant heat and threshold (in mA) to vocalize in response to tail shock. In pre-injection control tests, VS significantly increased both of these measures: overall mean tail flick latency (from 2.68±.09 *[s.e.m.]* sec. preprobing to 5.43±.16 sec. during probing *[203%↑]*), and the overall $\bar{X}$ vocalization threshold (from $\bar{X}$ = .21±.02 mA preprobing to $\bar{X}$ = .39±.03 mA during probing *[185%↑]*). The rats were then injected perispinally with 20 μL containing 10 or 40 μg phentolamine (Regitine HCl: n=7 and 8, respectively, 10 or 40 μg methysergide maleate (n=8 and 6, respectively) or 20 μL saline (n=7). Testing commenced 15 min. after completion of the injection. Results are reported as % of mean response (threshold or latency) of saline group during VS (*:p<.05). Vocalization threshold of saline group during VS: $\bar{X}$ = .31 mA = 100%; phentolamine; 10 μg: 108.7% ns; 40 μg: 37.1%*; methysergide: 10 μg: 50.0%*; 40 μg: 186/7% ns. Tail flick latency of saline group during VS: $\bar{X}$ = 4.91 sec. = 100%; phentolamine: 10 μg: 88.6% ns; 40 μg: 62.3%*; methysergide: 10 μg: 88.6% ns; 40 μg: 83.5% ns.

In a separate experiment, high pressure liquid chromatography using electrochemical detection was used to measure NE and 5-HT levels in superfusates (30 min. samples) of the spinal cord immediately preceding and during VS (1.5 min. VS on, 1.5 min. off; repeated for 30 min.) in 5 rats. VS increased the mean levels of NE from .01±.01 (s.e.m.) to .17±.03 ng/ml/10 min. and of 5-HT from .29±.12 to .48±.12 ng/ml/10 min. Thus the present experiments indicate that a spinal α-adrenergic mechanism mediates the analgesic effect of VS, and provide evidence that a serotoninergic mechanism may also be involved.

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152.32 SEROTONERGIC AND NORADRENERGIC UPTAKE DURING THE ESTROUS CYCLE IN LIMBIC AND HYPOTHALAMIC NUCLEAR REGIONS. Donald C. Meyer, Department of Physiology and Biophysics, University of Louisville School of Medicine, Louisville, Kentucky 40292

The role of hypothalamic and limbic aminergic systems in ovulation in the rat has been investigated by measuring the temporal changes in serotonergic and noradrenergic neuronal uptake during the estrous cycle. Using the pregnant mare serum (PMS) model of induced ovulation and an in vitro uptake system, significant ($p < .05$) decreases in serotonergic uptake were found during the mid to late light period in the amygdala during diestrus. This pattern was repeated during proestrus and estrus only in the amygdala, suggesting a consistent pattern of serotonergic uptake on every day of the estrous cycle. Significant changes in noradrenergic uptake also occurred in the amygdala during proestrus ($p < .05$) suggesting a possible interaction between serotonin and norepinephrine in regulating ovulation.

During proestrus serotonergic uptake in the median eminence and suprachiasmatic regions reaches peak values ($p < .05$) at the beginning of the critical period for luteinizing hormone release. This peak of serotonergic uptake in the suprachiasmatic region confirms previous data on cycling females and is probably related to photoneuroendocrine control mechanisms.

Diestrus serotonergic uptake in the median eminence shows maximum values during the mid light period and minimum values during the late light-early dark period ($p < .05$). These results in conjunction with the diestrus serotonergic uptake changes in the amygdala, suggest a role for these regions in ovulation control mechanisms prior to the critical period for luteinizing hormone release.

(This work was supported in part by BRSG grant number 531245 from the University of Louisville School of Medicine.)

152.33 OPPOSING ACTIONS OF TWO NOREPINEPHRINE (NE) CNS STRUCTURES IN THE REGULATION OF RHYTHMIC GROWTH HORMONE (GH) SECRETION. T.A. Day* and J.O. Willoughby. Centre for Neuroscience, Flinders University of South Australia, Bedford Park, South Australia 5042

Pituitary GH secretion is regulated by somatostatin (SRIF) and a postulated GH releasing factor (GRF) which are released from tubero-infundibular (TI) nerve terminals at the median eminence (ME) into the hypothalamo-pituitary portal circulation. Cell bodies of SRIF TI neurons are located in the preoptic/anterior hypothalamic area (PO/AHA). Hypothalamic deafferentation studies suggest that GRF TI neurons are located in the medio-basal hypothalamus. Previous studies have shown that NE is important in the regulation of GH secretion but have not specified its site of action. NE may act directly on SRIF or GRF cell bodies or on SRIF or GRF terminals in the ME. The present study examined the effects on GH secretion of 6 hydroxydopamine (6OHDA) destruction of catecholamine (CA) structures within the CNS, generally, or in the ME or PO/AHA, specifically. A CA-fluorescence technique was used to confirm destruction of CA structures in the respective studies.

Male Porton albino rats chronically implanted with right atrial cannulae were used. Radioimmunoassay of GH (NIAMDD materials) in plasma samples obtained by serial blood sampling of these undisturbed animals permitted construction of hormone profiles before and after treatments of the animals with (a) 6OHDA 600 µg intracerebroventricularly (icv) (b) 6OHDA 2 µg intracerebrally in the PO/AHA and (c) intravenous (iv) 6OHDA 50 mg/kg, a treatment which selectively destroys ME NE terminals because 6OHDA fails to penetrate the blood-brain barrier, which is absent at the ME, and ME DA neurons are resistant to this treatment.

One day after treatment GH secretion was almost completely suppressed by icv and PO/AHA 6OHDA but iv 6OHDA dramatically increased the frequency of GH secretory bursts. Recovery of GH secretory rhythms occurred in all groups within 1-3 weeks.

As DA is thought to have only a minor role in the regulation of GH release, suppression of GH following icv 6OHDA treatment favours the accepted facilitatory action of NE in GH secretion. GH suppression by PO/AHA 6OHDA indicates that it is NE in the PO/AHA which is facilitatory for GH, presumably by an inhibitory action on SRIF neurons. The increased frequency of GH peaks after iv 6OHDA indicates that ME NE is inhibitory to GH secretion. Others have shown that NE stimulates SRIF release from ME terminals incubated in vitro, suggesting that ME NE inhibits GH through an excitatory action on SRIF terminals.

Supported by the Australian Neurological Foundation and the South Australian Neurosurgical Research Foundation.

152.34 DIFFERENCES IN THE PROLACTIN-INDUCED ACTIVATION OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS IN MALE AND FEMALE RATS. K.T. Demarest and K.E. Moore. Dept. of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

Studies were undertaken to investigate factors responsible for the previously observed differences in the activity of tuberoinfundibular dopaminergic (TIDA) neurons in male and female rats (Demarest et al., Fed. Proc. 39(3): 980, 1980). The in vivo rate of dopamine (DA) synthesis, estimated by the accumulation of DOPA in the median eminence 30 min after the administration of a DOPA decarboxylase inhibitor (NSD 1015; 100 mg/kg, ip), was used as a biochemical index of TIDA neuronal activity. The rate of DOPA accumulation in the median eminence of female rats (16.8±0.9 ng/mg protein/30 min) was greater than that in male rats (5.6± 0.4). Previous reports indicate that increased concentrations of prolactin in serum or CSF cause a delayed activation of TIDA. Estradiol benzoate (25 µg/kg sc x 3 days), which increases serum prolactin, increased the rate of DOPA accumulation in the median eminence of both male and female rats. Since this effect was not observed in hypophysectomized rats it is probably not the result of a direct action of estrogen on TIDA neurons, but rather the result of the estrogen-induced increase in serum concentrations of prolactin. These results demonstrate that prolactin stimulates TIDA nerves in the female. It was unexpectedly found, however, that hypophysectomy per se dramatically reduced the rate of DOPA accumulation in the median eminence of female rats but not in male rats. TIDA neurons in the hypophysectomized female rats retain the capacity to respond to prolactin since the intracerebroventricular administration of this hormone (1 µg/10 µl) increased DOPA accumulation in the median eminence of hypophysectomized animals in the same way as it does in intact animals. The greater basal rate of DOPA accumulation in the median eminence of the female rat and the marked decrease in this rate following hypophysectomy may be due to the greater sensitivity of TIDA neurons to circulating prolactin in the female. To test this possibility the capacity of prolactin to stimulate TIDA neuronal activity was compared in male and female rats. Rat prolactin (0.1-10 µg) was infused into the lateral cerebroventricles of conscious castrate male and female rats via previously implanted cannulas. When measured 12 hr after prolactin administration DOPA accumulation in the median eminence was significantly increased at a lower dose in the female than in the male (minimal effective doses were 0.1 µg and 1 µg, respectively). These results indicate there is a marked difference in the sensitivity of TIDA to prolactin in male and female rats. (Supported by USPHS grant NS9174.)

152.35 EFFECT OF DEHYDRATION AND REHYDRATION ON TUBEROHYPOPHYSEAL DOPAMINERGIC NEURONS. R.H. Alper and K.E. Moore. Dept. of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

Tuberohypophyseal dopamine (DA) neurons originate in the arcuate nucleus of the hypothalamus and project to the neural and intermediate lobes of the pituitary (posterior pituitary). The purpose of the present study was to characterize some of the factors that regulate the activities of these neurons in the rat. For this purpose the rate of synthesis of dopamine was quantified by measuring the accumulation of DOPA 30 min after the inhibition of DOPA decarboxylase with NSD 1015 (100 mg/kg, ip). Three days of water deprivation did not alter the concentrations of DA or norepinephrine (NE) but selectively increased the rate of DOPA accumulation in the posterior pituitary (2.4 versus 1.3 ng DOPA/mg protein/30 min). Water deprivation did not alter DOPA accumulation in the striatum or median eminence, regions containing terminals of nigrostriatal and tuberoinfundibular DA nerves, respectively. The rate of DA and NE synthesis in the posterior pituitary was also calculated from the rate of decline of these amines following the administration of α-methyltyrosine (250 mg/kg, ip). Three days of water deprivation increased the rate of DA synthesis in the posterior pituitary (1.9 versus 1.1 ng DA/mg protein/hr) whereas NE synthesis was unaltered (0.2 ng NE/mg protein/hr for water deprived and control rats). Thus, the increased DOPA accumulation in the posterior pituitary of water deprived rats represents an increase in DA and not NE synthesis. The water deprivation-induced increase in tuberohypophyseal DA nerve activity was partially reversed in animals provided access to water for 12 hr and completely reversed in animals rehydrated for 24 or 48 hr.

Subcutaneous injections of polyethylene glycol (PEG) cause similar but more rapid increases in hematocrit and plasma vasopressin concentrations than those observed during water deprivation (Dunn et al., J. Clin. Invest. 52: 3212, 1973). Hypovolemia, as evidenced by an increase in hematocrit, was observed 3, 6 and 12 hr after PEG 4000 (4 g/kg, sc). At 12 hr, but not at 3 or 6 hr, after PEG 4000 DOPA accumulation was increased in the posterior pituitary but not in the striatum or median eminence. These results demonstrate that PEG 4000 and water deprivation selectively stimulate DA synthesis in tuberohypophyseal neurons and the effect of water deprivation is reversible by rehydration. (Supported by USPHS grants NS9174 and NS15911.)

152.36 LACTATION ALTERS THE ACTIVITY OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS. D.W. McKay*, K.T. Demarest, G.D. Riegle* and K.E. Moore (SPON: W.H. Lyness). Depts. of Animal Husbandry, Physiology, and Pharmacology and Toxicology, Michigan State University, E.Lansing, MI 48824.

Alterations in tuberoinfundibular dopaminergic (TIDA) neuronal activity may play a role in the regulation of prolactin secretion during lactation. Studies were undertaken to determine the activity of TIDA neurons, estimated by the accumulation of DOPA in the median eminence 30 min after the administration of a decarboxylase inhibitor (NSD 1015, 100 mg/kg, i.p.), in female rats during diestrus and lactation. All lactating animals were used 12 days post-partum and litters equalized to 8 pups at 7 days post-partum. After 4 hr of pup deprivation, the activity of TIDA neurons was determined in the following groups of lactating female rats: 1) pups were returned and allowed to suckle for 30 min during which time DOPA accumulation was measured (suckled), 2) pups were returned, allowed to suckle for 30 min and then removed for 30 min during which time DOPA accumulation was measured (post-suckled), 3) pups were not returned and DOPA accumulation was measured for 30 min (non-suckled).

In the lactating animals, suckling significantly reduced the rate of DOPA accumulation (6.3±0.5 ng DOPA/mg protein/30 min) when compared to the nonsuckled (9.9±0.5) or post-suckled groups (8.0±0.9). This suckling-induced decrease in TIDA neuronal activity may play a role in the mediation of the increased prolactin secretion during suckling. The rate of DOPA accumulation observed in the diestrous group (18.5±1.4) was significantly greater than that in any of the lactating groups. There were no significant differences in the rates of DOPA accumulation in brain regions (striatum, nucleus accumbens, olfactory tubercle) containing terminals of mesotelencephalic DA neurons. Since results of previous studies have demonstrated that TIDA neurons are activated during periods of increased serum concentrations of prolactin (see review Moore and Wuerthele, Prog. Neurobiol. 13: 325, 1979), the low rate of DOPA accumulation observed in the median eminence of lactating animals was unexpected. It would appear that TIDA neurons are somehow inhibited during lactation to allow the serum prolactin concentrations to increase. (Supported by USPHS grants HD 10521 and NS 9174.)

153.1 EXTRANUCLEAR INPUT TO NEURONS OF THE CAUDAL NEUROSECRETORY SYSTEM. R.M. Kriebel* and J.P. O'Brien* (SPON: J. Wells). Dept. of Anatomy & Neurobiology, University of Vermont, College of Medicine. Burlington, VT 05405

The caudal neurosecretory system provides a favorable structure in which to study the synaptic control of neurosecretory mechanisms. The neurosecretory neurons in the caudal system of the black molly are an isolated population of cells in the terminal aspect of the spinal cord. This group of neurosecretory cells can be deafferented from extranuclear input to examine the relationship of extranuclear and intranuclear synaptic control of neurosecretion. To identify the extranuclear synaptic input, surgical deafferentation was performed on sixteen animals by transection of the spinal cord rostral to the caudal system. Animals were allowed to survive 12, 24, 40 hours, and 7 days after the lesion. At each postlesion time, four animals were anesthetized in MS-222 and killed by decapitation. Five animals provided control data on the synaptic organization of the caudal neurosecretory system. The caudal system was fixed in vivo by a 2.5% glutaraldehyde, 2% paraformaldehyde, phosphate buffered solution (0.06M) and subsequently prepared for electron microscopy. In control tissue, synaptic terminals were observed on cell bodies and dendrites of the neurosecretory cells. Identification of the extranuclear synaptic input was determined by the dark reaction of synaptic boutons, preterminals, and axon bundles in the lesioned animals. Degeneration of small myelinated axon bundles was seen in the dorsolateral portion of the spinal cord at seven days after transection. At forty hours, degenerating synaptic boutons were found primarily in the vicinity of the neuronal soma and engulfed in thin glial processes. These results suggest that control of neurosecretory mechanisms by extranuclear input is at the level of the somal membrane. HRP tracer studies are currently being undertaken to determine the specific source of these terminals. (Supported by PHS 5429-16-19)

153.2 DYE-COUPLING BY GAP JUNCTIONS IN MAGNOCELLULAR NEUROENDOCRINE CELLS OF RAT HYPOTHALAMUS: EVIDENCE FOR ELECTROTONIC COUPLING. R. David Andrew, Brian A. MacVicar, F. Edward Dudek and Glenn I. Hatton. Dept. Zool., Univ. Toronto, Toronto, Ontario M5S 1A1, and Dept. Psychol. and Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Electrotonic coupling has been demonstrated between endocrine cells and between some neurons of the vertebrate brain. The morphological substrate of communication is gap junctions, channels which allow molecules of MW <1000 to pass between cells. It is not known if vertebrate neuroendocrine cells or hypothalamic neurons in general are coupled. We have examined, by two independent means, the possibility of electrotonic coupling in neuroendocrine cells of the rat paraventricular nucleus (PVN) and supraoptic nucleus (SON).

Coronal hypothalamic slices (500 μm) were cut and single cells in PVN and SON impaled with micropipettes containing 5% Lucifer Yellow (MW=453) in .33 M Li Citrate. Dye was injected with hyperpolarizing currents (7nA, 30-180 sec). Ten dye ejections (7nA, 180 sec) into extracellular space served as controls. Injected magnocellular neurosecretory cells (MNC's) of PVN or SON were identified by a minimum diameter of 15 × 15 μm and a large cytoplasm-to-nucleus ratio. Of 32 well-filled MNC's, 14 were dye-coupled to a second MNC (9 of 19 in PVN, 5 of 13 in SON). The majority of coupling appeared to be dendro-dendritic. In two cases, a third MNC was faintly stained. No intracellular uptake of dye was observed following extracellular ejections.

For electron microscopy, tissue blocks containing PVN or SON were dissected from fresh brains and immediately fixed. Freeze-fracture replicas displayed particle aggregates on P-face membrane of cells with cytoplasm containing 170-220 nm diameter neurosecretory vesicles. Furthermore, these aggregates were comprised of from 15 to 400 small diameter particles surrounded by a halo of non-particulate membrane, thus having a typical gap junction morphology.

We conclude that gap junctions provide the channels that dye-couple MNC's in PVN and SON and that, as such, these cells are probably electrotonically coupled. Coupling may facilitate the recruiting or synchronizing of peptidergic neurons which release neurohormone in a pulsatile fashion. Lucifer Yellow injections together with immunohistochemistry should identify the peptide contents of coupled MNC's.

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153.3 HYPOTHALAMIC ACCESSORY MAGNOCELLULAR VASOPRESSIN, OXYTOCIN AND NEUROPHYSIN NEURONS PROJECTING TO THE NEUROHYPOPHYSIS IN THE RAT. M. V. Sofroniew, U. Schrell*, W. Glasmann*, A. Weindl* and R. Wetzstein*. Dept. of Anatomy, Ludwig-Maximilians University, Pettenkoferstr. 11, 8000 Munich 2, FRG.

It is commonly known that vasopressin (VP), oxytocin (OT) and neurophysin (Nph) are produced by hypothalamic magnocellular neurons located in the supraoptic and paraventricular nuclei. However, in many mammalian species there is a large population of magnocellular VP, OT and Nph neurons outside of these nuclei, located in various groups in different parts of the hypothalamus. In this study we have examined the topography of these groups, as well as the extent of their projections to the neural lobe, using a new procedure allowing the parallel demonstration of the horseradish peroxidase (HRP) retrogradely transported by, and the peptide produced by, the same neurons. For topographical analysis, endogenous VP, OT and Nph were visualized using the immunoperoxidase technique. To analyze peptide-specific projections, HRP (0.4 μl, 15%) was injected into the neural lobe; after 48 hr, rats were sacrificied and hypothalami processed for 1.5 μm sections; HRP, VP, OT and Nph were then detected immunohistochemically in neighboring serial sections. In the rat hypothalamus, accessory magnocellular neurons can be divided into more than 8 separate groups. The groups are located in the medial preoptic area and medial anterior hypothalamus, behind the anterior commissure, near the rostral end of the stria terminalis, along the third ventricle, at several levels of the lateral hypothalamus, and in the posterior hypothalamus lateral to the fornix. Most groups contain both VP and OT neurons. The group just caudal to the anterior commissure consists of only OT neurons. The neighboring serial 1.5 μm sections showed that a large number of VP, OT and Nph neurons in the various groups had retrogradely transported HRP from the neural lobe. The presence of VP, OT and Nph neurons not transporting HRP may have been due to injection sites which did not always include the entire neural lobe. At present all magnocellular neurons in the accessory groups (or in the supraoptic and paraventricular nuclei) identified as transporting HRP from the neural lobe, have also stained positively for Nph and either VP or OT in the neighboring 1.5 μm sections. No neurons stained for both VP or OT. These findings indicate that there is an extensive network of magnocellular accessory neurons in the rat, and that a large portion of these neurons project to the neurohypophysis. These neurons may also contribute to the extrahypophyseal VP, OT and Nph projections. Past descriptions of their topography are incomplete, and a new and simpler nomenclature is warranted. Supported by the Deutsche Forschungsgemeinschaft (We 608/5).

153.4 THE HYPOTHALAMIC ARCUATE NUCLEUS OF RAT: A QUANTITATIVE GOLGI ANALYSIS. Anthony N. van den Pol and John R. Cassidy*. Sect. Neurosurgery, Yale Univ. Sch. Med., New Haven, Conn. 06510.

The arcuate nucleus (ARC) has classically been considered a loosely organized net of relatively simple neurons. To better understand the intrinsic organization of this nucleus, a computer-assisted analysis of Golgi impregnated material was undertaken. From our series of several hundred Golgi impregnated hypothalami, 11 brains from 8-10 day old rats were selected. High magnification camera lucida drawings were made of 1,686 neurons in ARC and additional cells in the adjacent hypothalamus for comparative purposes; coronal and horizontal sections were used. Neurons and their 3-dimensional position in the ARC were digitized (Summagraphics Digitizer) for computer storage and quantitative analysis. Neurons had one to four primary dendrites. The mean number of primary dendrites from 487 neurons drawn from coronal sections was 2.1±0.8 (SD). Medially, on horizontal sections, dendrites of arcuate neurons tended to run orthogonal to the tanycytes projecting from the wall of the third ventricle into ARC. The center of dendritic mass (in two dimensions) relative to the perikaryon of origin was examined; averaged over all areas of ARC, the percent of cells with the center of mass dorsolateral to the soma was 28%, dorsomedial 21%, ventromedial 17%, and ventrolateral 34%. When these data were grouped on the basis of perikaryal location, differences between ARC subpopulations were found: for instance dorsomedial cells were more likely to send dendrites dorsomedially (28%) than ventrolateral cells (14%) were. When the total length of a cell's dendritic arbor in single coronal sections was examined, neurons in the rostral 30% of ARC had 56% larger total dendritic length than cells in the caudal 30% of the nucleus. Axons originated from perikarya and primary dendrites; locally terminating collaterals, projections lateral and outside ARC, and projections ventrolateral into the median eminence were found. The initial trajectory of 249 axons originating from ARC cells was in a medial direction (62%). Impregnated axons were found in coronal sections which proceeded horizontally through the dorsal median eminence and gave off ventrally directed collaterals at regular intervals parallel to ventral tanycytes. Tanycytes represent a significant factor in the overall cellular organization of ARC. The height of the tanycyte cell body above the base of the brain was highly correlated (r=.95) with the position of the terminal process of the same tanycyte measured laterally from the ventricular wall. Taken together with additional analyses not described above the arcuate nucleus is found to have a significant organization which is revealed by quantitative assessment of large number of cells.

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153.5 SUCKLING INCREASES DIRECT CELL-CELL APPOSITION BETWEEN NEUROSECRETORY NEURONS IN THE RAT SUPRAOPTIC NUCLEUS. Glenn I. Hatton and Charles D. Tweedle, Neuroscience Program and Department of Anatomy, Michigan State University, East Lansing, MI 48824

Water deprivation for short periods of time (4-24 h) has been shown to increase the amount of direct soma-somatic contact between magnocellular neurosecretory cells (MNC's) of the supraoptic (SON) and circularis nuclei (Cell Tiss. Res. 1977, 181, 59-72). This increased contact was apparently due to withdrawal of fine glial processes from between the MNC somata. Rehydration reversed this effect, re-establishing control levels of cell-cell contact presumably by glial re-insertion.

Lactating rats were studied to determine whether another, naturally occurring stimulus complex known to physiologically activate hypothalamic MNC's would lead to similar changes in cell contact characteristics. Thin sections of SON from lactating and non-lactating rats (Holtzman strain) were quantitatively examined at the ultrastructural level. Lactating rats were allowed to nurse their young for 2 weeks prior to perfusion fixation with buffered aldehydes. To date, we have measured the % of cells in direct soma-somatic contact and the % total membrane in contact for samples of thin sections from these two treatment groups. In non-lactating rats the cells in contact = 4.6% and the amount of membrane in contact = 0.09% of total. These values in lactating rats were 42.8% and 7.2%, respectively. Thus, there was a 10-fold increase during lactation in the number of cells and a 70-fold increase in the amount of membrane involved in making soma-somatic appositions. The large increase in direct cell contact may be related to the fact that in lactating rats not only are oxytocin containing neurosecretory cells being activated by suckling, but other neurosecretory cells containing other peptides (e.g., vasopressin, enkephalin) may also be activated by dehydration which results from lactation.

This research was supported by NIH grant NS 09140 and by the College of Osteopathic Medicine of Michigan State University.

153.6 BRAIN CELLS OF THE MALE RHESUS MONKEY ACCUMULATE ^{3}H-TESTOSTERONE OR ITS METABOLITES. H.D. Rees and R.P. Michael. Dept. of Psychiatry, Emory Univ. Sch. of Med., Atlanta, GA 30322 and Georgia Mental Health Institute, Atlanta, Georgia 30306.

Androgens restore the potency of castrated male rhesus monkeys, and may also stimulate aggressive behavior. To help elucidate the neural mechanisms for the behavioral actions of testosterone in the primate, the distribution of testosterone target cells in the rhesus brain was studied by autoradiography. Two days after castration, a mature male rhesus monkey, weighing 9.6 kg, was tranquilized with ketamine (10 mg/kg) and injected intravenously with 2 mCi of ^{3}H-testosterone (5.8 µg) (New England Nuclear, 98.8 Ci/mmol). An hour later, the monkey was killed by an intravenous injection of Nembutal, and its brain was removed and frozen in liquified propane at -180°C. Frozen sections, 4 µm thick, were cut in a cryostat, thaw mounted on Kodak NTB3 emulsion-coated slides, and photographically developed after exposure for 10 months.

Radioactivity, representing ^{3}H-testosterone or its labelled matabolites, was concentrated in nuclei of neurons in several areas of the brain, including the medial and cortical amygdaloid nuclei, the accessory part of the basal amygdaloid nucleus, periamygdaloid cortex, suprachiasmatic preoptic nucleus, medial preoptic nucleus, anterior hypothalamic area, bed nucleus of the stria terminalis, ventromedial hypothalamic nucleus, and periaqueductal gray. All of these structures are known targets for testosterone in male rats (M. Sar and W.E. Stumpf, in Anatomical neuroendocrinology, Stumpf and Grant, eds, Karger: Basel, 1975), but androgen localization has not previously been reported for the brain of any primate species.

Since the brain of the rhesus monkey has the enzymatic capacity for the aromatization of androgens to estrogens (F. Flores et al., Neuroendocrinol., 11:177, 1973), as well as for 5α-reduction and 17β-dehydrogenation (L.J. Sholiton et al., Steroids, 24:537, 1974), further studies are necessary to characterize the chemical identity of the nuclear radioactivity visualized autoradiographically in the present study.

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153.7 THE EFFECT OF KAINIC ACID ON AROMATIZATION AND 5α-REDUCTION IN EMBRYONIC RAT HYPOTHALAMIC CULTURES. E.G. Livingston*, J.A. Canick*, D.E. Vaccaro*, K.J. Ryan*, and T.O. Fox, (SPON: S.Wieland), Depts. of Obstetrics and Gynecology, and Neuropathology, and Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, and Dept. of Neuroscience, Children's Hospital Medical Center, Boston, MA 02115.

Kainic acid, a potent neurotoxic agent (Olney et al, Brain Res. 77:507, 1974), was used in the investigation of cell classes responsible for aromatization and 5α-reduction of androgen in the hypothalamus. The conversions of [6,7-^{3}H]19-hydroxyandrostenedione to [^{3}H]estrone (aromatization) and [1β,2β-^{3}H]testosterone to [^{3}H]5α-dihydrotestosterone (5α-reduction) were studied in intact primary cell cultures of embryonic rat hypothalamus (Vaccaro & Messer, TCA Manual 3:361, 1977). To determine the effect of kainic acid on a non-neuronal class of cells, glutamine synthetase activity, localized to glial cells in the brain (Martinez-Hernandez et al, Science 195:1356, 1977), was measured in extracts of the cultured cells. All cultures were treated initially with 1β-D-arabinofuranosylcytosine (ara-C), which results in increased neuritic elongation and inhibition of the proliferation of non-neuronal cells. Seven days after plating, the cultures were exposed to kainic acid for an additional 8 days. The neuronal population was low in number and some of the remaining neurons had disintegrating processes in the kainic acid-treated cultures. The effect of kainic acid (K.A.) on enzyme activity was:

	Aromatase (pmol/culture/24hr) (mean±SEM, n=6)	5α-reductase	Glutamine synthetase (µg GHA/culture/hr) (mean, n=2)
Control	0.25±0.03	1.69±0.06	10.3
10^{-4}M K.A.	0.14±0.01	1.75±0.05	9.8
10^{-3}M K.A.	0.02±0.002	1.75±0.03	10.3

A time course experiment indicated that kainic acid (10^{-3}M) affects neuronal morphology and aromatization as early as two days after treatment. Kainic acid preferentially decreases aromatization without measureably affecting 5α-reductase. This is consistent with a predominant localization of aromatase in neurones.
(Supported by NIH grants HD 12337 to J.A.C. and HD 10818 to T.O. F.; a postdoctoral N.R.S.A., NINCDS, to D.E.V.; facilities of the Mental Retardation Res. Ctr., NICHD, at Children's Hospital Med. Ctr.)

153.8 EFFECT OF A POLYPEPTIDE (E_5) EXTRACTED FROM BOVINE PINEAL GLANDS ON PLASMA AND PITUITARY LEVELS OF LUTEINIZING HORMONE (LH) AND PROLACTIN IN NORMAL AND CASTRATED ADULT MALE RATS. M.K. Vaughan, L.Y. Johnson, P. Pevet*, C. Neacsu*, and R.J. Reiter. Department of Anatomy, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284, The Netherlands Institute for Brain Research, Amsterdam, The Netherlands, and Oncological Institute, Bucharest, Romania.

A highly purified polypeptide (E_5) from the bovine pineal gland blocks spermatogenesis in gonadotropin-treated male frogs (Rana esculenta) and inhibits uterine weight and P^{32} incorporation in gonadotropin-treated female mice (Neacsu, Rev. Roum. Physiol. 9: 161-169, 1972). In the present experiment, the effects of E_5 on plasma and pituitary levels of LH and prolactin in intact and castrated adult male rats were assessed. Forty-three adult male rats (225-275 g) were housed in a temperature and light-controlled room (14L:10D; lights on 0600) and provided with food and water ad libitum. Twenty-three rats were castrated using ether anesthesia; 20 rats were left intact. One hour following castration, the intact and castrated rats were divided into 4 treatment groups: intact + diluent (11 rats), intact + 5 µg E_5 (9 rats), castrate + diluent (12 rats) and castrate + 5 µg E_5 (11 rats). E_5 was diluted in 0.5% bovine serum albumin (BSA)-Ringers lactate diluent. Subcutaneous injections of 5 µg E_5 or diluent were administered every 12 hours for 5 injections. One hour following the last injection, the rats were rapidly decapitated and a trunk blood sample obtained. Anterior pituitaries were weighed and disrupted by sonication in 1% BSA-0.05M phosphate buffer. Plasma and pituitary samples were stored frozen until radioimmunoassay (RIA). RIA kits were provided by NIAMDD. As expected, castration caused a significant elevation in plasma LH ($p < 0.001$) and a concomitant decrease in pituitary LH ($p < 0.001$) when compared to the levels of diluent-treated intact rats. Both plasma ($p < 0.05$) and pituitary ($p < 0.01$) levels of LH were significantly depressed in E_5-treated intact rats compared to intact controls; E_5, however, had no effect on plasma or pituitary levels of LH in castrated rats. E_5-treated intact rats had a significantly lower ($p < 0.001$) level of pituitary prolactin than the corresponding diluent-treated intact animals; no effect was observed on plasma prolactin levels. In conclusion, the effects of E_5 administration are most evident on pituitary levels of LH and prolactin in intact rats; the exact mechanism by which this phenomeon is accomplished remains to be elucidated.
Supported by NSF grant PCM-77-05374 and NIH Center for Reproductive Biology (Bioassay Core) #P 30 HD 10202.

153.9 EFFECTS OF NEONATAL ALTERATIONS IN GONADAL STEROIDS ON CA1 HIPPOCAMPAL PYRAMIDAL CELLS: CHALLENGE WITH SEX HORMONES TO IN VITRO SLICE PREPARATIONS FROM ADULT RATS. Richard M. Vardaris, Timothy J. Teyler, and Craig T. Reiheld. Neurosci. Lab., Kent State U. and N.E.O.U.C.O.M., Kent, OH 44242.

In previous studies we have found that 17-beta-estradiol enhances throughput of the Shaffer collateral-CA1 synapse in slices from male hippocampus. Slices from females, however, show this response to testosterone. The female CA1 response to gonadal steroids varies with stage of estrous cycle. In addition we have found similar relationships for hippocampal CA1 cells with intact preparations.

In an effort to determine whether these gender-specific hippocampal response patterns are attributable to CNS differentiation or to circulating sex steroids, we are comparing the effects of gonadectomy at birth and in adulthood. For the present study, male and female rats were gonadectomized on Day 1 of life and then were injected with testosterone, 17-beta-estradiol, or a vehicle control solution. Both genders received each treatment.

Monosynaptic population responses were recorded from the CA1 cell body region of slices from the adult castrates. Control responses were obtained from slices that had equilibrated in steroid-free Ringer (Earle's medium). Responses following challenge with 10pM 17-beta-estradiol or testosterone were expressed as a percent of control amplitudes. Effects were evaluated 10 and 20 minutes after the administration of steroids.

The major result was that, after gonadectomy at birth, slices from both male and female adult hippocampus responded with increased synaptic throughput to challenge from either hormone. Steroid injections on Day 1 had inconsistent effects on responding. Studies involving different dosages and methods of hormone replacement are in progress as further attempts to investigate this problem.

153.10 IMMUNOHISTOCHEMICAL MAPPING OF NEUROACTIVE PEPTIDES IN THE CAT MEDIAN EMINENCE. Paul E Micevych and Robert P. Elde, Dept. of Anatomy, Univ. of Minnesota Medical School, Minneapolis, MN 55455

The effects of both exogenous and endogenous opiates on the neuroendocrine axis have been extensively documented by many authors. The localization of the enkephalins and opiate receptors to the median eminence (ME), as well as physiologic evidence support the concept that enkephalin is apparently acting at the level of terminals in the median eminence or the posterior pituitary. We had reported an extensive enkephalinergic system which overlapped the vasopressin-oxytocinergic system in the cat median eminence and posterior pituitary (Micevych, P. and Elde, R., J. Comp. Neurol., in press). We now expand these earlier immunohistochemical studies of the cat median eminence to demonstrate further areas of enkephalin-neuropeptide overlap and potential interaction in the median eminence.

Normal and colchicine treated cats (500 μg in the third ventricle, 48 hour survival) were perfused with 4% paraformaldehyde. Serial 10 μ cryostat sections were taken of the cat hypothalamus at 22 levels and stained with the indirect immunofluorescence method. Primary antisera directed against bovine neurophysin (NP), met-enkephalin (M-ENK), β-endorphin (β-END), α-MSH, somatostatin (SOM), luteinizing hormone releasing hormone (LHRH), and substance P (SP) were applied to adjacent sections and the pattern of staining for each antiserum was mapped.

Within the cat ME, very dense NP-like immunoreactivity was found in the internal layer and moderate to dense immunoreactivity was localized in the external layer abutting portal capillaries. The distribution of SOM and LHRH terminals and fibers in the ME appeared as a discrete band in the external layer of the lateral wings of the ME. Bright, punctate structures were scattered in the internal layer of the ME with anti-SOM sera. In contrast to the rat, scattered SP-like fibers and terminals were localized in the ME in the external layer near portal capillaries. No α-MSH or β-END was detected with antisera against these substances in the ME.

This overlap of neuropeptides may suggest physiologic interactions in the ME which may regulate hypophyseal function.

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153.11 PEPTIDE HORMONES AND THEIR PRECURSORS IN EXTRACTS OF BOVINE AND SHEEP PINEAL GLANDS. Jerry Vriend*, Patricia M. Hinkle*, Karl M. Knigge and Shirley A. Joseph* (SPON: L.A. Sternberger). The Neuroendocrine Unit and Department of Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Extracts of bovine and sheep pineal glands were fractionated on a Sephadex G-25 column (5 x 150 cm). Aliquots of lyophilized fractions were prepared for radioimmunoassay of LRF, TRF, and SRIF, for bioassay in an in vitro system of dispersed pituitary cells, and for further purification. Three 'peaks' of LRF immunoreactivity were detected and tested in bioassay. LH releasing activity was detected in only one peak. A second peak of material containing LRF immunoreactivity migrated on G-25 as if it contained an LRF-like peptide larger than LRF; a third peak of material migrated as if it contained an LRF-like peptide smaller than LRF. Evidence for two TRF-like substances was obtained using bioassay, radioimmunoassay, and radioreceptor assay. The two substances, apparently TRF and a TRF antagonist, were separated on the G-25 column. The material containing TRF antagonist activity displaced ^{3}H-TRF from pituitary cell membrane preparations in the TRF radioreceptor assay and inhibited TRF induced TSH release in cell culture. The TRF inhibitory factor (TRF-IF) was further purified on Sephadex G-10 and carboxymethylcellulose ion exchange columns. Copurification of anti-TRF bioactivity (in the dispersed pituitary cell system) and anti-TRF binding activity (in the radioreceptor assay) suggests that the inhibitory activity results from the binding of a TRF antagonist to receptors. TRF-IF did not copurify with SRIF or LRF, but did copurify with prolactin release inhibitory activity. Two peaks of SRIF immunoreactivity were detected by radioimmunoassay of G-25 fractions. Growth hormone releasing activity was detected in material obtained by subfractionation of G-25 fractions on LH-20. These results are interpreted as evidence for peptide hormones in the pineal gland. Larger molecular weight substances related to these hormones may be precursors with either no bioactivity or inhibitory activity.

(Supported by NIH Fellowship AM05995 and Program Project Grant NS15345.)

153.12 EFFECTS OF CENTRALLY AND PERIPHERALLY ADMINISTERED MAO INHIBITORS ON SEXUAL BEHAVIOR, MONOAMINES AND MAO ACTIVITY. V. Luine, E. Schmitt*, C. Paden*. The Rockefeller University, New York NY 10021.

The effects of inhibition of monoamine oxidase (MAO) on female sexual behavior was investigated. Adult female rats were given pargyline or clorgyline i.v., tested for lordosis responding, and then levels of serotonin (5HT), norepinephrine (NE), MAO A and MAO B were measured in the preoptic-hypothalamic area.

Rats were ovariectomized-adrenalectomized, and one week later they received 2.5μg of estradiol benzoate for 2 days. On the 3rd day, MAO inhibitors were given, followed 2h later by progesterone. Sexual behavior (lordosis quotient (LQ) and quality score (QS)) was tested 3-5h later. Dose-dependent decreases in LQ and QS were observed after MAO inhibitors. Inhibition of behavior was most closely correlated with the extent of MAO inhibition and with increases in levels of 5HT in the preoptic-hypothalamic area. A significant lowering of LQ and QS occurred when both forms of MAO were inhibited by approximately 90% and/or when levels of 5HT were increased twofold. When only the A form was inhibited, the QS was lowered but the LQ was not. These levels of MAO inhibition were associated with increases in 5HT levels which were less than twofold. Increases in NE levels after MAO inhibition did not correlate well with decreases in behavior. The same increases in NE levels were found in treated females which did and which did not show decreased lordosis.

In order to localize more precisely the sites within the preoptic-hypothalamic area which may be involved in MAO inhibitor dependent decreases in sexual behavior, double barrel cannulas were stereotaxically implanted within this area. One week later, estrogen + progesterone (E+P) dependent lordosis responding was measured. The following day inner cannulas containing 2mm of crystalline pargyline were inserted, P was given 2h later, and lordosis behavior was tested 3-5h after P. Placements dorsal to the lateral aspect of the ventromedial nucleus led to decreases in LQ and QS 5-7 and 29-31h after pargyline. Placements at a greater distance above the ventromedial nucleus (within or dorsal to dorsomedial nucleus) led to decreases in LQ and QS only at the longer time interval suggesting further that diffusion of the drug to the ventromedial nucleus area might be critical for inhibiting behavior. The LQ and QS of all females recovered to pre-pargyline values 53-55h after pargyline administration.

Results suggest that serotonin containing systems projecting into the vicinity of the ventromedial nucleus may exert regulatory control of female sexual receptivity in rats.

(Supported by PHS grant HD12011)

154.1 VISUAL PROJECTIONS TO LIMBIC CORTEX. Richard T. Robertson, Scott M. Thompson*, and Martine J. RoBards. Department of Anatomy, College of Medicine, University of California, Irvine, CA 92717.

A neural substrate for visual projections to limbic cortex has been demonstrated in the rat by the combined use of anterograde transneuronal and retrograde transport techniques. Retrograde transport of horseradish peroxidase was demonstrated in 6μm cryostat sections by conventional histochemical techniques using 3-3' -diaminobenzidine or the Hanker-Yates reagents. These sections were then processed for autoradiography to demonstrate anterograde transneuronal transport of ^{3}H-adenosine and/or ^{3}H-uridine. Neurons that were labelled both with intracellular peroxidase reaction product and with overlying silver grains were interpreted as receiving an afferent projection from the region injected with ^{3}H-adenosine/uridine, and sending an efferent projection to the region injected with peroxidase.

Intraocular injections of ^{3}H-adenosine/uridine combined with pressure or iontophoretic injections of peroxidase into the lateral dorsal nucleus of the thalamus resulted in double labelled cells in the pretectal complex. Double labelled cells were found in the nucleus of the optic tract, the posterior pretectal nucleus, and the pretectal olivary nucleus.

Pressure injections of ^{3}H-adenosine/uridine were made in the pretectal complex, involving the nucleus of the optic tract and the posterior pretectal nucleus. When these injections were combined with pressure injections of peroxidase into the retrosplenial cortex or the presubiculum, double labelled cells were seen throughout much of the lateral dorsal nucleus of the thalamus.

Two conclusions can be made from these studies. First, the combined use of anterograde transneuronal transport and retrograde transport techniques offers a unique approach to determining functional neural connections with the light microscope. Second, these data indicate a functional series of connections that could convey visual information to the limbic system. The system includes retinal projections to the pretectal complex, pretectal projections to the lateral dorsal nucleus of the thalamus, and lateral dorsal projections to limbic cortex.

Supported by NSF grant BNS 79-14223 and NIH grant NS 14267.

154.2 FUNCTIONAL CONNECTIONS OF THE AMYGDALA IN THE RAT DEMONSTRATED WITH THE (^{14}C)2-DEOXYGLUCOSE TECHNIQUE. B.S. Layton, S. David*, R.S. Poulsen*, C.J. Thompson* and Leo P. Renaud. Dept. of Neurology, Montreal General Hospital, Montreal, Canada.

A variety of morphological and electrophysiological techniques have been utilized to determine central connections of the amygdala from a structural and functional viewpoint. In the present experiments we have combined electrical stimulation in the rat amygdala with the (^{14}C)2-deoxyglucose (2-DG) autoradiographic method so as to trace its functional connections to various brain regions. Specific emphasis was directed to those connections of the amygdala with basal forebrain structures that have been examined with electrophysiological techniques. Pentobarbital anaesthetized Male Sprague-Dawley rats were implanted with monopolar or bipolar nichrome electrodes stereotaxically positioned in various amygdaloid nuclei and stimulated with current pulses (0.05msec duration 0.1-1.0mA) at frequencies of 2.0, 7.5, 10 and 20Hz. for 45 minutes. At the onset of stimulation, 150μCu/Kg body weight of 2-DG in 0.4 ml of normal saline was injected intravenously. After 45 minutes, the animals were sacrificed by decapitation and the brains were sectioned for autoradiographic and histological analysis. Our results indicate that electrical stimulation in the amygdala at frequencies greater than 2Hz enhanced 2-DG uptake in several areas ipsilateral to the stimulation site including the bed nucleus of the stria terminalis, preoptic area, ventromedial hypothalamus, head of the caudate nucleus and piriform cortex. Uptake was particularly dense over the stria terminalis pathway itself. These observations are in accord with known anatomical data on amygdalofugal projections to basal forebrain regions and provide information on changes in metabolic activity induced by electrical stimulation that can be correlated with the activity of single neurons evoked in these areas by stimulation in the amygdala. (Supported by the Canadian MRC).

154.3 Thalamic projections to the cingulate cortex of rabbits: Robert W. Sikes and Jon F. DeFrance, Dept. Anatomy & Neurobiology, Univ. of Texas Medical School, Houston, TX 77025

In their study of the cingulate cortex in rabbits, Rose and Woolsey (1948) described a topographical pattern of projection from the anterior thalamus in which the anterior medial nucleus (AM) projected to the anterior cingulate (LA), the anterior ventral nucleus projected to the posterior cingulate (CING), while the anterior dorsal nucleus (AD) projected to the retrosplenial cortex (RS). Recent studies in rats and cats have shown more overlap in the cortical projections of anterior thalamic nuclei and have described projections from thalamic nuclei not described in the Rose and Woolsey paper. This paper will reevaluate the thalamic projections to the cingulate cortex in rabbits using the retrograde horseradish peroxidase technique.

Adult New Zealand rabbits received small injections (10-100nL) of 25% horseradish peroxidase through micropipettes with a tip size of 10-50 mu. Injections were placed in the cortical fields defined by Rose and Woolsey. The tissue was processed by the tetramethylbenzidine technique (Mesulam, 1976).

Although the scheme of cortical projections by the anterior nuclei described by Rose and Woolsey was generally supported, important overlap was found. The projection from AM did terminate primarily in LA, but many neurons were labeled in this nucleus after injections into CING. Conversely, cells in AV were heavily labeled after injections into the CING and RS. These neurons were concentrated in the ventral part of the nucleus. Cells in the dorsal part of AV were labeled only by injections into the presubiculum or injections which entered the cingulum bundle. The AD had projections to much of the medial cortex caudal to the septal area, but appeared to have no projection to the dorso-lateral CING.

A major projection to the cingulate cortex also originated in the lateral thalamus (LP,LD). Neurons were heavily labeled in LP following all injections except for the most anterior ones. Neurons were also seen in LD, but in greatly reduced numbers.

Projections from the ventral thalamus (VA,VL,VM) terminate in LA. The primary projection field of VA may lie anterior to that of VL and VM which have a strong projection to the caudal LA.

Finally, neurons were labeled in the midline thalamic nuclei, especially in the mediodorsal nucleus (MD). Injections into LA produced labeled neurons in the lateral part of MD, and injections into the infralimbic area labeled neurons in the medial MD. The central and reunions nuclei also were occasionally labeled, but the topographical distribution is at this time uncertain.

Rose, J.E. and Woolsey, C.Nr, 1948, J. Comp. Neurol., 89:279
Mesulam, M. 1976 J. Histochem. Cytochem., 24: 1273

154.4 ALTERED LH RESPONSE TO ESTROGEN PLUS PROGESTERONE TREATMENT IN FEMALE RATS WITH DUAL LIMBIC SYSTEM LESIONS. Julia L.Tyler, Valer Csernus* and Roger A. Gorski. Departments of Psychology and Anatomy, UCLA, Los Angeles, California 90024.

Extrahypothalamic structures appear to modulate luteinizing hormone (LH) release. In this study we examined the potential functional interaction of 3 limbic structures: olfactory bulbs, amygdala, and septum, in the release of LH in response to estradiol benzoate (EB) and progesterone (P). Ovariectomized rats received 1 or 2 of these bilateral procedures: sham surgery (SHAM), bulbectomy (OB), amygdala lesions (AL) or septal lesions (SL). Operations were performed simultaneously or 10 weeks apart. Ten to 12 weeks after the second operation the influence of EB and P on LH titers was tested. Jugular blood samples (0.5ml) were removed from etherized rats at 1600-1700 h (lights on 0500-1900 h) on 5 consecutive days. The first sample was taken the day before EB injection. EB (20 ug) was injected sc at 1300 h and P (2 mg) was injected 72 h later. Analyses of variance were applied to the data, followed by Neuman-Keuls tests. Relative to their pre-EB levels, all rats showed negative feedback 5 h post-EB. There were no differences among groups in LH levels prior to EB or 5 h or 29 h post-EB. Rats given OB first and SL 10 weeks later showed higher LH levels 53 h post-EB compared to those of several other groups, but were not different from SHAM rat values. Thus, few lesion effects were detected prior to P injection. Lesions did significantly alter the magnitude of the P-induced LH surge. LH values 5 h post-P are listed below:

SHAM:	2623 ± 471 ng/ml	OB/SL:	2803 ± 378 ng/ml
OB:	1374 ± 393*	OB+SL:	2870 ± 426
AL:	1379 ± 427*	SL+OB:	2170 ± 489*
SL:	2739 ± 352	AL/SL:	3209 ± 288
OB/AL:	1366 ± 474*	AL+SL:	2763 ± 420
OB+AL:	1747 ± 304*	SL+AL:	1910 ± 183*
AL+OB:	1398 ± 304*		

*= significantly different from SHAM value. A "/" signifies simultaneous dual lesions of the types indicated; a "+" signifies that the indicated lesions were produced sequentially. It is clear that OB or AL decrease P-induced LH release in the EB-primed rat while SL alone does not. SL before OB (SL+OB group) or SL before AL (SL+AL group) does not prevent the OB or AL-induced deficit in the magnitude of the P-induced LH surge. However, SL could counteract the effects of OB or AL if it occurred after OB or AL or simultaneous to OB or AL. Thus, it is clear that the sequence of lesioning is a critical parameter although the mechanisms involved in lesion-induced alterations in LH control remain to be elucidated.

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154.5 NON-FORNIX SPREAD OF HIPPOCAMPAL AFTERDISCHARGES DEMONSTRATED BY THE 14C DEOXYGLUCOSE TECHNIQUE. Michel Kliot*, Mark Boytim*, and Charles E. Poletti* (SPON: E.I. Knudsen). Department of Neuro-Surgery, Massachusetts General Hospital, Boston, MA. 02114.

Non-fornix hippocampal efferent projections were investigated using the 14C Deoxyglucose (DG) technique in rats. In a previous study (Science 204:641-643,1979) we showed that only after-discharges (AD's) electrically initiated (.2-.4mA, 1-msec pulses, 16 per sec) in the ventral subicular cortex increased metabolism in the amygdala, hypothalamus, and basal forebrain. In the present series of experiments, AD's were triggered in the ventral subicular cortex of rats with either bilateral lesions of the fornix or fornix and stria terminalis combined.

Rats with complete lesions of the fornix displayed increased DG uptake in the ipsilateral hippocampal formation, amygdala, hypothalamus, and basal forebrain. This pattern differed from that found in unlesioned rats in three respects. First, the AD's did not spread to the contralateral side. Second, increased activity in the hypothalamus was confined primarily to the preoptic, ventromedial, and premammillary nuclei whereas in intact rats marked increases were also found in the anterior, lateral, dorsal, and posterior hypothalamic nuclei. And third, increased metabolism was not present in the dorsal portion of the lateral septum. As in unlesioned rats, increases were found in the ventral portion of the lateral septum and the nucleus accumbens, ventral putamen, nucleus of the lateral olfactory tract, and bed nucleus of the stria terminalis.

Rats with combined lesions of both the fornix and stria terminalis showed a similar pattern of increased glucose consumption in the ipsilateral hippocampal formation and amygdala. In contrast, the basal diencephalon showed only modest increases in activity restricted to the ipsilateral lateral preoptic region, nucleus accumbens, ventral putamen, nucleus of the lateral olfactory tract, and bed nucleus of the stria terminalis.

In controls with lesions but no electrical stimulation, only tissue injured by the surgery showed DG levels above those levels seen in nonstimulated intact animals.

These results demonstrate that AD's initiated in the rodent ventral hippocampal formation can spread to the amygdala, hypothalamus, and basal forebrain in the absence of the fornix. They also support the proposal that ventral hippocampal activity is relayed to the hypothalamus and basal forebrain via the amygdala and its efferent projections, the stria terminalis and ventral amygdalofugal pathway (Poletti and Sujatanond, J. Neurophysiol., in press; Morrison and Poletti, Brain Res., in press).

This research was supported by the NeuroResearch Foundation and BioMedical Research Support Grant 05486-16 of the Massachusetts General Hospital.

154.7 AMYGDALOID EFFERENTS TO THE BED NUCLEUS OF THE STRIA TERMINALIS AND THE MEDIAL PREOPTIC AREA CONTROLLING MALE HAMSTER SEXUAL BEHAVIOR: COMBINED HRP AND BEHAVIORAL ANALYSES. Michael N. Lehman and Sarah S. Winans. Neurosci. Prog. and Dept. Anat., Univ. Mich., Ann Arbor, MI 48109.

Recent evidence suggests that olfactory and vomeronasal information critical to male hamster mating behavior is processed in the medial nucleus of the amygdala, an androgen-binding brain area. This nucleus is a major source of efferents, via the stria terminalis (ST), to the bed nucleus of the stria terminalis (BNST) and the medial preoptic area (MPOA), two additional androgen-binding brain areas important in the control of male copulatory behavior. Because medial nucleus lesions but not ST destruction eliminates male hamster mating behavior (Neurosci. Abstr., 4:88), we hypothesized the existence of a non-strial route by which the medial nucleus influences the BNST or MPOA. To test this, we iontophoretically applied horseradish peroxidase (HRP) in the BNST or MPOA of male hamsters in which we surgically interrupted the ST, and control males with the ST intact. After 48 hours survival, the hamsters were perfused and the brains were processed using the tetramethylbenzidine procedure.

Histological analysis of HRP labelled brain sections demonstrated the existence of a ventral non-strial projection from the medial nucleus to the BNST, but not to MPOA. To test the functional significance of this pathway, we observed the mating behavior of male hamsters before and after either: a) bilateral parasagittal knife cuts placed between the amygdala and the BNST (n=8); b) a unilateral parasagittal knife cut with a contralateral medial nucleus lesion (n=7); or c) control knife cuts (n=5). After postoperative testing, we iontophoretically applied HRP in the BNST of these animals.

Procedures a) and b) completely eliminated mating behavior in 10 out of 15 animals. The remaining males showed impaired or absent mating in at least two of the three postoperative tests. Males with control knife cuts continued to mate normally. HRP analysis confirmed that the parasagittal knife cuts had interrupted the ventral pathway, and showed that the ST was intact in at least four of those males that displayed severe mating deficits. These results suggest the importance of the ventral pathway from the medial amygdaloid nucleus to the BNST as a route by which chemosensory information influences the display of male copulatory behavior.

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154.6 CYCLIC CHANGES IN HIPPOCAMPAL EVOKED RESPONSES DURING THE OVULATORY CYCLE IN RATS. L. F. Mercer, Jr. and N. Hagino. Dept. of Anatomy, University of Texas Health Science Center, San Antonio, Texas 78284.

Experiments were undertaken to examine the characteristics of hippocampal responses evoked by medial forebrain bundle (MFB) stimulation during stages of the ovulatory cycle of rats. In ten female rats (Timco Simonson), stimulating and recording electrodes were surgically implanted in the MFB at the level of the lateral preoptic area and dorsal hippocampus (area CA2), respectively, and were connected to an Amphenol connector to allow recording with unanesthetized preparations. An ovulatory sequence was determined for each animal by daily inspection of vaginal smears. Evoked response patterns were determined during metestrous, diestrous, proestrous, and estrous. Stimulation voltages were slowly increased until a reliable pattern was obtained from the hippocampus. In the evoked response, three peaks were identified having latencies of 8-12 msec (P_1), 18-24 msec (P_2) and 30-40 msec (P_3). P_1 showed the most reliable correlation with the vaginal status. P_1 was of lowest amplitude during metestrous and progressively increased during diestrous and proestrous with a subsequent decrease on estrous. Since the pattern of changes in P_1 paralleled the pattern of estrogen levels during the cycle, Experiment 2 examined the effect of exogenous estrogen during metestrous when the amplitude of P_1 was found to be lowest. Administration of 1 µg estradiol benzoate in oil, IM, on the day prior to metestrous produces a 45% greater amplitude of P_1 during metestrous than occurred in noninjucted controls. The findings of Experiment 2 supported the suggestion of an excitatory effect of estrogen on MFB-Septal-hippocampal system. The possibility of catecholamine involvement in the observed changes was investigated in Experiment 3 in which the evoked response pattern was observed after depletion of catecholamines with alpha methyl tyrosine (AMT 250 mg/kg, IM) on proestrous when the P_1 component is at its maximum. Injection of AMT eliminated the expected proestrous rise in emplitude of P_1 resulting in no significant difference from the amplitude during metestrous. The overall results of the study suggest that the excitability of the MFB-septal-hippocampal system is lowered by estrogen and requires the integrity of catecholamines for the process to occur. (Supported by NIH HD 10071)

155.1 NEURAL CONTROL OF CARDIOVASCULAR RESPONSES DURING FOOD AROUSAL IN APLYSIA. U. T. Koch,* J. Koester, and K.R. Weiss (SPON: W.L. Nastuk). Div. of Neurobiology and Behavior, Depts. of Anatomy, Physiology and Psychiatry, Columbia University, New York, N.Y. 10032.

Exposure of Aplysia to food stimuli produces an arousal state that is reflected in parallel increases in heart rate (30% above baseline) and in biting rate. Two-thirds of the heart rate increase is eliminated by cutting either the pericardial nerve (J. Comp. Physiol., 123:11) or the pleural-abdominal connectives, suggesting that this effect is primarily neurally mediated. With chronic recordings from intact animals we have found that blood pressure also increases during food arousal.

We have developed a preparation to study the cellular basis of the cardiovascular components of food arousal. The anterior region of the head, connected to the abdominal ganglion by the pleuro-abdominal connectives, was dissected free. The pericardial nerve connections to the heart and the abdominal aorta were also maintained. The animal was kept at 0 to 2° C. throughout the dissection. During experiments the head was held by a soft, inflatable collar, and perfused through the anterior aorta with culture medium. The lips and tentacles protruded into a separate chamber, filled with artificial seawater. Seaweed extract was introduced into this chamber to elicit food arousal. Biting movements were monitored with a strain gauge attached to the buccal mass. We recorded intracellularly the activity of the LB_{VC} vasoconstrictor motorneurons, and the RB_{HE} heart excitor neuron. Activity of the LB_{VC} cells reduces blood flow to the visceral organs, and firing RB_{HE} increases the strength and frequency of heart beat.

During arousal biting movements were elicited, and the firing rates of both cell types increased significantly. The mean spike rate increase was 35% for RB_{HE} ($N=5$, $p < .05$) and 105% ($N=7$, $p < .01$) for LB_{VC} during stimulation. The firing pattern of the LB_{VC} cells changed from a steady rate to a pronounced bursting pattern that was phase-locked to the biting movements. The increase in RB_{HE} firing rate during arousal was a tonic change with no link to the biting cycle.

These results are consistent with the hypothesis that LB_{VC} and RB_{HE} cells contribute to the changes in heart rate and blood pressure that occur during food arousal. If so, the overall effects of this neural activity may be to increase blood flow, with a shift in distribution from the viscera to the head. Such a response may be required to increase O_2 supply and support the hydrostatic skeleton of the animal during the head extension and biting movements that occur during food arousal.

As in the case of the biting component of food arousal, it appears that the phasic cardiovascular changes during arousal are mediated primarily by cholinergic cells (LB_{VC}), whereas the tonic effects are primarily mediated by an increase in cyclic AMP produced by a serotonergic modulatory cell (RB_{HE}) (Br. Res., 177:388; J. Neurophysiol., 42:791).

155.2 MORPHOLOGY AND TONIC ACTIVITY OF THE LASHING VENTRAL NERVE CORD IN A SATURNIID MOTH. James J. Cole and Frances V. McCann. Dept. of Physiology, Dartmouth Med. School, Hanover, NH 03755.

Hyalophora cecropia is a moth having a flexible ventral nerve cord (vnc) which is constantly in left-right oscillation at 1 cm intervals along its length, with an amplitude of 2-4 mm and a frequency of 1-3 Hz at physiological temperatures.

The abdominal vnc is a series of four ganglia communicating by paired interganglionic connectives having unmyelinated axons. It is profusely invested with branching tracheae, and sends out ganglionic roots that innervate the abdominal body walls and viscera. The contractile cells directly responsible for vnc motility are bilateral sheets of muscle fibers attached dorsally to connective tissue overlying the neural elements. The lashing fibers comprise the muscular component of the ventral diaphragm. The fibers on left and right sides of the vnc contract alternately. Each ganglion has its locally coordinated pair of fiber sheets whose combined activity pulls the segments of the vnc from side to side in a serpentine fashion. Putative functions of lashing include facilitation of gas exchange between tracheal air and neural tissue, and generation of a caudal flow of hemolymph in the ventral sinus (Hessel,J.H., Ann.Ent.Soc.Am.62:353, 1969).

The fibers of the lashing muscle resemble at a histological level the alary muscle fibers of the heart. The rate of oscillation of the vnc approximates the rate of the heartbeat. Histology and ultrastructure, however, have not provided evidence of synapses onto the heart or alary muscles. Tonic units exist in the vnc which have firing frequencies similar to the heartbeat, but the heart will continue to beat in isolation.

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155.3 NEURAL CARDIAC MODULATION IN A PULMONATE GASTROPOD LIMAX MAXIMUS. D.S.Grega and D.J.Prior. School of Biol. Sciences, Univ. of Kentucky, Lexington, KY 40506.

The myogenic heart of the garden slug Limax maximus is innervated via the visceral nerve (VN) by neurons in the visceral ganglion. Stimulation of the intact VN with a suction electrode has both chronotropic and inotropic effects on the heart. Heart activity was monitored with a force transducer; the parameters measured were heart (HR), contraction amplitude (Amp) and inter-contraction interval (ICI) to determine immediate inhibition due to stimulation. A single stimulus pulse of 1 ms duration evokes a brief immediate inhibition of heart contraction indicated by a 68% increase in ICI followed by reestablishment of the control HR with a 55% increase in Amp. With trains of pulses at various frequencies (0.5 to 100 Hz) low frequency stimulation (0.5 to 10 Hz) was most effective in increasing Amp and evoking immediate inhibition of heart contraction. Moderate increases in ventricular pressure were achieved in two ways. Increasing peripheral resistance with a ligature of the aorta and increasing heart volume with an injection of saline via the cut aorta both result in a slight 1 to 3% decrease in Amp and HR which returned to control values when the pressure was eliminated. Thus, the increase in Amp after initial inhibition of heart contraction is probably neuronally mediated and not due to volume stress associated with accumulation of blood in the heart. Unlike VN stimulation, topical stimulation of discrete areas of the visceral ganglion with fine-tipped suction electrodes cause both cardiac inhibition and excitation (increase in HR and Amp). Thresholds for cardiac excitation were observed to be higher than those for inhibition in accord with that reported for other pulmonates. Cobalt backfills of VN toward the ganglion reveal the position of possible cardioregulatory neuron cell bodies. These soma generally were located toward the periphery of the dorsal surface of the ganglion in areas which,when topically stimulated excite or inhibit heart activity. Preliminary pharmacological studies were done by drug application on the isolated,beating heart with the central nervous system removed. A low concentration (10^{-7}M) of serotonin results in an immediate,transient decrease in Amp followed by a decrease in HR. Application of acetylcholine (10^{-7}M) evokes an immediate inhibition of heart contraction followed by an increase in HR and Amp.

155.4 MOTONEURONS INNERVATING ANTERIOR AORTA OF APLYSIA. J.E. Blankenship, M. Sawada* and D.H. McAdoo. Marine Biomedical Institute, Univ. Tx. Med. Br., Galveston, TX 77550.

Three new motoneurons in the abdominal ganglion of Aplysia californica have been identified which make monosynaptic connections with muscle fibers in the wall of the anterior aorta but not in other aortic vessels. Two of these cells, designated RD_{AAI}, are inhibitory, and one is excitatory, RD_{AAE}. The cell bodies lie near cell R2 and send their axons to the aorta via the vulvar nerve. Neurons and muscle fibers were impaled with microelectrodes, the vulvar nerve was recorded or stimulated with suction electrodes, and muscle tension in the aortic wall was recorded by a micro-strain gauge. Various transmitters or pharmacologic blocking agents were applied to the bathing solution, and in some experiments the ionic composition of the bath was altered to determine which ions were involved in junctional current. Muscle fibers of the aorta had a mean resting potential of -62 mV. Muscle fibers could have any combination of at least three spontaneous junctional potentials (diffuse, polyneuronal innervation): a very brief ejp, a slower ejp, and an ijp. The motoneuron producing the fast ejp is unidentified, but these responses are blocked by 10^{-4} M d-tubocurarine (dTC). The other ejp is produced directly by cell RD_{AAE}. This response could be mimicked by μM amounts of serotonin (5HT) and was almost completely blocked by 10^{-7} to 10^{-5} M bromo-D-lysergic acid. It was also partially blocked by atropine, but unaffected by dTC. The amplitude of this ejp was reduced when extracellular Na was reduced to 80% of normal. The ijp was produced by either of the two cells RD_{AAI} which are larger than RD_{AAE} and have lower thresholds to extracellular stimulation of the vulvar nerve. The ijp's are completely and reversibly blocked by 10^{-4} M dTC and are mimicked by acetylcholine (ACh). The ijp is reduced in 1/10 normal Cl and increased in 1/10 normal K and is believed to be due to an increase in conductance to both ions. RD_{AAE} firing or bath applied 5HT cause muscle depolarization and increased contractions, while RD_{AAI} activity or bath applied ACh produce muscle hyperpolarization and relaxation. Motoneuron activity partly underlies spontaneous oscillatory contractions of the aortic wall. Muscle fibers only rarely show spike-like responses; these do not overshoot the zero potential but do have a small afterhyperpolarization. Both types of motoneuron-induced jp's show moderate facilitation with repetitive firing. The motoneurons do not synaptically interact with one another, but bursts of activity in interneuron II drive RD_{AAI} and cause pronounced muscle relaxation while RD_{AAE} is simultaneously inhibited.

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156.1 PREMOVEMENT POSTURAL MUSCLE ACTIVITY DURING RAPID UNILATERAL ARM FLEXION IN CEREBRAL PALSIED AND NORMAL PERSONS. W.A. Lee, Neuromuscular Laboratory, University of Texas, Austin, Texas, 78712

Activity in postural (posterior thigh: biceps femoris) muscles has been reported to precede arm movement in a rapid unilateral arm flexion task in neurologically unimpaired persons (1,2). The pattern of neuromuscular activity which has been described in normal subjects consists of: onset of biceps femoris activity ipsilateral to the arm raised; onset of the primary agonist (anterior deltoid); onset of contralateral biceps femoris; and finally arm movement (reaction time, RT). It has been hypothesized that the premovement biceps femoris activity may preset stiffness of the trunk support system to counteract perturbations of balance expected to accompany the voluntary arm flexion. Were this the case, persons with some neurological dysfunction like cerebral palsy who have imbalance as one motoric symptom might be predicted to produce neuromuscular patterns with reduced or delayed biceps femoris activity, as compared with the pattern from normal subjects.

In the present report, data on the complex neuromuscular patternof rapid arm flexion are described for three adult, ambulatory cerebral palsied (CP) persons; these are compared with previously reported data (2) on normal subjects' behavior. Each subject performed 30 left and 30 right rapid flexions of one arm from hip to shoulder level while in a standing posture, under simple RT conditions. Means and standard deviations of response component latencies (ipsilateral biceps, anterior deltoid, contralateral biceps, and RT) and intercomponent correlation coefficients for individual subjects are reported. Correlations among components were high and significant for CP subjects, in a manner similar to those for normal subjects, indicating a similarly tight linkage of postural and task components for the two groups. However, the temporal order of the pattern differed for the CP subjects, with a delay observed in either contralateral or ipsilateral biceps femoris (or both) relative to anterior deltoid onset. The data are consistent with the hypothesis that premovement biceps femoris activity observed in standing subjects for the rapid arm flexion task may serve some function in controlling balance. Because the delays were specific to biceps femoris, it is plausible that the postural and task components are controlled via parallel pathways although both components are part of the voluntary response of rapid arm flexion.

References
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156.2 THE ORGANIZATION AND REORGANIZATION OF SPEECH MOVEMENTS. R. Netsell, R.Kent* and J. Abbs*. Boys Town Institute, Omaha, NE 68131 and University of Wisconsin, Madison, 53706.

X-ray motion pictures from three subjects were used to study lip and tongue movements during speaking conditions in which the jaw was held in a fixed position (the upper and lower incisors being separated by 8, 16, and 24 mm) or was free to assume its normal movement pattern. The data on movement reorganization in the lips and tongue were drawn from the jaw-fixed conditions, where, in some cases, the lips and tongue were required to more than double their range of movement in order to produce normal speech.

For the most part, the subjects successfully reorganized the lip and tongue movements to closely approximate the temporal and spatial targets achieved in the control (jaw-free) condition. These precise adjustments were made without conscious mediation, i.e., subjects reported no awareness of the movement reorganization. For productions of single vowel sounds, the tongue adjustments were made before vocalization, i.e., before the subject had auditory feedback about the adequacy of the adjustment. In jaw-fixed sentence productions, subjects maintained the temporal organization of the control condition by increasing the velocity of movements. For example, to achieve lip closure for p in the word heap for the 24 mm. jaw-fixed condition, the lower lip more than doubled its velocity over the control condition. Other data displays demonstrate which aspects of these temporal/spatial goals are achieved with high accuracy and which are more variable.

Two hypotheses could explain the reorganization of these speech movements. With the jaw held in the fixed positions, subjects may have (1) selected different muscles to achieve the observed vocal tract adjustments, or (2) increased contractile force to the same muscles used in the control (jaw-free) condition which, in turn, increased the observed range and velocities of movement. In the absence of EMG data on individual muscle activity, the latter hypothesis is more parsimonious and consistent with recent modelling approaches to tongue movements during speech.

From the vowel data, it is hypothesized that spatial representations for these vocal tract shapes exist in the brain and can be reconstructed through intero- and exteroceptive feedback of tongue and oral cavity contacts. Auditory feedback is not necessary in these instances. The use of auditory feedback in the ongoing control of speech movements might be clarified by observing the reorganization task under the conditions of auditory masking.

156.3 REDUCTION OF THE LENGTH-THRESHOLD OF THE MYOTATIC REFLEX BY VOLUNTARY MUSCLE CONTRACTION. Gyan C. Agarwal, Gerald L. Gottlieb and Robert J. Jaeger. Department of Physiology, Rush College of Health Sciences, 1753 W. Congress Parkway. Chicago, Illinois 60612.

Torques were applied to the human ankle joint to first plantarflex the foot and, after a specified delay of 50 ms to 750 ms, rapidly dorsiflex it. The initial plantarflexion shortened the soleus muscle and the subsequent dorsiflexion restretched it, evoking a myotatic reflex.

The latency of the soleus myotatic reflex is not sensitive to the rate of muscle stretch under many circumstances (Gottlieb & Agarwal, J. Neurophysiol., 1979) but under conditions where the muscle is shortened before stretching, an increase in latency is observed which can be accounted for by the existence of a threshold process within the reflex arc of the form of equation 1.

$$f(x) = x + \tau \dot{x} \;;\; f(x) > \Delta \qquad = 0 \;;\; f(x) < \Delta \qquad \text{eq. 1}$$

In this equation, no reflex output occurs until the function f(x), where x is the change in muscle length, exceeds Δ. This model predicts that reflex latency (t_r) during constant velocity stretch (γ) will be described by equation 2 and the stretch (x_t) during that subthreshold period by equation 3.

$$t_r = t_c + \left(\frac{\Delta}{\gamma} - \tau\right) \qquad \text{eq. 2}$$

$$x_t = \Delta - \gamma\tau \qquad \text{eq. 3}$$

(t_c is conduction time for a tendon-jerk reflex)
These predictions were verified experimentally. The hypothesized site of the threshold is the muscle spindle membrane where length changes are transduced to action potentials.

Voluntary plantarflexion reduces the reflex threshold. This suggests that coactivation of skeletomotor and fusimotor pathways prevents spindle unloading during voluntary muscle shortening and thereby maintains the integrity of the reflex pathway during voluntary movements. (Supported by NIH grants NS-12877, NS-00196 and NSF grant ENG-7608754).

156.4 INFLUENCE OF TONIC LABYRINTHINE REFLEX UPON SOLEUS MOTONEURON EXCITABILITY IN MAN. C.W.Y. Chan, K.E. Kearney, and I. Levine. School of Physical and Occupational Therapy, Biomechanican Engineering and Aviation Medical Research Units, McGill University, Montreal, Quebec, Canada.

Although animal studies have demonstrated that vestibulospinal influences can reach the lumbar cord, and through their modulation of spinal mechanisms, contribute in a systematic way to the control of posture, such effects have not been examined in man. The objective of the present study was therefore to determine how tonic labyrinthine reflexes, evoked by changes in the static position of the head and body relative to gravity, modulate the excitability of human soleus motoneurons.

Subjects were blindfolded and fixed to a circular bed in supine position by means of body straps, as well as partial casts at the neck and legs. Extreme care was taken to ensure a constant angle between head and body to eliminate neck afferent inputs. The excitability of soleus motoneurons, as reflected by the H reflex, was then measured as a function or orientation of the head with respect to gravity, by rotating the bed over a range of 180° in steps of 15° or 30°. Stimulus intensity for the H reflex was carefully adjusted to elicit a small m response and an H reflex usually no larger than 70% of the maximum m response. At each orientation, 25 responses were recorded and later analysed to determine the "mean" and "standard deviation" of the peak-to-peak amplitude of H and m components. Responses were rejected if there was a significant variation in m response indicating a change in stimulus conditions.

Seven subjects were examined to date. In one subject, the H reflex was facilitated when the head was tilted forward and backward from the vertical position. The opposite was found in another subject. However, in 5 out of 7 subjects, there was no significant difference in the amplitude of the H reflex as a function of changes in static head orientation with respect to gravity. This consistent finding indicates that tonic labyrinthine reflexes probably exert negligible influence on the excitability of human soleus motoneurons. The two exceptions mentioned earlier could presumable be explained on the basis of different cutaneous inputs, a possibility being currently investigated.

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156.5 VOLUNTARILY INDUCED DIFFERENTIAL ALTERATION IN FORCE THRESHOLD OF SINGLE MOTOR UNITS OF THE HUMAN VASTUS LATERALIS. S. A. Cremer*, R. J. Gregor*, M. Mirsky*, and V. R. Edgerton (SPON: J. Keesey). Neuromotor Control Laboratory, University of California, Los Angeles, California 90024.

It has been shown that the force threshold (FT) of single motor units within a muscle can be differentially activated via skin afferents (Datta and Stephens, Neuroscience Abstracts, 1979). The possibility of differentially altering the force thresholds of single motor units (SMUs) by voluntarily modifying the type of muscle effort was investigated in this study.

Bipolar fine wire (50μm) electrodes were inserted into the vastus lateralis muscle (VL) for recording of SMU action potentials. Surface EMGs of the biceps femoris and vastus lateralis were also recorded. The subject's knee remained at a 90° angle with the ankle fixed at 110° and the foot secured to a force platform to record the three components of the isometric force produced by contraction of the knee extensors. The subject received auditory and visual feedback of the action potentials and visual feedback of the resultant vector of the forces produced in the X-Y plane of the force platform. The force thresholds of the SMUs were determined for a slow (approx. 10N/s) smooth ascending and descending force ramp and for the same ramp rate, but with voluntary oscillations (alternating contraction-relaxation of the knee extensors). These oscillations produced at a rate of about 6-12 Hz, resulted in a fluctuation in force of 2.5-5.0 N at the platform. Firing frequencies both with and without voluntary oscillations were determined at maintained force levels.

Thirty-one SMUs were studied in three healthy subjects. The SMUs had force thresholds in the smooth ramps up to approximately 25% of maximum voluntary contraction (MVC). During oscillation, the FT increased more than 5% of MVC in 22% of the SMUs, while 6.5% had a lower FT during oscillatory efforts. The largest increase in the FT of a SMU was 40% of MVC. Two pairs of SMUs were observed to exchange rank in the recruitment order. Thirteen per cent of the SMUs varied in FT by greater than 5% of MVC on the ascending vs. descending portion of the ramps with oscillations. At a maintained steady force level 42% of the SMUs decreased firing frequency by 20% (e.g. from 10 to 8 Hz) to 100% (cessation of firing) during oscillation. These data suggest that the input to the motoneuronal pool can be selectively influenced voluntarily during a rapid but low amplitude oscillating movement.

156.6 SPEED OF TENSION DEVELOPMENT IN ISOMETRIC CONTRACTIONS OF A HUMAN HAND MUSCLE, R. G. Miller, A. Mirka and M. Maxfield*. Department of Neurology, University of Oregon Health Sciences Center, Portland, Oregon 97201.

This study compares the amplitude and rate of tension development during both electrical stimulation and voluntary activation of a human hand muscle. The contractile responses from the first dorsal interosseous muscle of 14 normal human subjects were studied to provide the experimental data.

Under each experimental condition, there was a linear relationship between force generation and speed of contraction over the force range studied. This linear relationship between the speed and force of contraction, as the proportion of active motor units varied, implies that these two parameters are similarly related in motor units of various sizes.

In fast voluntary contractions, the rate of rise of tension at any given force level was higher than that observed in response to repetitive stimuli delivered to the motor nerve. Even at 200 and 500 impulses per second at maximal intensity, the speed of tension development was less than that found in fast voluntary contractions. The importance of asynchronous impulses and optimum discharge sequencing in generating a faster speed of tension development in fast voluntary contractions is underscored by these findings.

156.7 MODIFIABILITY OF HUMAN LONG LATENCY (90-100 ms) MUSCLE RESPONSES TO POSTURAL PERTURBATIONS BY EXPECTANCY. M. Woollacott, O. Marin and L. Nashner. Neurological Sci. Inst., Good Samaritan Hosp., Portland, OR 97209.

The EMG activity of four leg muscles [gastrocnemius (G), tibialis anterior (T), hamstrings (H) and quadriceps (Q)] was recorded while freely standing humans were subjected to anterior or posterior horizontal displacements of the platform on which they stood.

In order to determine the effects of expectancy on postural responses, latencies of rectified and integrated EMGs were compared for trials in which a) the perturbation was unexpected and unidentified, b) the perturbation was preceeded by a tone cue (500 ms in advance) but information on the direction of the perturbation was not given, and c) the perturbation was preceeded by a tone cue (500 msec in advance) which identified the perturbation direction by its pitch. EMG response latencies to unexpected anterior platform translations (causing posterior sway and stretch of T) were 88 ± 4 msec in the tibialis anterior. With a 500 msec advance warning cue, latencies were reduced to 74 ± 12 msec for anterior translations. With 500 msec advanced warning and identification of the direction of movement the latencies were reduced further to 61 ± 9 msec.

In order to determine the effects of repetition on postural responses, a series of 20 posterior platform displacements was given (intertrial intervals were 15 sec). In the subjects tested, the long latency EMG response in the stretched G muscle decreased in latency from 100 msec to 71 msec and decreased in amplitude to 50% of initial values. After 20 trials, an unexpected anterior translation was given. The subject was thrown off balance and required assistance for postural support.

These results indicate that long latency postural responses can be reduced in amplitude and latency by preparatory processes, and that these processes may reduce the effectiveness of antagonist postural pathways.

156.8 SYNCHRONIZATION OF HUMAN MOTOR UNITS DURING STATIC AND RAMP-FORCE ISOMETRIC CONTRACTIONS. Carl G. Kukulka* and Brenda Bigland-Ritchie*. (Spon: Charles F. Stevens). John B. Pierce Foundation, New Haven, CT. 06519 and Quinnipiac College, Hamden, CT. 06518.

It is generally accepted that motor neurons discharge asynchronously in voluntary contractions of unfatigued muscle. We have found, however, that at the time of first recruitment, pairs of units recorded from a single intramuscular electrode frequently demonstrate quite high degrees of synchrony even at low force levels.

Bipolar, fine wire recordings of single motor units were obtained during both static force and ramp isometric contractions of human biceps brachii and first dorsal interosseus muscles. Units were recruited between 1 and 30% maximum voluntary contraction (MVC), and each was identified by its characteristic recruitment force, spike shape and amplitude. All pairs of units which were clearly identifiable for sufficient time (5-60 sec.) were tested for cross-correlation of their firing rates. Of 10 pairs from biceps brachii, 3 showed prominent synchronization which could generally be detected visually in the raw data as well as by clearly defined peaks in their cross correlograms; 3 showed less well defined peaks; and 4 were clearly asynchronous. Of the 4 pairs analyzed from first dorsal interosseus, 2 displayed similar prominent peaks. As a measure of this synchronization the area of each peak was expressed as a percentage of the total area of the correlogram (ie. 100% = total synchrony). For well synchronized units, these varied from 20-60% (mean = 38%), with lower values for the rest. Firing frequencies varied from 8-12/sec. Some pairs of highly synchronized units had nearly identical frequencies, while others did not. For well synchronized pairs the difference between the recruitment forces for each unit was less than 5% MVC. This suggested that synchrony might result from a common excitatory input to neurons of similar size and excitability, yet asynchronous pairs with similarly close recruitment threshold were also observed. Several pairs of units, well synchronized when first recruited, remained so when responding to increasing excitatory drive during slow force ramps, but lost their synchrony during subsequent static contractions at constant force.

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156.9 VELOCITY DEPENDENT CENTRALLY PROGRAMMED HUMAN VOLUNTARY ACTIVITY. B. T. Shahani, R. R. Young, and J. L. Harrison*. Clinical Neurophysiology Laboratories, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

The central programmes subserving the execution of fast voluntary flexion and extension movements of human limbs during a visual matching task produce a triphasic EMG pattern (Ag1: the initial agonist burst, Ag2: the second agonist burst with a silent period Ag1-Ag2 interposed between them and An1: the antagonist burst) in a pair of antagonist muscles whereas slower "smooth" movements are produced by continuous EMG activity only in the agonist. The present study was undertaken to determine whether or not the transition from continuous EMG activity to a discrete "burst" pattern is velocity dependent. Gradual changes in linear velocity were studied using 5 healthy subjects, 22-34 years old. In different individuals, continuous agonist EMG activity seen with slower velocities (< 30 cm/sec.) was transformed into a two-burst pattern at a velocity of 35-50 cm/sec. An1 appeared only with even greater velocity (greater than 55 cm/sec.) Durations of Ag1 and Ag1-Ag2 interval both decreased with increasing velocity, reaching, at approximately 120 cm/sec., the normal range (Ag1 = 86 $\pm$ 19 msec., Ag1-Ag2 = 66 $\pm$ 29 msec., N = 32). The triphasic EMG pattern seen with human ballistic movements appears to be velocity dependent. Changes in these EMG parameters produced by increasing angular velocity at a joint are appropriate for bringing the distal limb to the target with speed and precision.

156.10 TWO TYPES OF POSTURAL ADJUSTMENT TO LIMB FLEXION IN THE CAT. J.M. Macpherson*, M. Dufossé* and J. Massion* (SPON: J.H.J. Allum) Dépt. Neurophysiol. Générale, C.N.R.S., 13274 Marseille, FRANCE.

Previous studies in the cat have shown that the postural adjustment to flexion of a limb can differ, depending on how the limb flexion is elicited.[1] By dropping the platform supporting one forelimb, or by perturbing that limb to cause loss of support, a diagonal pattern of support is elicited such that the pressure exerted by the contralateral forelimb and ipsilateral hindlimb is increased, while that exerted by the contralateral hindlimb is decreased. In contrast, the postural adjustment to a conditioned raising of the forelimb is non-diagonal, with increased pressure exerted by the contralateral forelimb but no reciprocal type of change for the hindlimbs.

This study was undertaken to elaborate the biomechanical and electromyographic (EMG) differences between these two patterns of postural adjustment. The cats were unrestrained, and stood with each limb on a small platform. The pressure exerted by each limb in a vertical direction was recorded on-line. To measure movements of the vertebral column, fine rods were implanted in the spines of vertebrae at three levels (approx. T1, T12 and L5). Movements of the cat as well as the video display of limb forces were recorded on film. In another series of experiments with the same cats, the EMGs of limb extensors were recorded on-line along with the forces.

When postural adjustment was diagonal, the vertebral column remained relatively rigid, i.e. no lateral displacement in space, and very little or no torsion or rotation. The triceps surae muscles of the hindlimbs responded in a reciprocal manner: the muscle of the hindlimb which increased its pressure was activated whereas the other was inhibited. Both muscles responded before the onset of force change in the corresponding limb.

During conditioned movement of a forelimb with a non-diagonal force pattern, there was a marked lateral flexion of the forequarters of the cat toward the side of the supporting forelimb. The triceps muscles of the hindlimbs showed no reciprocal response, corresponding to the lack of reciprocal change in pressure exerted by the hindlimbs.

The diagonal pattern is closer to that seen during locomotion (e.g. trotting), however the non-diagonal pattern appears to be the more stable one for single limb flexion. The elaboration of these different mechanisms of adjustment to limb flexion provides an important basis for studying the central control of postural change.

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156.11 AGONIST-ANTAGONIST COUPLING IN RAPID LIMB MOVEMENT. C. Ghez and J. Martin*. Div. of Neurobiol. & Behav., Depts. of Physiology & Neurology, Columbia P&S, New York, N.Y. 10032.

EMG activity associated with rapid limb movements exhibits a characteristic "three burst pattern". The first burst is in the agonist muscle (AG1) and accelerates the limb. Next, during the dynamic phase of movement, a burst of activity occurs in the antagonist (ANT) which may decelerate the limb. Finally, a burst of unknown significance is observed in the agonist (AG2). The present study was undertaken to determine if ANT and AG2 reflect the action of a central program or if they represent stretch reflexes evoked during the evolving movement. To answer these questions it is necessary to dissociate the intended force produced by the subjects from the ensuing limb displacement.

Cats were trained (using a compensatory display) to apply force isometrically or to position the lever of a torque motor controlled manipulandum to match a target level which was stepped at random times. Under isometric conditions, angular displacement of the limb was prevented by an electromechanical brake which could be released on random trials during the animal's response (when dF/dt exceeded a threshold corresponding with the end of the first agonist burst). The torque motor was used to generate elastic and viscous loads to control limb trajectory. Thus, we could dissociate centrally programmed muscular events from their intended mechanical consequences.

Whereas AG1 was present under both isometric and isotonic conditions, ANT and AG2 required limb displacement and were time locked to movement parameters. ANT occurred within 15 msec following the onset of acceleration; its magnitude varied linearly with this parameter and inversely with AG1. Thus, passive displacements stretching the antagonist elicited responses with similar latencies but of greatest magnitude. AG2 was only present in underdamped movements with terminal oscillations and typically occurred at the initial position overshoot as the velocity recrossed zero. Its magnitude was a function of both limb deceleration and of the intended force.

These data suggest that both ANT and AG2 represent stretch reflexes whose amplitudes are modulated by descending commands. Reciprocal mechanisms operating at a spinal level could account for the transient reduction of the antagonist stretch reflex as a function of the intended force. The increased sensitivity of the agonist stretch reflex, compatible with alpha gamma co-activation, suggests that acceleration sensitivity of muscle spindles can provide a predictive signal to dampen mechanical oscillations which ensue from rapid movement of a mass against elastic forces.

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156.12 LUMBAR BACK MUSCLE ACTIVITY DURING WALKING AND GALLOPING IN DECEREBRATE CATS. Zomlefer, M. and Rossignol, S. Centre de recherche en sciences neurologiques, département de physiologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

Electromyograms (EMG's) were recorded from trunk muscles in pre-collicularly decerebrated cats moving on a treadmill during stimulation of the mesencephalic locomotor region. Bipolar intramuscular electrodes were introduced bilaterally into the Multifidi (Mf) and Longissimus (Lo) muscles along the lumbar spine, as well as into the hindlimb extensors vasti lateralii (VL). During slow and fast walking, at speeds ranging from 0.55 to 1.15 m/s, all the lumbar back muscles exhibited two separate bursts in each step cycle. These bursts occurred within periods of overlapping activity in the two VL's, with burst duration decreasing and mean amplitude increasing as the cat walked faster. In general, the larger of the two bursts in the back muscle on one side was linked to the onset of the VL on the same side. These EMG patterns from decerebrate walking cats are consistent with those reported earlier (Carlson et al., Acta physiol. scand., 165: 251-253, 1979) from chronic cats during treadmill and overground walking. In several animals, it was also possible to elicit good sequences of galloping at belt speeds from about 2.0 m/s to 2.9 m/s. Angular movements of the spine are clearly much more pronounced in galloping than in walking (Goslow et al., J. Morphol., 141: 1-42, 1973). During galloping, the lumbar back musculature underwent a dramatic shift in its activation pattern, to yield only a single large burst in each step cycle. All back EMG's were synchronously initiated about 50 ms before the co-contraction of the VL's, with burst durations of the order of 150-225 ms. Hence, the Mf and Lo become active prior to the hindlimb stance phase and remain active for about the first third of this phase. Whereas in the above cited studies it was apparent that the back muscles mainly serve to control trunk stiffness during slow and fast walking, the present work indicates that during gallop the back muscles may also be used to extend the spine prior to and during the initial part of the stance phase.

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156.13 EFFECTS OF THE GLOBUS PALLIDUS ON RAPID ARM MOVEMENT IN MONKEYS
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The role of basal ganglia output via the globus pallidus was examined in monkeys trained to elevate the arm in a forced reaction time task. Normal pallidal output was disrupted using either microstimulation or a kainic acid-produced lesion.

Microstimulation in the globus pallidus contralateral to arm movement with currents as low as 25 μA during random trials resulted in an increase in the movement duration. As stimulus intensity was increased up to 100 μA, there was a graded prolongation of movement duration, which finally exceeded control values by up to 100 msec. The reaction time following the target light onset, however, was minimally affected. Stimulus trains as short as 25 msec initiated at varying times during the task were most effective if applied 50-150 msec prior to movement onset. Stimulation early during the reaction time or late during movement execution did not lengthen movement times. Stimulation was effective only when applied in the area where typical high frequency pallidal unit activity was recorded, and the firing of many pallidal units in these same tracks covaried with task execution. Prolongation of movement was not caused by stimulation in areas surrounding the globus pallidus.

EMG activity recorded in six arm and paraspinal muscles was depressed and prolonged by the stimulation. Details of somatotopic and temporal effects of stimulation on EMG patterns are being investigated.

A lesion of the globus pallidus produced by injecting kainic acid during contralateral task execution also resulted in profound slowing of the arm movement with little effect on the reaction time. This effect persisted during the ten day survival period following the lesion. The changes in EMG activity underlying the prolonged movements, however, appeared to be different when the slowing was caused by a lesion, instead of by microstimulation.

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156.14 HOW IS THE DIRECTION OF A BALLISTIC WRIST MOVEMENT PROGRAMMED?
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The wrist joint is capable of movements along two axes: flexion-extension and radial-ulnar deviation. How does a single muscle which acts across this complex joint participate in generating ballistic movements in different directions? In order to answer this question, we monitored the activity of extensor carpi radialis (ECR) with intramuscular electrodes while human subjects performed 20 degree ballistic wrist movements in 8-12 different directions. When subjects performed a ballistic movement which required equal combinations of radial deviation and extension, there was a single, >60 msec. long, burst of ECR activity beginning prior to movement onset (=agonist burst). Later phases of ECR activity were observed, but will not be described here. When subjects performed a movement in the opposite direction (equal combinations of ulnar deviation and flexion), there was a single, >50 msec. long, burst of ECR activity at or just after movement onset (=antagonist burst). The antagonist burst was present only when subjects were required to terminate their movements accurately.

ECR displayed two initial phases of activity when subjects performed movements orthogonal to those described above (ulnar deviation + extension or radial deviation + flexion). One burst of activity occurred prior to movement onset and the second occurred at or just after movement onset. These two bursts occurred at time intervals similar to the agonist and antagonist bursts described above. In addition, the second burst, like the antagonist burst, was present only when accurate termination of movement was required. Furthermore, the amplitude of the two bursts in ECR varied with the direction of movement. The first burst increased and the second burst decreased in amplitude as the subject moved in directions approaching radial deviation-extension (agonist burst direction). Opposite changes in burst amplitude were observed as the subject moved in directions approaching ulnar deviation-flexion (antagonist burst direction). Therefore, we believe that these two bursts are equivalent to the agonist and antagonist bursts described above. We conclude that the trajectory of an accurate ballistic movement of the wrist is determined by modulating the amplitude of agonist and antagonist bursts in single muscles.

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156.15 COMPUTER SIMULATION OF THE PERFORMANCE OF A WEIGHT-LIFTING TASK BY NORMAL AND DEAFFERENTED MONKEYS.
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Performance on a weight-lifting task of deafferented monkeys approaches that of normal monkeys (Wylie and Tyner, Soc.Neurosci. Abstr. 4:308, 1978). Among the possible interpretations of these results is the possibility that the mechanical properties of the muscle-joint system are such that to perform the task at the observed levels, the animal need not modulate the neural signals driving the muscles in response to the external load. To assess this possibility, I have developed a mathematical model embodying the mechanics of the physical apparatus and the muscle-limb system. The model is based on a model of horizontal rotation of the head (Bizzi, Dev, Morasso and Polit, J. Neurophysiol. 41:542-556, 1978) with terms added to reflect the action of gravity on the system.

In the experimental paradigm, flexion of the forearm in the vertical plane rotated an arm restraint and lifted different test weights vertically. The model is the equation of motion of the system in which an applied torque is equated to the sum of a series of terms representing each of the resistances to movement along any specified trajectory. The resistances incorporated in the model are the rotational inertia of the monkey's arm and of the arm restraint, the translational inertia of the test mass, the gravitational forces acting on the test mass and the monkey's arm, and the internal viscous and elastic resistances of the muscles acting across the elbow. The model is a lumped parameter model in which the resistance of each component is idealized as a constant multiplied by the appropriate variable of motion.

To explore the potential role of the non-linear properties of muscle, in particular the viscous-like dependence of force on the velocity of shortening and the spring-like dependence of force on the length of the muscle, the parameters reflecting these two properties were independently varied to study their effects on the behavior of the system. This is a heuristic exercise in that both the viscosity and elasticity of muscle are dependent upon the level of activation of the muscle which is a time dependent function. Therefore, viscosity and elasticity are neither independent of each other nor constant. Nevertheless, results from the model focus attention on the importance of these properties in movement and the dangers of drawing inferences about compensatory responses without taking these properties into account.

Results from computer simulations indicate that the entire range of observed performances by monkeys can be simulated using a command signal which does not change with the imposed load. EMG recordings from both groups indicate active compensation contributes to the performance of monkeys.

156.16 THE PAW SHAKE REFLEX IN CHRONIC CAT.
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Chronic spinal cats transected at T_{12}, at a young age, exhibit several normal reflexes (Forssberg, Acta Physiol. Scand. Suppl. 474, 1979). Using cats which were spinalized at 2 or 12 wks, and allowed to recover for 6 mth, we studied hindlimb shakes elicited by sticking tape to the plantar pads. This cutaneous reflex is abolished when the pads are made insentient with xylocaine (0.2%) injections. EMG from soleus (SOL), lateral gastrocnemius (LG) and tibialis anterior (TA) was coordinated with 16 mm high speed film (100 fr/s), and 63 records from 6 spinal cats were analyzed.

A shake record consisted of 12 cycles, on average, with alternating ankle flexion-extension (F-E) occuring at 11 c/s, or with a paw shake interval of 88 ms. The average displacement at the ankle was 42 deg for both F and E, while peak velocities of 25 rad/s were common for both movements.

LG-EMG bursts,initiated at least 35 ms prior to peak F, had burst duration times (BDT) of 22 ms and interburst intervals of 66 ms. Neither the cycles times, ankle kinematics, nor LG-EMG differed significantly from normal cats (Smith, et al, J. Neurophysiol. 43: 612, 1980). The EMG of the SOL and LG coincided when both were active; however, the BDT of the SOL was slightly longer (31 ms). In normal cats the SOL is not recruited during paw shaking; however, in those spinal cats with a fast-contracting SOL (CT: <50 ms), the SOL was active (see Smith, et al, Neuroscience Abst. #1304, 1979).

The TA exhibited the longest BDT (38 ms), being initiated prior to peak extension and terminating 20 ms after. When the LG acted to decelerate ankle flexion, its EMG activity appeared, on average, 10 ms before the EMG activity of the TA ceased. When the TA acted to decelerate extension, no such coactivation was observed. Others have reported coactivation of antagonistic muscles during rapid movements (Lestienne, Exp. Brain Res. 35: 407, 1979), as well as asymmetrical co-contraction during reciprocal E-F movements (Norman & Komi, Acta Physiol. Scand. 106: 241, 1979).

Although the ankle kinematics and EMG during paw shaking are relatively normal, differences do exist. For instance, the reflex cannot always be elicited in chronic spinal cats, and the failure rate is about 20%. In addition, rather than abduction preceding the movement, the hip is fully extended at the beginning and then fully flexed at the termination of the reflex. With the hip in flexion, the knee and ankle are held rigidly in extension with the digits abducted. Also the reflex can be obtained with spinal cat held suspended; this position is not conducive to eliciting the reflex in intact cats. NIH Grant 10423.

156.17 "FATIGUE PROPERTIES" OF HINDLIMB MOTONEURONS OF THE CAT. D. Kernell* and A.W. Monster☒. Dept. of Neurophysiology, Jan Swammerdam Institute, University of Amsterdam, The Netherlands.

It is well known that the motor units of a mixed hindlimb muscle may differ markedly from each other with respect to their resistance to contractile fatigue. We have investigated whether the motoneurons innervating muscle fibers with different fatigue resistance would show any consistent differences with respect to their ability to produce a steady discharge in response to constant stimulation. The experiments were performed on cats anesthetized with pentobarbitone. M.gastrocnemius medialis (GM) was attached to an isometric force transducer. GM motoneurons were penetrated with single-barrelled micro-electrodes filled with potassium citrate. For measurements of the twitch and the fatigue resistance (ref.1) of a muscle unit, its motoneuron was directly activated by 1 ms pulses of injected current. The "late adaptation" (ref.2) of the motoneuron was determined by measuring the decline of firing rate during direct stimulation with a step of steady injected current which lasted 240 sec. Earlier studies have indicated that damage inflicted by the microelectrode may make motoneurons unable to produce a maintained discharge in response to steady stimulation (ref.2). For the present investigation, we only used neurons that possessed healthy-looking spikes of > 55 mV, and which were capable of tonic firing during at least a few seconds of constant stimulation. Thus far, we have measured the "motoneuronal fatigue"(late adaptation) in response to various stimulus intensities in a total of 24 GM motoneurons. In 18 of these cells we applied a test current of 5 nA above the threshold for rhythmic firing. The resulting discharge lasted for 124±95 sec ($\bar{x}$±SD; range 10 - 240 sec); its duration showed no significant correlation to the "fatigue index" (ref.1) of the muscle unit (r=0.14, n=16). Among cells capable of firing during > 2 min of constant stimulation, adaptation was faster during the first minute of maintained firing than later on. During an initial period of the discharge (typically some tens of seconds) the decline of discharge rate could usually be approximated by an exponential function. Preliminary results suggest that the time constant of this initial neuronal rate decrease tends to be longer for units with a slow twitch and a high fatigue resistance than for faster and more fatiguable ones.

References: 1/ Burke, R.E., D.N. Levine, P.Tsairis and F.E.Zajac, J.Physiol.(Lond.) 234, 723, 1973.
2/ Kernell, D., Acta physiol.scand. 65, 65, 1965.

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156.18 THE EFFECT OF UNILATERAL FASTIGIAL LESIONS ON STATIC AND DYNAMIC RESPONSES OF MUSCLE SPINDLE PRIMARY AFFERENTS IN THE CAT. D. Kornhauser*, M.B. Bromberg and S. Gilman. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48109 and Neurosurgery Section, V.A. Medical Center, East Orange, NJ 07019.

The responses of single gastrocneimus muscle spindle primary afferents to static and dynamic extension of the gastrocneimus muscle were recorded before and after lesions of the fastigial nucleus. Sixty-five units were recorded in 15 cats. In all animals, the lesion involved the rostral pole of the fastigial nucleus on the side of the recording. The fastigial lesions depressed significantly the responses to static extension of the spindle primaries and the extent of the lesion was a significant factor in this effect. Lesions involving 15 to 50% of the nucleus had no significant effect but when the lesion involved 51% or more of the fastigial nucleus, a significant decrease of response occurred. The lesion interrupted projections from both the ipsilateral and contralateral fastigial nucleus since efferent fibers from the caudal pole of the contralateral fastigial nucleus pass through the rostral pole of the ipsilateral fastigial nucleus en route to the brainstem.

The dynamic sensitivity of the muscle spindle primaries was assessed by measuring the afferent response at the immediate termination of a ramp stretch performed at 5, 30 and 100 mm per second. Even lesions damaging 76% or more of the fastigial nucleus resulted only in a slight and non-significant decrease in the peak frequency of the response at all three velocities of stretch in comparison to control levels. De-efferentation by ventral root section had a pronounced effect on the peak frequency response to stretching at 5 and 30 mm per second and no effect on the response to stretching at 100 mm per second.

Thus, lesions involving at least 51% of the fastigial nucleus, including its rostral pole, decrease the responses to static extension but not to dynamic extension of muscle spindle afferents in an extensor muscle on the ipsilateral side of the body. The lesion includes projections from the caudal pole of the contralateral fastigial nucleus. We conclude that the fastigial nuclei maintain a tonic facilitatory influence upon muscle spindle primary afferents for their responses to static extension. This influence is probably mediated to the spinal cord via the spinal projections of the vestibular and reticular nuclei. The vestibulo spinal projections make contact primarily with static fusimotor neurons which are responsible for the spindle afferent responses. Supported in part by USPHS Grant NS 10612.

156.19 REGIONAL GLUCOSE METABOLISM IN THE DECEREBRATE CAT. R. Watts*, E. B. Montgomery, Jr.*, W. Landau and R. C. Collins. Dept. of Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

[^{14}C]-2-deoxyglucose (2-DG) autoradiography was used to study changes in functional metabolism in the brainstem and cerebellum of cats in response to decerebration. Two cats were decerebrated by blunt intercollicular transection and 2 by the anemic method (ligation of both carotid arteries and the basilar artery at mid-pontine level). The decerebrate cats were maintained in a resting prone position with the extremities and head held extended during the injection of 2-DG two hours after recovery from ether anesthesia. Two control cats were maintained in a resting prone position with their extremities casted in extension, head freely movable and were injected with 2-DG two hours after recovery from ether anesthesia. Arterial blood gases and blood pressure were maintained within physiological ranges.

Cats anemically decerebrated (AD) differed behaviorally from cats decerebrated by transection (TD). The AD cats displayed lability of blood pressure and respirations, more "running" and other atypical behavior, and more vigorous extensor hypertonus. The brainstem rostral to the basilar artery ligation in the anemic preparations showed marked variability in the pattern of 2-DG uptake. However, where there was uptake it was mottled and not confined to specific structures as compared to the more caudal structures or to control cats. This suggests that this pattern of uptake in the rostral brainstem was due to ischemia whereas the specific patterns seen in structures caudal to the rostral pons were not.

Hyperglycemia following decerebration compromised quantitative analysis. Changes in functional metabolism were compared as a ratio of tissue 2-DG concentration of various areas to the tissue 2-DG concentration of the central gray area of the spinal cord in each cat. Preliminary results of changes in functional metabolism as compared to control cats showed: 1) increase in the medial and inferior vestibular and lateral reticular nuclei in both AD and TD cats; 2) increase in dentate, interpositus and fastigial nuclei in AD cats; and 3) decrease in principle inferior olivary nuclei (IOP), whereas there was no change in medial (IOM) and dorsal (IOD) accessory olivary nuclei in both AD and TD cats. The increase in functional metabolism of the deep cerebellar nuclei in AD cats is consistent with the loss of inhibitory input from ischemic cerebellar cortex. The decrease in functional metabolism in IOP without change in IOM or IOD is consistent with the dependence of IOP on descending inputs from rostral structures whereas IOM and IOD are more dependent on ascending spinal inputs.

156.20 QUANTITATIVE ANALYSIS of NORMAL and REGENERATING BEHAVIORS in the SEA LAMPREY. Joseph Ayers, Gail A. Carpenter, Scott Currie and James C. Kinch. Marine Science Institute and Dept. of Mathematics, Northeastern University, Boston, MA 02115

Lamprey behaviors have been analyzed by stop-frame analysis of motion pictures. These films provide numerical data for physiological models of motor pattern generation and spinal cord regeneration. The experiments have been performed on normal ammocoete larvae, recently transformed adults, and ammocoetes recovering from complete transection of the spinal cord. The images from each frame are digitized on a magnetic tablet and analyzed by computer algorithms which determine, for example, curvature as a function of distance along the body. Further analysis determines the repetition frequency, amplitude, direction, and rate of propagation of flexion waves; the phase lag between flexions on opposite sides; and the speed and direction of motion. With this method it is possible to specify behaviors by a small number of parameters, computed automatically from the digitized data, and to compare the behaviors with cellular correlates in reduced preparations. Several behaviors have been studied, including: swimming, turning, burrowing, aversive withdrawal, forward escape, and forward and backward crawling.

A similar analysis has been performed on ammocoetes regenerating from spinal cord transection and the chronology of recovery has been traced. Spinalized animals eventually display most of the above behaviors, with some deficits. Abnormal behaviors such as coiling and shaking occur during recovery. The contribution of passive mechanical propagation is computed by analysis of body movements produced by mechanical head oscillations of dead and acutely transected ammocoetes.

The lamprey has recently become an important model system (Rovainen, C. M., Physiol. Rev. 59:1007, 1979) and the present research provides standardized criteria for systematic studies of behavior patterns and their underlying cellular mechanisms.

157.1 ABNORMALITIES IN THE EMG PATTERN OF ELBOW FLEXIONS DURING CEREBELLAR DYSMETRIA. J. Hore and T. Vilis. Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

To identify the basic features of cerebellar dysmetria we compared EMG patterns of the same monkeys during normal elbow flexion movements and during dysmetric movements. Reversible cerebellar lesions were produced by cooling through sheaths placed lateral to dentate and through interpositus. This technique allowed us to compare normal and abnormal EMG activity of biceps and triceps recorded through the same intramuscular electrodes. Cebus monkeys were trained to make prompt and accurate movements between visual targets. Movements of 40 degrees of arc were made in about 300 msec. A small load was placed on the handle to accentuate antagonist triceps activity.

Under normal conditions well-trained monkeys initiated movements by a decrease in triceps and a phasic burst in biceps. This phasic burst had two components: the first was of constant duration (30-40 msec) and, on average, was of constant amplitude for different sized movements. The amplitude and duration of the second component was related to the size of the movement. During these control movements the antagonist triceps commenced discharging tonically at a level appropriate for the new flexed position. The time of onset of triceps was fixed to the start of the movement, but variable in relation to the end of the movement. Thus the antagonist EMG activity did not play a major role in stopping the movement. These monkey movements would appear to be equivalent to the slow category of human arm movements (Lestienne, 1979) in that braking forces were predominantly generated by visco-elastic muscle properties.

During cerebellar cooling monkeys displayed both hypometria and hypermetria in consecutive movements followed in both cases by a tremor at 3-4 Hz. In these dysmetric movements there was a delay in onset of the phasic biceps EMG by about 50 msec and there was no clear cut first component. A major change was observed in antagonist activity during dysmetria. In normal movements onset of the tonic triceps activity occurred at a variable time to the end of the movement. During dysmetria there was a phasic burst in triceps which was fixed in relation to the end of the movement. When this burst occurred early in the movement it caused hypometria, while when it occurred late in the movement it allowed hypermetria. This burst in triceps initiated the terminal tremor.

Thus one major difference between these normal well-trained arm movements and dysmetric movements is that in the former case they appear to be terminated primarily by visco-elastic forces, while in the latter case they are terminated by a burst of activity in the antagonist muscle.

Lestienne, F. (1979). Exp. Brain Res. 35, 407-418.

157.2 PURKINJE CELL INHIBITION DURING THE CO-ACTIVATION OF ANTAGONIST MUSCLES IN ISOMETRIC PREHENSION. Allan M. Smith and Daniel Bourbonnais. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada.

Recordings of cerebellar Purkinje cell discharge in the awake animal has been shown to be related to various parameters of agonist muscle activity such as the duration, the direction and the velocity of limb displacement. Nevertheless, it is still unexplained how the excitation of these inhibitory neurons is related to motor performance. One possibility is that the Purkinje cells are related to the inhibition of antagonist muscles. It was decided to test this hypothesis in a situation requiring the co-contraction of mechanically opposing muscles. Monkeys were trained to exert and maintain a precisely controlled force between the thumb and forefinger for a fruit juice reward. Extensive recordings from the 24 muscles of the wrist and fingers active during the maintained precision grip demonstrated that all muscles show a similar pattern of co-activation. Purkinje cells were identified by the presence of climbing fiber discharge in extracellular recordings. All other units were considered unidentified neurons. To date, about 85% of the unidentified neurons increased discharge frequency during grasping. In contrast, 71% of the Purkinje cells were inhibited during the application of prehensile force. Purkinje cell discharge frequency for 3 neurons was inversely related to the rate at which finger pressure increased. One of these neurons also had a significant negative correlation between discharge and prehensile force. The inhibition of Purkinje cell discharge appeared to coincide with the co-contraction in the flexor and extensor muscles of the wrist and fingers. In one Purkinje cell a clear excitation was observed when extensor digitorum contracted to produce isotonic extension of the fingers, but when this same muscle contracted simultaneously with its antagonist, the Purkinje cell discharge was inhibited. These results are interpreted as suggesting that Purkinje cells are excited by stretch of antagonist muscles or the central command to stretch these muscles. The inhibition generated in the deep nuclear cells is presumed to further relax the antagonists. However, in movements requiring the co-contraction of antagonists, the Purkinje cells themselves are inhibited, probably as a result of convergent excitation from both agonists and antagonists onto basket or stellate cells.

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157.3 REDUCTION OF POSTSYNAPTIC ACTIVITY ENHANCES PARALLEL FIBER FREQUENCY FOLLOWING. R. C. Malenka, J. D. Kocsis* and S. G. Waxman*. Dept. of Neurology, Stanford Sch. of Med. and Veterans Administration Med. Ctr., Palo Alto, CA 94304

It has been reported that axon excitability can be affected by (1) previous impulse conduction along the same axon (2) ephaptic interactions between axons or (3) electrical or extracellular ionic changes associated with postsynaptic activity. In this study we examine the frequency following capabilities of the parallel fibers (Pfs) in the presence and absence of postsynaptic activity.

Rat cerebella were continuously superfused with normal Ringer solution (NS). Local microstimulation of the Pfs was employed and the on-beam Pf field potential was recorded within 50 µm of the cerebellar surface. In addition the extracellular DC slow potential (SP) was recorded. The latency and amplitude changes of consecutive Pf responses to stimulus trains (10-80 Hz) were analyzed. Postsynaptic activity was manipulated either by superfusion with Mn^{2+}, Mg^{2+}, or 4-aminopyridine (4-AP); or by giving a conditioning stimulus train.

In NS consecutive Pf responses to stimulus trains revealed an initial supernormal period followed by a gradual transition into subnormality as determined by latency and amplitude variation. A pronounced negative SP was present during these trains. Both the Pf amplitude reduction and the SP amplitude were frequency dependent. In the absence of postsynaptic activity, the Pfs showed enhanced frequency following as evidenced by a much reduced amplitude cut-off during stimulus trains. In the presence of 4-AP the postsynaptic potential was increased as was the SP amplitude and conduction block occurred after the first or second volley of the stimulus train. Addition of Mg^{2+} to the 4-AP superfusate abolished the postsynaptic potential, reduced the SP amplitude and restored the frequency following properties of the Pfs.

These results suggest that changes associated with postsynaptic activity in the cerebellar cortex may dampen the frequency following capabilities of the afferent Pfs. Other investigators (Nicholson et al, J. Neurophysiol. 41, 1978) have shown significant changes in extracellular ionic concentrations due to postsynaptic activity during tetanic stimulation. Our results suggest that these changes may influence Pf excitability. A morphological channel may be available for ionic movement through the extracellular space since the Pfs are extremely fine, non-myelinated axons with few intervening glial elements. (Supported in part by the NIH (RR-5353 and NS-15320), the Veterans Administration, and the National Multiple Sclerosis Society (RG-1231).)

157.4 IMPULSE ENTRAINMENT: A TIMING MECHANISM FOR PARALLEL-FIBER ACTIVATION OF PURKINJE CELLS? K.L. Cummins*and J.D. Kocsis*. (SPON: L. Masukawa). Dept. of Neurology, Stanford Univ. Med. Ctr., and V.A. Med. Ctr., Palo Alto, CA. 94305.

The fine-caliber parallel fibers (Pfs) of the cerebellar cortex propagate action potentials over a wide range of conduction velocities (CVs), depending on the time since the previous impulse. This dependence of CV on spike interval may have physiologic significance by controlling the precise spacing of these neural signals. In the simple case of two propagating action potentials, the recovery of CV of the second impulse in the pair is characterized by three distinct periods; CV is increased during the supernormal period (SNP) and decreased during the relative refractory (RRP) and subnormal periods. A result of this recovery process is "entrainment" whereby impulses initiated over a wide range of initial intervals converge to the same interspike interval after propagation (Kocsis et al, Exper. Neurol., 65:230, 1979).

We have studied the phenomenon of entrainment using both computer simulation and experimental methods. The simulations used a simple parametric model to generate the spatial and temporal properties of impulse propagation from the relationship between instantaneous CV of the second impulse and the interval between the impulses. Using this model the three phases of recovery were studied and impulses were shown to converge to one of three intervals at infinite propagation distance. For finite propagation the simulations demonstrated that manifestation of entrainment was dependent upon the particular recovery curve, conduction distance, and the initial interval between impulses.

When recovery data from rat Pfs were used, the model accurately predicted the spatial and temporal properties of Pf interspike interval during conduction, and demonstrated that convergence toward entrainment occurred at physiological distances for propagation during the RRP and early SNP. This result is discussed in terms of the timing of Pf activation of Purkinje cells.

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157.5 LIDOCAINE DIFFERENTIALLY BLOCKS FAST AND SLOWLY INACTIVATING SODIUM CONDUCTANCE IN PURKINJE CELLS: AN IN VITRO STUDY IN GUINEA PIG CEREBELLUM USING IONTOPHORETIC GLUTAMIC ACID. M. Sugimori* and R. Llinás (SPON: C. Nicholson). Dept. Physiology & Biophys., New York Univ. Med. Ctr., 500 First Ave., New York 10016.

Cerebellar slices obtained from guinea pig cerebellum were utilized following the experimental techniques of Llinas and Sugimori (J. Physiol. 305: 1980 in press). Intrasomatic and intradendritic recordings were obtained while a second electrode filled with glutamic acid was kept in the vicinity of the impaled neuron. Extracellular iontophoretic injection of glutamic acid produced a fast depolarization capable of activating both sodium and calcium-dependent action potentials. This depolarization could be reversed by direct modification of the membrane potential level to 0 mV, suggesting that in these cells glutamic acid produces simultaneous conductance to both sodium and potassium. Following application of cadmium, cobalt, manganese or D-600, voltage-dependent calcium spikes could be blocked and the non-inactivating sodium action potential (Sugimori and Llinas, Soc. Neurosci. Abst. 5: 107, 1979) was evoked by either direct or glutamic acid depolarization. This voltage-dependent plateau conductance generated trains of action potentials which often reached inactivation level. Further studies have indicated that lidocaine at low concentrations (10^{-5} to 10^{-4} M) can produce a blockage of the fast sodium action potentials but does not block the slowly rising sodium-dependent plateau depolarizations. Addition of tetrodotoxin to the bath, however, blocked both the fast and the non-inactivating sodium conductances. The differential effect of lidocaine on the inactivation and non-inactivation of sodium conductance is in agreement with recent experiments, suggesting that local anesthetics block sodium conductances in their inactivated states by increasing drug binding when the h gate is closed (Cahalan, Biophys. J. 23: 285, 1978). While the present study does not address this question directly, it is at least consistent with the fact that sodium channels which do not show inactivation are less susceptible to certain local anesthetics. (Supported by USPHS grant NS-13742 from NINCDS)

157.6 DISCHARGE PATTERNS OF DENTATE NEURONS DURING BALLISTIC ARM MOVEMENTS IN THE MONKEY. G. Spidalieri* and Y. Lamarre. Centre de recherche en sciences neurologiques, Fac. de méd., Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.

The cerebellum is presumably involved in the initiation of some fast ballistic movements. Previous observations showed that dentate lesion can delay the initiation of arm movements that are triggered by light and sound but not of those triggered by somesthetic stimuli. This led us to examine the activity of dentate neurons in monkeys trained to execute the same arm movements (flexion or extension) in response to randomly presented light signals, pure tones, or small elbow displacements. The majority of neurons recorded in the dentate increased or decreased their rate of firing at least 100 msec before the onset of elbow displacement (mean 121 ms) while in the interpose nucleus, change of neuronal activity occurred later (mean 75 ms). Clear reciprocal pattern of activity with flexion and extension was seen only in the interpose nucleus. The pattern of discharge of many neurons varied according to the modality of the triggering stimulus. This was seen mainly in the posterior half of the dentate (40% of the neurons) where several units changed their activity only when the movement was triggered by light and/or sound. These data provide further evidence in favor of the hypothesis that the lateral cerebellum is particularly involved in the initiation of arm movements triggered by teleceptive input signals. (Supported by the MRC of Canada)

157.7 CHANGES IN SACCADE DURATION DURING CEREBELLAR DYSMETRIA. T. Vilis and J. Hore. Departments of Ophthalmology and Physiology, University of Western Ontario, London, Canada N6A 5C1.

The accuracy of saccadic eye movements made by four trained Cebus monkeys was studied during reversible lesions produced by cryoprobes implanted between the fastigial and interpositus nuclei (medial probe) or lateral to the dentate nucleus (lateral probe). Cooling through the lateral probe did not impair the accuracy of vertical or horizontal saccades. However cooling through the medial probe produced a dysmetria whose magnitude was dependent on the position of the eye and on the direction of the saccade (c.f. Ritchie, 1976). While hypermetric saccades primarily occurred for centripetal saccades and hypometric saccades predominated in the centrifugal direction, examples of both hypometric centripetal and hypermetric centrifugal saccades were noted. The duration of dysmetric saccades was related to their amplitude; hypermetric saccades had longer durations while hypometric saccades had shorter durations compared to controls between the same target displacements.

The dysmetria seen for a horizontal or a vertical target displacement corresponded to the dysmetria observed in the equivalent horizontal or vertical components of saccades resulting from an oblique target displacement. For example if a purely horizontal saccade was hypometric, the equivalent horizontal component of an oblique saccade directed to the same final position was also hypometric. Moreover the trajectories of these oblique saccades remained essentially straight during medial probe cooling even when the dysmetria in the two components was unequal. Thus, for example, when the magnitude of one component became hypermetric while the other remained normal during cooling, the duration of both components increased. This implies that the mechanism which produces dysmetria in one component must interact with the gaze center that determines the duration of the other component.

Cooling the cerebellar nuclei through either the lateral or medial probes did not alter the time of onset (reaction time) of saccades to a randomly timed step change in target position. This result differs from that found for limb movements where cerebellar dysmetria was associated with increased reaction times (Holmes, 1939).

These results provide evidence that the cerebellum through the medial nuclei normally plays a role in terminating but not in initiating saccades.

(Supported by MRC of Canada - MA-5978)

Ritchie, L. J. Neurophysiol. 39: 1246-1256 (1976)
Holmes, G. Brain 62: 1-30 (1939)

157.8 TONIC RESPONSES TO GALVANIC VESTIBULAR STIMULI ARE FOUND IN 'NON-VESTIBULAR' PARTS OF THE CEREBELLUM. David W. Jensen. Dept. of Otorhinolaryngology and Program in Neuroscience, Baylor College of Medicine, Houston, Texas 77030.

Experiments have begun with the goal of measuring the changes induced in the spontaneous neural activity in response to direct currents applied to the labyrinth. Guinea pigs were semichronically prepared, including implantation of an Ag-AgCl ball electrode on one round window membrane for d.c. stimulation. The next day the animals were checked for the presence of spontaneous nystagmus and/or body postural asymmetries. No asymmetries were found in 4/4 animals, and the thresholds for producing body postural asymmetry and eye nystagmus were from 0.1 to 0.2 mA. Anodal and cathodal polarizations of one ear induced postural reactions similar to those that would occur if instead the ipsi- or contralateral ears respectively were ablated.

Three of the implanted animals were prepared for single unit recording from the posterior vermal cerebellar cortex during light ketamine anesthesia (30 mg/Kg/hr) and local anesthesia of would edges with 1% Lidocaine with 1:100,000 epinephrine. The ongoing activity of Purkinje cell-type unitary potentials was measured during unilateral anodal and cathodal d.c. stimulations of 0.3 mA or less in amplitude and 5-10 seconds in duration. The stimulating reference electrode was in the ipsilateral pinna, in the proximal portion and removed from the facial nerve. Preliminary recordings have thus far revealed several new observations regarding vestibular input to the cerebellum: 1) Tonic vestibular influences are demonstrable in "non-vestibular" cerebellar cortex, and 2) For most cells, unilateral anodal and cathodal vestibular polarizations of equal absolute magnitude produced responses that are: a) not opposite in the direction or time course of ongoing modulations, and b) not equal in the absolute values of modulations.

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157.9 COORDINATE AXES OF THE VISUAL CLIMBING FIBER INPUT TO THE FLOCCULUS. C. Leonard*, W. Graf* & J.I. Simpson, Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016

Cells of the dorsal cap (DC) of the inferior olive project as climbing fibers to the contralateral flocculus and transmit direction and speed information about movement of large portions of the visual world. The spatial organization of the receptive fields (RFs) of DC cells was investigated in anesthetized, paralyzed rabbits by extracellularly recording unit responses to large-field, rotational visual stimuli. For all cells the majority of the RF is above the horizontal plane. Some cells are driven exclusively from one eye; others are driven from both eyes, but more effectively from one eye than the other. The RF organization for the dominant or exclusive eye can be used to assign cells to one of three categories according to the orientation in space of the axis about which rotation of the visual world produces best modulation. The categories are termed Vertical Axis, Anterior (45°) Axis and Posterior (135°) Axis. The cells of each category occupy distinct zones within the DC. Vertical Axis cells are driven only from the contralateral eye (re: recording site) and are best excited by horizontal movement in the temporo-nasal direction. The RF extends along the horizon from the nose (0°) up to 160° posteriorly. Anterior (45°) Axis cells are divided into two sub-classes: one driven only from the ipsilateral eye and the other driven from both eyes, but with ipsilateral dominance. The ipsilateral RF has two parts, each with a different preferred direction. In the anterior part (from 0° to approx. 45° along the horizon) the preferred direction is up and somewhat backward; in the posterior part (from approx. 45° to at least 160°) the preferred direction is down and somewhat backward. Thus, the optimal stimulus is rotation of the visual world about an axis at approx. 45° to the midline. For binocular Anterior (45°) Axis cells, the RFs are axially symmetric about the midline. The Posterior (135°) Axis cells are driven from both eyes, but with contralateral dominance. The contralateral RF extends from 0° to approx. 135° posteriorly; the preferred direction is up and somewhat backward. The ipsilateral RF is centrally symmetric to the contralateral RF with respect to the intersection of the RFs with the horizontal plane. The ipsilateral preferred direction is down and somewhat backward and is thus in concert with the contralateral preferred direction for rotation about the common principal axis running from 45° ipsilateral to 135° contralateral These findings indicate that the principal axes of the floccular visual climbing fiber inputs are oriented similarly to the semicircular canal principal axes. Supported by USPHS Grant NS-13742 and DFG Grant GR-688/1.

157.10 VISUAL PROPERTIES OF NEURONS IN THE PARAFLOCCULUS OF THE RAT CEREBELLUM. Richard A. Burne and Donald J. Woodward. Department of Cell Biology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The parafloccular lobule was recently identified as a cerebellar visual area in the rat by the anatomical and electrophysiological demonstration of inputs from the visual cortex and superior colliculus via the basilar pontine and reticular nuclei (Burne and Woodward, 1978, Neurosci. Abst. 4:64). As part of the general aim of determining the functional significance of the parafloccular visual input, the current study was undertaken to characterize the response properties of visually sensitive cells in the paraflocculus.

Single unit recordings of neuronal cell activity in unanesthetized, immobilized rats were obtained, and peri-event raster and histogram analyses were employed to determine the response properties following the presentation of computer-controlled visual stimuli. Of 32 identified parafloccular Purkinje (P) cells, 23 cells showed evidence of pure excitatory, inhibitory or mixed excitatory-inhibitory mossy fiber (MF) and/or climbing fiber (CF) inputs. MF mediated excitatory responses were also elicited in 13 additional cells which did not exhibit the typical climbing fiber bursts that are characteristic of Purkinje cells. MF mediated and CF burst responses were evoked in Purkinje cells by flashed on-off stationary bars or spots of light or by visual stimuli moving at 50-800°/sec at specific orientations. CF burst responses preceded or occurred coincident with simple spike responses. Parafloccular neurons excited by stimuli in the nasal field were often inhibited by visual presentations in the temporal field, suggesting the presence of a visuotopic map in the paraflocculus. Overall, the spatial-temporal properties of visual units in the paraflocculus are grossly similar to the highly specialized units identified in parallel studies of rat visual cortex (Parnavelas et al., Neurosci. Abstr. 1980).

In summary, responses of visually sensitive parafloccular cells were elicited by stationary and moving stimuli, and showed evidence for dirtectional, velocity and positional sensitivities. Our hypothesis is that the paraflocculus may integrate information received by the visual cortex concerning ongoing events in visual space into the control of motor patterns.
(Supported by NSF BNS77-01174 and a grant from the Biological Humanics Foundation)

157.11 PARAFLOCCULUS AND POSTERIOR VERMIS: AUDITORY AREAS OF THE CEREBELLUM. A. Azizi, R.A. Burne and D.J. Woodward. Dept. of Physiol., Univ of Tx Hlth Sci Ctr at Dallas, Dallas, Tx 75235.

Previously we presented evidence suggesting the existence of an auditory cortical input to the paraflocculus of the cerebellum. The present study was undertaken with the aim of determining the electrophysiological response properties of the sound sensitive neurons within the paraflocculus and midvermis of the rat cerebellum as determined by: (1) electrical stimulation of the auditory cortex and inferior colliculus and (2) presentation of controlled, time locked tones to the animal.

Single unit recordings were obtained from 105 identified parafloccular and 98 midvermal (lobules VIa-VIIb) Purkinje cells in halothane anesthetized rats. Post-stimulus time histogram (PSTH) analysis of 23 parafloccular Purkinje cells showed evidence of excitatory and/or inhibitory mossy fiber inputs following electrical stimulation of the auditory cortex (double pulses, 0.2 msec duration, -.05 to -.8 mA, 1-20 HZ), with latencies ranging from 6-13 msecs. No climbing fiber activation or response to tectal stimulation was observed. Fourteen midvermal units (13.9%) elicited inhibitory and/or excitatory responses with latencies ranging from 4-25 msec upon stimulation of the inferior colliculus. No responses were observed following the auditory cortical stimulation. These observations suggest that the auditory input to the midvermis and the paraflocculus may be from different sources.

Single unit recordings of an additional 11 units in the paraflocculus in unanesthetized immobilized rats showed evidence through PSTH analysis, for excitatory or inhibitory mossy fiber and climbing fiber inputs following presentation of tones in the auditory field. Simple and complex spike responses were elicited following presentation of tone bursts composed of white noise 500-1000 pulses/sec and intensities of 65-80 db. (background noise intensity was 58 db.). Two types of responses were evident, units with either long (70-85 msec) latencies or units with short (20-25 msec) latencies. The data suggest the existence of two different sources of auditory input to the paraflocculus. In addition, some units showed evidence of facilitation or occlusion when a combined auditory and moving visual stimuli were presented to the animal.

In summary, these observations argue for the presence of an auditory input to the paraflocculus. Our hypothesis is that the paraflocculus operates in parallel with the classical auditory-visual midvermal area in mediating visual-auditory sensorimotor integration by the cerebellum. Supported by NSF 77-01174 to DJW and an award from the Biological Humanics Foundation.

157.12 FRACTURED AND ORGANIZED SOMATOTOPY OF TACTILE AREAS OF CAT CEREBELLAR HEMISPHERES. Jeffrey Kassel, Georgia Shambes, and Wally Welker, Dept. of Neurophysiology, Univ. Wisconsin, Madison, WI 53706.

Snider's pioneering studies of tactile responses in the cerebella of cats and monkeys suggested that posterior regions of the cerebellar hemispheres receive somatotopically organized projections. However, recent studies in rats, using high-density microelectrode mapping techniques, have shown that tactile projections to the granule cell (GC) layer of the cerebellar hemispheres are somatotopically fractured (Shambes et al., BBE 15, 94-140, 1978). We decided, therefore, to reexamine the organization of tactile projections to the cerebellar hemispheres of cats using microelectrode micromapping methods.

Mechanical stimulation of cutaneous surfaces evokes multiple-unit responses in the cerebellar GC layer in ketamine or barbiturate anesthetized cats. Facial structures project to the more medial folia of posterior crus II and to several folia of the anterior paramedian lobule (PML), while the forelimb projects to anterior and posterior folia of PML. All projections are from tactile receptive fields (RF's) on the ipsilateral head and forelimb. We have not yet observed cerebellar responses to light tactile stimulation of the trunk or hindlimbs.

Cerebellar folia (in crus II & PML) receiving facial inputs display an array of patch-like projections similar to the somatotopically fractured tactile representation previously described in rats. Within single patches, tactile projections are somatotopically organized, but adjacent patches receive projections from noncontiguous body regions. Within a single folium, a particular facial region may project to two or three spatially separated patches. Patches with facial RF's are small, usually less than 1 mm^2. Forelimb patches in PML are larger, typically extending the entire length and breadth of a folial crown.

These data confirm Snider's original report of the locations of tactile projections to the cat cerebellar hemispheres, but reveal a more complex pattern of organization than previously believed. Together with our previously reported data for rats and unpublished observations in gray squirrels, these studies suggest that a mosaic pattern of patchy projections is a common feature of the organization of tactile inputs to the GC layer of the mammalian cerebellum. (Supported by NSF grant BNS-16230 and NIH grant 5 F32 NS06047.)

157.13 THE CEREBELLO-TECTAL PROJECTION IN THE HOODED RAT. A. Frankfurter and R. M. Beckstead. Depts. of Neurosurgery & Anatomy, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

Projections from the deep cerebellar nuclei to the superior colliculi have been documented. However, neither the location nor morphology of the cells of origin of these projections, nor their laminar termination in the superior colliculi have been studied in detail. The present study was designed to address these issues.

Unilateral deposits of horseradish peroxidase were electrophoretically delivered into the superior colliculi of hooded rats. The present results indicate that almost all of the labeled neurons are located within a ventral and lateral, crescent-shaped sector of the deep cerebellar nuclei contralateral to the injection site, which extends across cytoarchitectonic boundaries, and includes the large and small cell divisions of the lateral nucleus and nucleus interpositus. All of the labeled cells seem to be of uniform size and morphology. This projection is clearly topographic. Injections into the anterior half of the superior colliculus result in labeling of neurons which are located in a more anterior sector than those observed following injections into the posterior half of the superior colliculus. Labeled neurons were never observed within either medial segments of the lateral nucleus or medial segments of nucleus interpositus. Infrequently, one or two labeled neurons were observed in the medial nucleus contralateral to the injection site. No evidence for an ipsilateral projection has been observed. The available data also suggest that the laminar termination of these neurons within the contralateral superior colliculus is in the stratum griseum intermediale, since the largest number of labeled neurons were always observed when the pipette tip was centered within this lamina. A detailed analysis of this basic organization is currently in progress.

157.14 A CORRELATED AUTORADIOGRAPHIC AND EM DEGENERATION STUDY OF THE PROJECTION OF THE SUPERIOR COLLICULUS TO THE BASILAR PONTINE NUCLEI IN THE RAT. C.B. Watt*, R.A. Burne and G.A. Mihailoff. (SPON: R. Galosy). Dept. Cell Biology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, Tx 75235.

As part of ongoing studies concerning the synaptic organization of the rat basilar pontine nuclei (BPN), an attempt was made to 1) investigate the pattern of organization in the tectopontine projection system using light microscopic autoradiography and 2) to utilize the electron microscopic degeneration technique to establish the identity of tectopontine axonal boutons and characterize their synaptic relationships within the BPN. Initially, ^{3}H-Leucine was stereotaxically delivered to the superior colliculus of Long-Evans rats utilizing either pressure or electrophoretic injection systems. After a 24 hour survival period, frozen sections were processed routinely for autoradiography and allowed to expose for five weeks. Upon examination of autoradiograms, the majority of tectopontine axons were found to terminate ipsilaterally within the lateral pontine region throughout much of the middle 3/5 of the basilar pons. The heaviest labeling was concentrated in the dorsolateral pontine region, while a less dense termination zone was observed in the ventrolateral portion of the BPN. The superior colliculus also gave rise to a sparse, contralateral pontine projection. The latter terminal zone was situated within a restricted portion of the contralateral, dorsomedial pontine region. Following bilateral electrolytic superior collicular lesions, degenerating axon terminals were identified within those pontine regions which received a tectal input as determined in previous autoradiography studies. Tectopontine axonal boutons (.8-2.2 µm) exhibited early dark degenerative changes following survival periods from 2 to 4 days. With longer survival times (4-7days) degenerating boutons became more electron dense and shrunken while being engulfed by glial elements. Tectopontine axon terminals contained small, round synaptic vesicles and formed asymmetric synaptic contacts with small diameter dendritic profiles. These observations when correlated with previous autoradiography studies provide anatomical evidence suggesting a functional connection between the superior colliculus and the basilar pontine nuclei in the rat.

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157.15 AUTORADIOGRAPHIC AND EM DEGENERATION EVIDENCE FOR AXONAL SPROUTING IN THE CORTICOPONTINE SYSTEM OF THE RAT. G.A. Mihailoff and A.J. Castro. Dept. Cell Biology, The Univ. Texas Hlth. Sci. Ctr., Dallas, Tx. 75235 and Anatomy Dept., Loyola Univ. Stritch Sch. of Medicine, Maywood, Il1. 60153.

Several previous accounts in the literature employing silver degeneration techniques have reported the existence of sprouting in the corticopontine and cerebellopontine systems when either system is interrupted in the neonatal rat brain (1-4 days postnatal). We have sought to confirm these findings in regard to the corticopontine system, utilizing the orthograde transport method (autoradiography) and to investigate the possible synaptic remodeling which might occur in the pontine gray in this situation using electron microscopy. Newborn (1-4 days of age) Long-Evans rats received large frontal cortex ablations by aspiration under hypothermic anesthesia. Several weeks (10-13) later, when the animals had attained a weight of 250-350 gm, one group received a ^{3}H-Leucine injection in sensorimotor cortex contralateral to the neonatal lesion while another group was subjected to a large sensorimotor (SM) lesion in that (contralateral) hemisphere. The autoradiographic studies demonstrated the normal corticopontine projection zones within the pontine gray ipsilateral to the leucine injection and also revealed axonal and terminal labeling in the contralateral pontine gray where the normal corticopontine input had been removed by the neonatal lesion. The implication here is that the contralateral labeling is demonstrating the sprouted corticopontine axons. Recently, similar findings have been obtained in animals which received an adult SM lesion followed 3-79 days later by a ^{3}H-Leucine injection in the opposite hemisphere suggesting that the capacity for sprouting might be retained by mature corticopontine axons. In the group of animals subjected to an SM lesion long after degenerating elements resulting from the neonatal ablations should have been cleared, the presence of numerous fragmented axons and degenerating synaptic boutons was evident in the pontine gray neuropil contralateral to the second (adult) SM lesion. Interestingly, the degenerating boutons included both the electron dense and filamentous varieties and frequently it was noted that what appeared to be a postsynaptic locus (membrane specialization) was apposed to a glial process as if the postsynaptic profile had been abandoned by its presynaptic cortical bouton. The combination of autoradiographic and EM studies thus support the notion that sprouting of cortical axons has occurred across the midline into the pontine gray which had been cortically deafferented shortly after birth.

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157.16 LIGHT AND ELECTRON MICROSCOPIC ANALYSES OF PURKINJE CELL AXON COLLATERALS: AN INTRACELLULAR HRP STUDY IN THE CAT. G.A. Bishop, J.S. King, and R.A. McCrea. Dept. of Anatomy, The Ohio State University, Columbus, Ohio 43210; and Dept. of Physiology and Biophysics, New York University, New York, New York.

Purkinje (Pk) cells of the intermediate anterior lobe and paramedian lobule in the cat cerebellum were intracellularly injected with horseradish peroxidase (HRP). The axon collaterals of 24 injected Pk cells were reconstructed from serial sections using a light microscope and drawing tube. In one animal, data were obtained on the ultrastructural characteristics of the collaterals. One to three collaterals arose from Pk cell axons either as they traversed the granule cell layer or as they entered the white matter immediately subjacent to this layer. In most cases, these collaterals remained unbranched as they coursed through the granule cell layer toward the Pk cell somata. On reaching the Pk cell layer, each branch arborized to form a plexus which extended 200-1000 µm in the sagittal plane and 200-400 µm in the frontal plane surrounding the somata of adjacent unlabeled Pk cells. This ganglionic plexus was characterized by a series of round or irregularly shaped enlargements (0.5 - 1.5 µm in diameter) connected by thin axonal strands. Two distribution patterns were formed by the collateral plexuses. In most cases, collaterals extended bidirectionally in the sagittal plane relative to the soma of origin. However, in some cases, collaterals extended only unidirectionally in the same plane. Some beaded branches from the ganglionic plexus extended into the molecular layer and coursed among the dendrites of the parent cell. Electron microscopic analysis of collaterals in the ganglionic plexus demonstrated that they lost their myelin sheath just prior to forming a series of boutons en passant, in some cases with a single post synaptic element. The boutons were filled with pleomorphic vesicles and formed symmetric junctions of variable lengths. Evidence for an autaptic contact between a labeled Pk cell dendrite and its own collateral was also obtained. In the granule cell layer finely myelinated and unmyelinated collaterals immediately surrounded the glomeruli. These data confirm and extend earlier descriptions of these collaterals derived from correlative Golgi-electron microscopic observations. (Chan-Palay, 1971: Z. Anat. Entwickl. - Gesch. 134:200). (Supported by USPHS NS-08798).

157.17 THE ORGANIZATION OF OLIVO AND PONTOCEREBELLAR PROJECTIONS TO THE PYRAMIS AND COPULA PYRAMIDIS: SAGITTAL ORGANIZATION IN THE RAT. L.M. Eisenman. Department of Anatomy, Thomas Jefferson University, Philadelphia, PA. 19107.

Retrograde transport of horseradish peroxidase was used to study the organization of the projections from the inferior olivary complex and pontine nuclei to the pyramis (Lobule VIII) and copula pyramidis in the rat. Small injections of horseradish peroxidase (20-30 nl of a 10 % solution) were made in different sagittal regions of this cerebellar lobule. Retrogradely labelled cells were seen in numerous brainstem nuclei including the inferior olivary complex, pontine nuclei, lateral reticular nucleus, perihypoglossal nuclei, extermal cuneate nucleus and others. Detailed study of the labelled cells seen in the inferior olivary complex indicates that sagittal zones in the pyramis and copula pyramis receive afferents from different regions of the caudal inferior olive. Labelled cells in the pontine nuclei in these same cases appear to indicate the existance of a sagittal organization in this projection system too. It does appear somewhat different from that coming from the inferior olivary complex in that wider regions of the pyramis and copula pyramidis receive projections from similar pontine locations. Three sagittal zones in this cerebellar lobule could be distinguished as receiving a projection from different regions of the pons where as six zones of varying width receive input from different regions of the inferior olivary complex. Chan-Palay et al, Exp. Brain Res., 30: 561, 1977) have shown that the sagittal zones of olivary terminations in the rat are narrow. The present data suggests that some of these adjacent sagittal zones receive input from similar regions of the inferior olivary complex. Whether a similar relationship exists in the ponto-cerebellar projection remains to be determined.

157.18 LENGTH OF PARALLEL FIBERS IN VARIOUS LOBULES OF THE CEREBELLAR CORTEX OF THE CAT. AN AUTORADIOGRAPHIC STUDY. J. Courville, H. Kitahara. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, H3C 3J7.

A recent study (Brand, Dahl and Mugnaini, Exp. Br. Res. 26: 39-58, 1976) using an anterograde degeneration method demonstrated that the total length of parallel fibers approximates 6 mm. The authors also indicated that parallel fibers cover the whole extent of those folia which are shorter than that length. For the present study, 0.02-0.5 µl injections of concentrated radioactive L-leucine (250 µCi/µl) were placed subcortically in the following regions of the cat cerebellar cortex: lobules VI and VIII, Crus I and II and paramedian lobule. Survival time was 24 hours in all animals except for one cat held for 48 hours; the observed length of the fibers remained the same. Optimal labeling was obtained with injections centered in the white matter and extending into the granular layer. Sections were cut in a plane approximately tangent to the surface and the complete series, extending from the surface through the whole thickness of the folia, were studied. Trigonometric reconstructions of the actual length of parallel fibers could thus be achieved even when their long axis did not coincide with the plane of section. The present observations confirmed that the parallel fibers extending through the superficial part of the molecular layer, originate from cells in the upper part of the granular layer. These are a little longer than those fibers which run in the deeper portion of the molecular layer. The latter stem from more deeply situated granule cells. The maximum length of one branch of the bifurcating fibers is approximately 1.8 mm in lobule VI, 2.0 mm in Crus I and II, 1.2 mm in the paramedian lobule and 1.3 mm in lobule VIII. Granular cells positioned centrally along the long axis of the folia have two branches of equal length while those located near the ends have one short and one long branch. Provided that synaptic contacts are distributed regularly along the length of these fibers, it can be suggested that centrally situated granular elements contribute relatively more connections with the arborizations of Purkinje cells, than those which are located distally. Evidence was also obtained that the orientation of the fibers is at times not strictly parallel to the long axis of the folia in regions where the cerebellar cortex presents sharp curvatures such as at the bottom of a sulcus.

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157.20 THE ORGANIZATION OF CEREBELLAR CORTICONUCLEAR FIBERS IN OPOSSUM: EVIDENCE OF ZONES IN ANTERIOR LOBE. P. Satrulee*, J.L. Culberson and D.E. Haines. Department of Anatomy, West Virginia Univ. Sch. of Med., Morgantown, WV 26506.

Although more than three longitudinal zones have been described in the cerebellar cortex of cat and primate, no effort has been made to elucidate the presence and/or arrangement of zones in a more generalized mammal, such as the North American opossum, Didelphis virginiana. In a series of adult opossums, small lesions were made in the cerebellum by applying the heated tip of a teasing needle to the cortex of a single folium or lobule. Following 4-9 days survival animals were killed by transcardiac perfusion with 0.9% heparinized saline followed by 10% formalin. Brainstem and cerebelli were sectioned at 40 µm in parasagittal and coronal planes, and impregnated using the Fink-Heimer method. In opossum, there appear to be 6 ipsilateral zones: two comprising the vermis (A,B) three in the intermediate area (C_1, C_2, C_3) and at least one in the lateral cortex (D). Following damage to zone B of lobule V, degenerated fibers are found primarily in rostrodorsal portions of the lateral vestibular nucleus (LVN). In contrast, only fibers of passage and sparse preterminal debris are present in the medial cerebellar nucleus (NM). Damage restricted to zone A of lobule V produced greater amounts of debris in NM and relatively fewer numbers of degenerated axons in LVN. Similar medio-lateral patterns were seen following injury of lobule IV. Lesions involving the lateral aspect of lobule III produce preterminal debris in the vestibular complex and the anterior and posterior interposed nuclei. This suggests that there are zones B (lateral vermis) and C_{1-3} (paravermal area) in lobule III. The midline portion of this lobule projects primarily to NM. The results of this study provide data concerning the arrangement of zones in the anterior lobe cortex of a generalized mammal. Zones A and B are proportionately larger than those seen in other mammals such as ferret, rabbit and bushbaby and zones C_{1-3} appear to overlap extensively. From indirect evidence, zone D is probably very narrow. The individual cortical zones become progressively narrower in more rostral lobules and, collectively, converge towards the midline. Although zones in the anterior lobe of opossum are not sharply demarcated there is an obvious medio-lateral organization.

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157.21 MORPHOGENESIS OF THE PURKINJE CELL IN THE OPOSSUM: L.C. Laxson and J.S. King, Departments of Anatomy and Surgery, The Ohio State University, College of Medicine, Columbus, Ohio 43210.

Cerebellar cortical development was studied in pouch young opossums from birth (12 days after conception) to weaning (postnatal day 90) using Golgi and Nissl preparations. Purkinje (Pk) cells were analyzed throughout the vermis in parasagittal sections. At birth the cerebellum is present as an undifferentiated plate of cells with numerous mitotic figures in the ventricular zone. Pk cells cannot be distinguished nor has the external germinal layer (EGL) appeared. At day 10 the cerebellum has enlarged and the EGL is evident as a thin covering of cells over the entire cortical surface. Pk cells have not formed a distinct band at this age.

By 21 days the Pk cells are in a zone 4-5 cells deep beneath a thin molecular layer. The EGL has increased in thickness and rudimentary folia are developing. In Golgi impregnations Pk cells have a long apical process extending toward the pial surface and a thickened varicose axon coursing toward the deep cerebellar nuclei. The soma and proximal portions of the apical process exhibit lateral processes with irregular bulbous expansions.

During the next two weeks (21-35 days) the folia resemble those of the adult, the EGL increases in depth, and the Pk cell zone is reduced to a thickness of 1-2 cells. Golgi impregnated Pk cells at postnatal day 35 have a stellate appearance with numerous long fine perisomatic processes radiating from an elongated soma. These neurons typically lack the long apical process seen at day 21. Recurrent collaterals were observed arising from Pk cell axons within the granule cell layer.

The Pk cells are in a monolayer by day 49 and the EGL has now decreased in depth. In Golgi preparations a range of Pk cell morphology is seen. The least mature forms have some perisomatic processes and 1-3 thick apical dendrites with club-like appendages. In the more mature Pk cells perisomatic processes are reduced in number and length. A single apical dendrite gives rise to numerous branches which are covered by filopodia and sessile spines. Between day 49 and day 90 the Purkinje cell attains its adult appearance.

Data obtained from Golgi impregnations suggest that the spatial orientation of Pk cells occurs during or prior to the fusiform stage of development (day 21). Later developmental changes (days 35-90) correspond to previous descriptions of Pk ontogeny. (Supported by USPHS NS-08798; we are indebted to Dr. K. Morest for the use of his Golgi collection).

158.1 MODIFICATION OF OPTOKINETIC TRACKING (OKN) BY MAINTAINED VISION REVERSAL IN NORMAL AND STROBE REARED CATS. G. Melvill Jones, G. Mandl & M. Cynader*. Dept. of Physiol. McGill Univ., Montreal; Dept. of Psychol.* Dalhousie Univ., Halifax; Canada.

The present experiments were undertaken to determine whether OKN would be modified in subjects whose vestibulo-ocular reflex (VOR) gain had been attenuated by maintained vision reversal. Since it has been suggested that VOR adaptation and OKN tracking are activated by the vector of retinal image slip, both normal animals and animals whose complement of direction selective visual cells in cortex and superior colliculus (SC) had been reduced by strobe rearing, were employed.

METHODS. One normal and 2 strobe reared (8 Hz) cats were chronically prepared with a head fixation implant and a scleral eye coil for measurement of eye movements. OKN stimulation was achieved by first gradually accelerating (subthreshold for VOR) the animal in the dark up to a steady angular velocity of 20°/sec. This steady state was continued in the dark for a further 2 min. The lights were then suddenly switched on providing the rotating animal with a view of the laboratory environment in the absence of vestibular stimulation. Monocular OKN (temporal or nasal) was recorded for 50 secs with the visual field (no prism) restricted to that available during optical vision reversal. VOR gain attenuation of 60-90% was established by means of left-right reversing dove prisms having a binocular visual field of 50° solid angle.

RESULTS. Mean slow phase eye angular velocity was measured over consecutive 5 sec periods, from which was derived the cumulative slow phase eye position (CEP) over the whole 50 sec period of OKN stimulation. In all conditions and all animals, nasal stimuli produced larger CEP values than temporal stimuli. The 2 strobe reared animals performed better in strobe light than the normal control animal. However, normal light was always more effective than strobe light, independently of rearing conditions. After adaptive attenuation of VOR gain, CEP values for both nasal and temporal OKN were significantly reduced in all animals, in both normal and stroboscopic (8 Hz) light. The fact that strobe reared cats produced both nasal and temporal OKN implies that direction sensitive visual detectors in cortex and SC are not necessarily involved in generating these responses. Since the strobe reared cats produced adaptive attenuation in VOR and OKN, such detectors also seem unnecessary for these adaptive processes. The concurrent adaptation of VOR and OKN suggests a sharing of adaptive mechanisms in common neural circuits.

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158.2 ELEVATED GAIN OF OPTOKINETIC NYSTAGMUS WITH PROLONGED EXPOSURE. Terry Fauvel*, L. R. Harris* and M. Cynader (SPON: K. Reverley). Dept. of Psychol., Dalhousie Univ., Halifax, N. S. B3H 4J1.

Compensation for motion of the retinal image during head and eye movement is achieved by optokinetic and vestibulo-ocular reflexes. When the optokinetic response (OKN) is studied in isolation by rotating a striped drum around the animal, many investigators have found that the velocity of the slow phase of OKN matches the velocity of the drum, thus compensating for its motion. We have found, however, that prolonged exposure to a rotating drum results in eye velocities which may exceed drum velocities by 30-50%.

We recorded eye movements in cats whose heads were held fixed with implanted tubes, using the magnetic search coil technique of Fuchs and Robinson (J. Appl. Physiol., 21: 1068, 1966). A striped drum (stripe period 20°) was rotated around the animal for 30 minutes at a velocity of 6°/sec. Slow phase OKN velocity rose rapidly over the first 5-10 seconds to match that of the drum, but with further exposure (varying from 2-20 minutes), eye velocity rose beyond that required for image stabilization so that the gain of OKN (defined as eye velocity/drum velocity) rose to 1.3 or more. This elevation of gain persisted throughout the 30 minute recording session.

To determine whether the increased gain represented addition of a constant bias velocity to the OKN or whether eye velocity maintained a constant ratio with drum velocity, we increased drum velocity to 9°/sec, 13.5°/sec and then 26°/sec in steps after an initial viewing period of 30 minutes. In this situation, eye velocity exceeded drum velocity by a constant percentage (i.e., gain stayed constant at 1.3-1.5) regardless of the speed of the drum. Reduction of drum velocity from 26°/sec to 0 in several decremental steps also resulted in a constant OKN gain of 1.3-1.5 regardless of drum speed. When the drum stopped rotating, OKN ceased as well.

This phenomenon was observed with binocular viewing and also when one eye viewed the drum moving medially (i.e., leftward with the right eye open). With lateral motion, results were much less consistant. The mechanisms underlying this anomalous enhanced OKN gain followed prolonged exposure remain unknown. (This research supported by USPHS Grant EY02248 and grants from MRC and NSERC of Canada.)

158.3 EFFECT OF LONG-TERM CHANGES IN THE VESTIBULO-OCULAR REFLEX (VOR) ON OPTOKINETIC RESPONSES (OKR) IN MONKEY. S.G. Lisberger, F.A. Miles, L.M. Optican*, and B.B. Eighmy*. Lab. of Neurophysiology, NIMH, Bethesda, MD 20205

Recordings from the primate medial vestibular nucleus (MVN) reveal that secondary vestibular neurons discharge in relation to optokinetic stimulation (Waespe & Henn, Exp.Brain Res. 1977), but show no manifestation of adaptive changes in VOR gain (Lisberger & Miles, J.Neurophysiol. 1980). This suggests that the modifiable elements subserving VOR gain control lie downstream from the MVN and may be shared by signals driving OKR. If the above is true, changes in the gain of the VOR should cause, as a secondary consequence, parallel changes in at least some aspect of the OKR. Adaptive changes in VOR gain were achieved by having animals wear either x2-telescopic spectacles or goggles providing a visual field that always moves with the head (equivalent to x0-telescopic spectacles). VOR and OKR tests were applied at 1-3 day intervals. VOR gain was measured as peak-to-peak eye velocity divided by peak-to-peak head velocity during sinusoidal, whole-body oscillation in total darkness. Horizontal OKR were elicited by seating the animal inside a full-field striped cylinder that rotated at constant velocity; the resulting nystagmus consists of smooth tracking eye movements in the direction of stripe movement (interrupted by resetting saccades). After sudden illumination of the moving stripes, the velocity of the tracking eye movements shows an initial rapid rise (lasting a few hundred msec) followed by a more gradual increase (lasting 10 to 15 sec); these rapid and gradual events are believed to represent two separate components of the OKR. After the illumination goes out, there is an initial rapid decrease in smooth eye velocity followed by a long-lasting afterresponse (OKAN), the initial velocity of which is assumed to be an index of the level achieved by the gradual component of the prior OKR. Six-fold changes in VOR gain (0.29 to 1.80) were associated with parallel, 4.5-fold changes in the initial eye velocity of OKAN, but little or no change in two measures of the rapid component of OKR (eye acceleration in the first 100 msec and peak eye velocity achieved in the first sec). That the rapid and gradual events of OKR are differentially affected by changes in VOR gain supports the idea that they represent separate components mediated by separate neural pathways. Furthermore, these findings imply that the modifiable elements subserving VOR gain changes lie after the point at which the pathways mediating the gradual component of the OKR (OKAN) and the VOR converge, but before the point at which the pathways mediating the rapid changes in eye velocity and the VOR converge. A computer simulation of such a model predicted even the quantitative details of the experimental observations.

158.4 THE "ERROR SIGNAL" SUBSERVING LONG-TERM ADAPTIVE GAIN CONTROL IN THE VESTIBULO-OCULAR REFLEX (VOR) IN MONKEY. F.A. Miles and S.G. Lisberger, Lab. Neurophysiology, NIMH, Bethesda, MD 20205

While the process underlying adaptive VOR gain changes depends ultimately on the association of visual and vestibular inputs, in theory any reliable central correlates of the same would suffice. In fish and rabbit, for example, others have shown that VOR gain changes depend on the association of retinal slip and eye velocity. In both these species, prolonged sinusoidal optokinetic stimulation without head oscillation induces increases in VOR gain; in rabbit, passive oscillation with reversed vision causes increases or decreases in VOR gain depending upon whether the eye movements during adaptation were or were not reversed. We now report that, in monkey, these same experiments yield quite different answers: VOR gain was unaffected by sinusoidal optokinetic stimulation alone, even though the retinal slip and eye velocity were similar to those effective during passive oscillation with telescopic spectacles; passive oscillation with reversed vision always produced decreases in VOR gain. These results imply that, in monkey, eye velocity per se is irrelevant and the adaptive process must use some direct measure of head velocity. In monkey, as in other species, passive oscillation with telescopic spectacles produces changes in VOR gain specific to the adapting frequency. That the mechanisms involved are similar to those at work during active head turns can be seen by adapting monkeys to telescopic spectacles with passive oscillation alternating between 0.2 and 2.0 Hz. This produces equal VOR gain changes at all frequencies (0.1 to 4.0 Hz) and parallel changes in optokinetic responses; both are also true of adaptation with active head turns.

We have also found that VOR gain changes can be induced in the absence of peripheral retinal slip. Eye movement was recorded in a monkey rewarded for fixating a small light in totally dark surroundings. During fixation the light and monkey were oscillated in the horizontal plane either in-phase or 180° out-of-phase, simulating the eye-head movement relationship normally associated with low- or high-gain spectacles. Eight hours of coordinated tracking caused adaptive VOR gain changes as large as 50% of those seen after equivalent passive oscillation in spectacles. That foveal pursuit during head movement is sufficient to elicit VOR gain changes implies that central correlates of pursuit might furnish part of the error signal guiding the adaptive process. Since 1) Purkinje cells in the flocculus discharge in relation to pursuit, 2) floccular lesions severely disrupt the adaptive process (Optican et al, Soc. Neurosci. 1980), and 3) the changes in Purkinje cell vestibular responsiveness accompanying VOR adaptation imply that the flocculus is not the site of the modifications, we suggest that the flocculus exerts an inductive effect on modifiable elements located elsewhere.

158.5 OCULOMOTOR DEFICITS IN MONKEYS WITH FLOCCULAR LESIONS.
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We studied the effects of bilateral flocculectomy in two monkeys. The optokinetic response (OKR) and adaptive gain control of the vestibulo-ocular reflex (VOR) were studied with the methods of Lisberger et al. (Neurosci. Abstr., 1980), and adaptive control of the pulse-step ratio of saccades was studied as in Optican & Miles (Neurosci. Abstr., 1979). 1) The tracking moiety of the normal OKR has rapid and gradual components. After flocculectomy, the rapid component was considerably attenuated (open-loop gain, measured as eye acceleration divided by retinal slip, was only 25% of normal), while the gradual component achieved nearly normal amplitude but over a much longer time course. Thus, saturation of the gradual component (as indicated by the maximum initial velocity of the after-nystagmus, OKAN) was almost normal, while the "charging" time constant of OKAN (determined by the methods of Cohen et al., J. Physiol., 1977) was two to four times normal. 2) Before flocculectomy, one day of passive oscillation at 0.2 Hz. with x0 spectacles caused the VOR gain (eye velocity divided by head velocity) to drop from 1.00 to 0.49 in one animal, and 0.98 to 0.55 in the other. After flocculectomy, the VOR of both monkeys had low gain (0.92 and 0.76, which persisted for four months) and showed reduced adaptability: e.g., even after 2 days of passive oscillation at 0.2 Hz. with x0 spectacles, the gains only fell to 0.78 and 0.64, respectively. 3) The animals' ability to adapt to optically-imposed post-saccadic retinal slip was estimated from changes in the pulse-step mismatch (PSM), defined as amplitude of the post-saccadic ocular drift divided by magnitude of the antecedent saccade. Exponential slip imposed in the direction of the antecedent saccade (with a 50 msec time constant and 50% of the saccade's amplitude) resulted in large, adaptive changes in the PSM before the lesion (from -3% to -70% in one animal and -1% to -50% in the other), while afterwards these changes were considerably reduced (from -2% to -7% , and +1% to -5%, respectively).

The reduction in the rapid component of OKR after flocculectomy supports the hypothesis that the flocculus acts to boost the gain of visual following mechanisms (cf., Zee et al., Neurosci. Abstr., 1978). The reduced adaptability of the VOR gain and the saccadic pulse-step ratio is consistent with the hypothesis that the flocculus provides the visually-based "error signal" guiding the underlying adaptive processes (Miles & Lisberger, Neurosci. Abstr., 1980).

158.6 INFLUENCE OF OPTICAL REVERSAL ON EYE-HEAD ORIENTING MECHANISMS.
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When the head is passively and suddenly moved, a reflexive rapid eye movement (here called the initial quick phase) occurs with a short latency. The purpose of this movement is to point the gaze axis in the direction of head motion (Melvill Jones, Aerospace Med. 35:316-332, 1974). The initial quick phase is of particular interest because Barnes (J. Physiol. 287:127-147, 1979) has shown that it is part of one of the major motor strategies used in carrying out a normal eye-head orienting response towards a visual target. The aim of the present experiments was to examine whether, and how, the initial quick phase is modified during the period of adaptation to reversing prisms. The results were obtained from a cat subjected to continuous vision reversal for a two week period, at the end of which the gain in the dark of the vestibulo-ocular reflex had decreased to a value of 0.25. Throughout the adaptive period the cat was placed on a horizontally rotating table and subjected to velocity steps ranging from 15 to 100°/sec.

Results: In the normal unadapted cat the amplitude of the initial quick phase increased with head velocity. This observation agrees with that of Chun and Robinson (Biol. Cybernetics, 28:209-221, 1978). As the time of adaptation increased, the amplitude of the initial quick phase decreased for each head velocity tested. The fact that slow phase velocity also simultaneously decreased suggested that the initial quick phase amplitude might be proportional to slow phase velocity. However, analysis revealed that the decrease in amplitude of the initial quick phase was, over a large range of head velocities (30 - 100°/sec), larger than that predicted by the decrease in slow phase velocity. The major cause underlying the amplitude decrease appears to be an adaptive change in the quick phase mechanism itself.

158.7 DEFICITS IN SPATIAL LOCALIZATION FOLLOWING MONOCULAR PARALYSIS IN ADULT CATS.
J. M. S. Winterkorn. Dept. of Anatomy, Cornell Univ. Med. Coll., New York, N.Y. 10021.

The effect of immobilizing an eye on performance of a simple visual task by adult cats was studied. Visual perimetry was conducted in an apparatus consisting of a semicircular table with a radius of 50 cm surrounded by an opaque black screen. The cat was positioned facing the screen and trained to fixate on a light located at an aperture in the screen at 0°. Other lights, located in apertures in the screen at 15° intervals from Left 90° to Right 90°, could be illuminated at variable intensities and for variable intervals.

Experimentally naive, intact adult cats were trained to respond to the onset of a light at any position on the perimeter by orienting to it and retrieving a piece of dry cat food from a small black cup located beneath the light. Fixation and eye movements were observed through a peep hole near the central fixation point. Accuracy of orientation responses was evaluated separately by two observers. The cats' visual fields were determined binocularly and monocularly using an opaque scleral lens.

After stable preoperative performances were achieved, cats were subjected to monocular paralysis by transection of the third, fourth and sixth cranial nerves. From the first postoperative day on, visual perimetry was carried out at least twice weekly for up to twelve weeks.

Postoperatively, the extent of the visual field was not observed to change significantly. Observations were made of the cats' orientation to either a steady or briefly flashed light presented through each eye from 90° ipsilateral to 30°-45° contralateral. However, when the stimuli presented were brief flashes, the accuracy of orientation responses mediated by the immobilized eye deteriorated significantly-- especially to ipsilateral stimuli presented at 60°-90°. When a light was flashed in these locations, the cat turned its head in the general direction of the light, indicating an intact visual field, but the cat then frequently approached a food cup beneath a neighboring stimulus, most often 15°-45° medial to the flashed stimulus. Thus, when using the immobilized eye, the cat had a diminished ability to maintain the localization of a short flash of light in the monocular field.

Similar difficulty in accurately localizing visual stimuli has been reported in human patients with paralytic strabismus.

158.8 BINOCULAR COUNTERROLLING IN HUMANS DURING SUSTAINED BODY TILT.
Shirley G. Diamond and Charles H. Markham. Reed Neurological Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

To examine the belief that ocular counterrolling (CR), a utricular reflex, is maintained at a steady, conjugate level during sustained body tilt, normal subjects were securely strapped into a rotating chair, the head position stabilized by a bite bar. Subjects were then rolled about their naso-occipital axes in separate trials to 30°, 60° and 90°, at a velocity of 3°/sec, with acceleration below the threshold of the semicircular canals. A camera mounted on the rotating chair photographed both eyes at each 10° during roll, and at each 10 sec while the subject was held for 10 min in the tilted position. A control trial of 10 min in upright position was also studied.

CR in both eyes was measured independently using a dual projector system (Diamond et al., Acta Otolaryngologica 87:490-498, 1979) in which mechanical accuracy is 1 minute of arc and practical accuracy is 15 minutes of arc.

Results showed the eyes moved in a smooth, conjugate manner during the roll from and back to the upright position. During the sustained tilt, however, the eyes were unstable with respect to both amount of CR and conjugate movement. Variations in CR during the 10 min of each tilt position ranged up to 4° in a single subject. At 30° tilt, CR ranged from 3.5° to 7.5°; and at both 60° and 90° tilt, CR ranged from 6° to 9.5°. Fluctuations in CR did not appear to be related to time.

The downward eye consistently rolled more than did the upward eye, with this effect more pronounced the greater the degree of tilt. Disconjugate movements similarly were more evident with increasing tilt. At 30°, 23% of the observations were disconjugate compared to the previous observation of the two eyes. At 60°, 35% were disconjugate, and at 90°, 47% were. This is consistent with our previous findings in dynamic CR that sensitivity increases the closer the position is to upright.

The observations in the upright position showed that small torsional movements are present in the eyes in the absence of tilt stimulation. Variations during 10 min ranged up to 1.5° Disconjugate movements were seen in 23% of the observations, the same amount as in the 30° tilt.

These studies suggest that each eye receives input from each utricle, and that in a given tilted position the planes of sensitivity may be slightly different in the two utricles, resulting in differing behavior in the two eyes.

158.9 VARIATIONS IN SACCADIC EYE MOVEMENT STEREOTYPES IN THE LITERATURE - A HYPOTHESIS. P.E. Hallett, Department of Physiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada.

The saccadic system is regarded as being slavishly servomechanistic only at the lowest motor level. Each saccade is assumed to arise from a new 'decision' as to the next spatial goal, which reflects not just the immediate spatial scene but also memories and diverse factors. The 'decision' delay is very variable in duration and is usually triggered by a change in the scene or by the prior saccade. A new decision may 'cancel' an on-going decision in an all-or-none fashion at an early stage, or modify the amplitude of an inevitable response at a late stage ('compromise'). The very variable latency data in the literature can be reduced to a few temporal stereotypes. The observed stereotype in a given individual and task is hypothetically selected by variations in the source of the trigger for the decision delay, the value of the mean decision delay relative to the fixed delays, and the respective advantages of cancellation or compromise.

158.10 BEHAVIOR OF EYE MOVEMENT NEURONS IN POSTERIOR PARIETAL CORTEX DURING CONTROL OF EYE POSITION IN TOTAL DARKNESS. Stephen Whittaker*, Gregory Moulton*, and Alex Skavenski. Dept. of Psychology, Northeastern University, Boston, MA 02115.

Substantial behavioral evidence from people indicates that information about eye position combines with retinal image position information of a target to determine the target location relative to the head. Thus, responses, such as a hammerblow, may be accurately directed at the target. Existing evidence from lesion experiments as well as single neuron recordings suggests that portions of posterior parietal cortex (PPC) are involved in selecting and localizing visible targets in space. Cells in this area have been found to have firing rates related to the retinal locus of stimulation. Although others have found the activity of cells related to eye and arm movement directed at visible targets, such relations have not been quantitatively studied in the absence of retinal stimulation.

In the present experiment, we identified two classes of cells related to eye position in total darkness. Single neurons were isolated in PPC near the intraparietal sulcus of intact, alert macaca nemistrina. Monkeys were trained to fixate a target lamp and maintain that eye position for periods when the target had been removed from view (in complete darkness) by giving reinforcing squirts of juice when the eye position was within $2\frac{1}{2}^{o}$ of the target position. The target position was systematically varied. Eye position, stimulus position and retinal image position of the target could be defined (within 15 min arc) over a 30^{o} X 40^{o} area. For one class of neurons the relation between eye position and concurrent discharge rate of each cell increased monotonically with eye position in a particular direction during steady fixation of a visible target and maintainance of the eye position in complete darkness. During control of eye position in the dark, another class of eye position related cells, "error cells", systematically increased discharge rate as actual eye position deviated from the eye position that matched the locus of the intermittently lit target. This activity is likely to be derived from a comparison of absolute eye position information with some stored analogue of the target position or "correct" eye position. The existence of this comparator mechanism and neural analogues quantitatively related to retinal error, eye position, and eye position error further suggests that PPC is involved in visual localization.

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158.11 VERTICAL OPTOKINETIC NYSTAGMUS DEFICIT FOLLOWING LESION OF PARIETO-OCCIPITAL ASSOCIATION CORTEX IN RHESUS MONKEY. Jay W. McLaren and James C. Lynch, Department of Physiology, Mayo Clinic and Foundation, Rochester, Minnesota 55901.

Unilateral damage of parieto-occipital cortex in humans and monkeys usually causes asymmetric horizontal optokinetic nystagmus (OKN) with slow phase velocity diminished toward the side of the lesion. The effects of cortical lesion on vertical OKN has not been studied extensively, although some clinical reports have suggested that deficits follow bilateral hemispheric lesions.

In the present study, vertical OKN was measured in 5 rhesus monkeys before and after unilateral and bilateral lesions of parieto-occipital cortex. OKN was induced by projecting a pattern of alternating black and white 5° wide stripes onto a 52° square tangent screen in front of the monkey. The pattern could be moved upward or downward at velocities ranging from 30°/sec to 160°/sec. Eye position was measured with chronic electrooculographic (EOG) electrodes. EOG signals were digitized and stored by a small computer for later analysis. Five monkeys had unilateral cortical ablations. After at least 2 weeks of testing, 3 of these monkeys received a similar lesion in the other hemisphere. Two monkeys had lesions limited to the inferior parietal lobule; 3 had lesions which included the inferior parietal lobule and immediately adjacent prestriate cortex and superior parietal lobule. Eye velocity during each OKN slow phase was measured by the computer, and the mean slow phase velocity during 27 seconds of stimulation was calculated for each stimulus velocity. Mean eye velocity during a 5-day period immediately preceding cortical lesion was compared with mean eye velocity during the 5 days immediately following unilateral or bilateral lesion.

Before lesion, no monkey showed consistent slow phase velocity asymmetry between upward and downward stimulus conditions. OKN efficiency (mean eye velocity/stimulus velocity) was lower than that of horizontal OKN induced by a full-field drum, but was comparable to that of horizontal OKN induced by projected stimuli. All 3 monkeys which had bilateral lesions showed significant ($p < .005$) reductions in mean slow phase velocity in both directions. Four monkeys were tested before and after unilateral lesions; only one showed any reduction of slow phase velocity following lesion, and then only in some stimulus conditions.

These results suggest that parieto-occipital cortex is important to normal vertical OKN, and that the parieto-occipital cortex of a single hemisphere can support both upward and downward slow phase eye movement.

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158.12 FRONTAL EYE FIELD AFFERENTS FROM THE BRAINSTEM IN THE CAT. Gary J. Pascuzzo* and Leslie C. Skeen (SPON: J. Maxwell). Institute for Neuroscience and Department of Psychology, University of Delaware, Newark, DE. 19711

Microelectrode recordings have shown that some neurons in the cat frontal eye fields (FEF) discharge before, during, or after certain types of eye movements (Guitton & Mandl, '78). The specificity and timing of these neuronal discharges indicate that the cat FEF may receive inputs from structures that are directly involved in the control of eye movements. With the goal of discovering the anatomical bases of these eye-movement related discharges, we have been examining the connections of physiologically defined areas in the cat FEF.

The boundaries of FEF areas were defined in cats under light chloral hydrate anesthesia (60-80 mg/kg) while monitoring their eye movements during intracortical microelectrode stimulation. In FEF areas where minimal stimulation (25-50 μA) consistently elicited constant-amplitude eye movements, small amounts of horseradish peroxidase (HRP; Sigma VI, 10-60%) were injected either by pressure (0.05-0.2 μl) or by iontophoresis (1-5 μA square wave pulses, 4-16 min.). After surviving for two days, the animals were perfused and sections (30-48 μm) from their brains were processed using a modified tetramethyl benzidine procedure (Mesulam, '78).

All injections restricted to physiologically defined FEF areas reveal retrogradely labeled cells in regions of the infratentorial brainstem that have been implicated in the modulation of oculomotor functions. Cases with very small injections have labeled neurons in the raphe nuclei and locus coeruleus. Both structures exhibit bilateral labeling although the cells ipsilateral to the injection site are much more prominent. These structures have been implicated in several broadly defined oculomotor functions including visual attention and rapid eye movements during sleep (Jouvet, '67; Goldberg & Wurtz, '72; Chu & Bloom, '74). Clusters of labeled cells are also present in the central grey of the midbrain and in the reticular formation immediately lateral to the medial longitudinal fasciculus. These areas have been implicated in vergence movements (Carpenter, '71). In cases with somewhat larger injections, but still restricted to the FEF, the above pattern of labeling is more pronounced and additional labeled cells appear more caudally in the paramedial pontine reticular formation. Several studies have demonstrated the importance of this region in the control of horizontal eye movements (Highstein et al., '75, '76; Graybiel, '77).

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158.13 THALAMIC NEURONS RELATED TO HEAD AND EYE MOVEMENTS. H. Maldonado and J. Schlag. Dept. of Anatomy and BRI, UCLA, Los Angeles, CA 90024.

Neurons of the thalamic internal medullary lamina (IML) in cat are known to discharge in relation with eye movements. Since electrical stimulation of the IML elicits head as well as eye movements, unit recordings were made in alert cats able to turn their heads in an horizontal plane. The animals were implanted with silver-silver chloride electrodes for recording the horizontal and vertical electrooculogram (EOG). Horizontal head rotations were measured with a low torque potentiometer mounted on a head holder. Tungsten microelectrodes were stereotaxically driven through the dura. EOG, head position and unit activity were simultaneously recorded on magnetic tape and polygraph paper.

130 units in the IML region were found active with either head movements (31%), eye movements (34%) or both (35%). These figures include phasic discharges related to movements and tonic firings related to positions. Most head-movement units had a direction preference, usually for the contralateral side. They paused during movements in the off- direction. Some cells discharged with passive as well as active head movements and they had the same directional preference in both cases. Cells active with both head and eye movements showed phasic discharges with ocular saccades which occurred without head turning. Their directional preference was the same for both eye movements alone or head-eye combined rotations. Increases of firing related to active head movements started from 0 to 100 msec before the movements.

Some of these units also responded to visual stimuli. The finding of cells related to head, eye movements and visual stimulation gives further support to the role of IML in targeting. (Supported by USPHS Grant NS 04955). Dr. Maldonado is a fellow from CONACYT #16005.

158.14 PREOCULOMOTOR BRAINSTEM NEURONS RECORDED DURING EYE-HEAD COORDINATION. D. A. Whittington*, F. Lestienne* and E. Rizzi. Dept. of Psychology, M.I.T., Cambridge, MA 02139.

Single unit recordings from the brainstem of four monkeys have provided information on the behavior of preoculomotor neurons during coordinated eye-head movements. Of the generally recognized classes of preoculomotor cells, some acted in a manner entirely consistent with previous studies. For instance, tonic cells always fired in tight correlation with eye position, independent of head movements. On the other hand, some burst and pause cells showed behavior not demonstrated in previous head-fixed experiments.

Within the category of medium lead burst cells having a fairly constant burst rate, we found two quite distinct subgroups. When the head was restrained, these two groups were indistinguishable, both having a strong correlation between the number of spikes in a burst and the size of the associated saccadic gaze shift. However, when the head was free to move, this population of bursters could be subdivided into two classes. This subdivision was related to the fact that saccades during coordinated eye-head movements contribute only part of the total gaze shift; the rest being supplied by the head. The first class included cells whose firing activity was closely related to the size of the saccadic eye movements, while in the second class of bursters, the firing was correlated with the total gaze shifts, including the head-movement contribution. We have tentatively characterized these latter cells as being upstream in preoculomotor processing at a level where the total shift in gaze is specified. Following this schema, the first class of bursters is conceived to be further downstream toward the oculomotor plant at a point where the contribution of the head has been subtracted out of the total gaze shift, presumably via the vestibulo-ocular reflex.

With respect to pause cells, we have recorded from a number of subgroups. The subgroups which were not vestibularly modulated did not display any head-related activity. In contrast, some pausing cells, whose tonic component was vestibularly modulated displayed head-related pauses that were a function of the magnitude and direction of head velocity. Although it has been proposed that pausing cells may be an important link in saccade generation, our results indicate that this property cannot be attributed to all pausing cells. (Research supported by NS09343, NGR 22-009-798 and EY02621.)

158.15 CONTROL OF EYE MOVEMENTS IN RAT SUPERIOR COLLICULUS. J. McHaffie* and B.E. Stein (SPON: A.J. Szumski). Dept. of Physiology, Medical College of Virginia, Richmond, VA 23298.

The superior colliculus plays an important role in sensorimotor integration, and it is generally assumed that this structure is integral in visual scanning. The activity of many cells in the superior colliculus of monkeys and cats has been closely related to the generation and control of eye movements. Electrical stimulation of the colliculus in these animals has been shown to evoke contralateral saccadic eye movements whose directions and amplitudes are predictable on the basis of the visuotopic map in this structure. However, it is often assumed that rodents, unlike monkeys and cats, make few saccades, and visual orientation occurs by head rather than by eye movement. The possibility that a fundamental species difference in the organization of the colliculus could underlie these differences prompted the present experiments.

Seven urethane anesthetized Sprague-Dawley rats were used. Immobilization of the head was accomplished by attaching a chronically implanted bolt to a modified stereotaxic head holder. Mirrors were glued to the anesthetized corneas and light beams were reflected from these mirrors onto a translucent hemisphere. Eye movements were indicated by displacements of the reflected spots of light. The visual cortex was aspirated exposing the dorsal surface of the colliculus. Electrical stimulation (70 msec trains, 0.1 msec pulses, 200 pps, 10-700 μA) of the colliculus evoked conjugate, contraversive saccadic eye movements. Closely spaced electrode penetrations were made over the collicular surface, and a map of evoked eye movements was constructed. Stimulation of medial sites yielded movements with upward components (as great as 30° above the horizontal axis), and lateral sites evoked movements with downward components (as great as 80° below the horizontal axis). Most of the eye movement map of the colliculus was devoted to saccadic movements which were nearly horizontal. The amplitude of any saccade varied directly with stimulus intensity and with stimulus location. Medial and caudal sites in the colliculus generally produced the largest saccades and movements of up to 15° of excursion were noted. The lowest thresholds for eye movements were in the intermediate and deeper laminae. Stimulation here also evoked vibrissae, ear, neck, and body movements. Although no systematic study of these other movements was made, they too appeared to be topographically organized. While a preference for orientation by head or eye movement may vary among primate, carnivore and rodent, the fundamental aspects of eye movement organization in the colliculus appear to be substantially the same for these different species. (Supported by grant NS 15912.)

158.16 CONNECTIONS OF A PREMOTOR REGION IN THE LATERAL MIDBRAIN RELATED TO CONTROL OF THE PINNAE. Craig K. Henkel, Dept. Anatomy, Bowman Gray Sch. Med., Winston-Salem, NC 27103.

That pinna movements in the cat are elicited by electrical stimulation of the superior colliculus may indicate that auditory modulation of motor activity occurs in a structure typically identified as a visual reflex center. However, in a recent study we showed that colliculuar control of the pinnae can be mediated through connections with a paralemniscal zone in the lateral midbrain tegmentum (Henkel and Edwards, '78), and it is not known what other brainstem and possibly auditory inputs may modulate activity at this premotor level. The present study is an attempt to identify the afferent connections of the midbrain paralemniscal zone in the cat, using the retrograde horseradish peroxidase tracing method. Preliminary results after HRP was deposited in the lateral midbrain indicate that the paralemniscal zone receives connections from numerous brainstem sources. In the ipsilateral subthalamus, a few cells were labeled in the zona incerta and fields of Forel. In the midbrain, small cells (16-25 μ) were labeled on the side of the deposit in the periaqueductal gray matter that in many cases were similar to those in the stratum griseum profundum. In the posterior and anterior pretectal nuclei, a smaller number of cells was also labeled. Starting in its rostral part, labeled cells in the cuneiform nucleus were sparse but became more numerous in caudal areas adjacent to the inferior colliculus. A commissural connection from the contralateral paralemniscal zone was also present. In the pons, labeled cells were present bilaterally in the ventral part of nucleus pontis oralis, but especially overlying the ipsilateral superior olivary complex. Finally, in the medulla an aggregation of cells (24-37 μ) was labeled in the contralateral nucleus prepositus hypoglossi. It remains to be shown whether labeled neurons found in the dorsal cochlear nucleus resulted from labeling the lateral lemniscus at the HRP deposit. In evaluating these afferent connections, it is noteworthy that the paralemniscal region does not receive a predominance of sensory inputs, but rather appears to be modulated by activity in other motor areas. It is particularly of interest that several of these regions contain units that appear to code information related to head and eye movements. Supported in part by BRS grant RR 05404.

158.17 NEURONAL ACTIVITY IN THE NUCLEUS RETICULARIS TEGMENTI PONTIS IN ALERT MONKEY. E.L. Keller and W. Crandall. The Smith-Kettlewell Institute of Visual Sciences, San Francisco, CA 94115.

The nucleus reticularis tegmenti pontis (NRTP) is a relatively large pontine reticular nucleus appearing as a dorsal extension of the pontine nuclei proper. It has been differentiated from these latter nuclei on an anatomical basis, however, its functional role is almost completely unknown. It has been most widely described as a precerebellar reticular nucleus, but on the basis of its known sources of afferent connections, a role for NRTP in the control of eye movements or gaze seems likely. It has also been suggested that NRTP forms a relay site for the visual pathways mediating the optokinetic (OK) modulations of vestibular nucleus cells. Therefore we studied the activity of neurons located in NRTP in alert monkeys trained to fixate a small visual target. Within the medial extent of NRTP two major classes of cell responses were found. A small fraction (less than 10%) of the cells showed a spontaneous discharge that could be modulated by the motion of a large-field, OK drum. Since the animal would fixate during drum rotation, we could relate the neural responses to actual retinal slip velocity of the OK stimuli. All cells saturated at stimulus velocities of 1 to 2 deg/sec which is well below the effective velocities reported to modulate vestibular neuron discharge in monkey. A majority of NRTP neurons were related to saccadic eye movements. Most of these latter cells (33 of 62) were silent except for intense bursts associated with (and preceding in most cases) certain saccadic eye movements. Some cells showed movement fields similar to those reported in the deeper layers of the superior colliculus. There was no spatial organization of the movement fields within the structure. Other neurons located in this nucleus showed a spontaneous discharge during fixation which either burst (7 cells) or paused (5 cells) during saccades. A small number of neurons (7 cells) showed a burst-tonic discharge similar to oculomotor neurons.

158.18 RESPONSES OF VESTIBULAR NUCLEI CELLS DURING VERTICAL VESTIBULAR AND PURSUIT EYE MOVEMENTS. R. D. Tomlinson* and D. A. Robinson. The Wilmer Institute, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Recent recordings made in the MLF (medial longitudinal fasciculus) of awake primates have demonstrated a class of cells carrying head velocity, smooth pursuit eye velocity, and eye position signals from the vestibular nucleus to the oculomotor nucleus (J.Neurophysiol. 39: 1135, 1976; J.Neurophysiol. 41: 245, 1978). These cells have been reported to not change their vestibular modulation depth when the animal cancels its VOR (vestibulo-ocular reflex) by fixating a target moving with the head. This observation is at odds with the generally accepted notion that VOR cancellation is accomplished by adding on an equal and opposite smooth pursuit signal in order to eliminate the unwanted vestibular drive.

One of the above studies observed only the VOR while the other studied only pursuit. The present study reinvestigated those observations by using both stimuli in the same animal. Recordings were made from the vestibular nuclei of rhesus monkeys. The animals were trained to follow a dot which was rear projected onto a tangent screen located 1 m away. Horizontal and vertical target position was controlled by mirror galvanometers. Eye movements were measured by the search coil-magnetic field method. Single cells in the vestibular nuclei were tested in four different conditions: 1) various positions of fixation; 2) smooth pursuit tracking; 3) vertical VOR in darkness; 4) vertical VOR cancellation. Eye, head, and target position and neuron firing were digitized and stored on magnetic tape for further analysis.

Discharge rate-position and rate-velocity curves were plotted for each unit and vestibular sensitivity was calculated both during VOR in darkness and VOR cancellation. Typically, pursuit and vestibular sensitivities were about 0.6 and 1.0 (spike/sec)/(degree/sec) respectively. Firing rate modulation during vertical sinusoidal head rotation (0.8 Hz, 60 deg/sec peak velocity) in darkness was compared with that obtained during VOR cancellation for cells with pursuit velocity signals. No significant differences in modulation depth were noted between the two test conditions. If, for example, a cell was modulated just as strongly during pursuit as during the VOR in the dark, then, during cancellation, the pursuit and vestibular commands should cancel and the modulation should decrease to zero. However, the modulation did not decrease at all. Thus, the hypothesis that VOR cancellation is accomplished by a simple addition of pursuit and vestibular commands on central neurons is incorrect. If pursuit and cancellation are related it must be in a rather complicated manner.

158.19 ARE FAST LATERAL HEAD MOVEMENTS MEDIATED BY PONTINE RETICULOSPINAL FIBERS? David W. Sirkin, Barbara Ann Rogowski*, Barbara E. Schlumpf*, and Albert S. Feng. Program in Neural and Behavioral Biology, Dept. of Physiology and Biophysics, and Dept. of Psychology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801.

Lesions of the pontine reticular formation (PRF) cause a loss of ipsilaterally directed horizontal fast eye movements (voluntary saccades as well as quick phases of nystagmus). A previous study in the rat (Sirkin et al., Soc. Neurosci. Abstr. 4:615, 1978; Exp. Neurol., in press, 1980) and preliminary results in the cat (Siegel, J.M. and Sirkin, in progress), showed that PRF lesions have effects on head movements that are analogous to the effects on eye movements: fast head movements to the ipsilateral side, notably quick phases of head nystagmus, are abolished. Since the PRF's control over eye movements is believed to be mediated by a direct projection to the motor nuclei (especially the homolateral abducens), we thought the same might be true of its control over head movements. The PRF is known to be a source of supraspinal fibers innervating the ventral horn. In this study we assess the role of these fibers.

We first verified with the autoradiographic method that the site of effective PRF lesions gives rise to reticulospinal fibers. [^{3}H]-leucine, injected using the same stereotaxic coordinates used for lesions in the earlier study, labeled strongly a projection to the cord, largely homolaterally. In another series of animals we made lesions to interrupt this projection in the medulla, where it runs in the medial longitudinal bundle, and begins to sweep ventrolaterally over the dorsal surface of the medial part of the inferior olive. These lesions caused deficits in fast ipsilaterally directed head movements similar to those caused by PRF lesions. Histological analysis with the Fink and Heimer method revealed degenerating reticulospinal axons descending from the sites of behaviorally effective lesions. A control lesion placed more laterally did not cause degeneration of reticulospinal axons, and had no observable effects on lateral head movements. These results suggest that pontine reticulospinal fibers play an essential role in mediating fast lateral head movements, thus providing a specific function for one supraspinal system.

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158.20 AFFERENT PROJECTIONS TO THE MONKEY FLOCCULUS. T.P.Langer*,A.F. Fuchs, M.C.Chubb*, and C.Scudder*. Dept. of Physiology and Biophysics and Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195.

The flocculus has been shown to participate in vestibular and oculomotor behaviors. In interpreting these studies and initiating others the anatomical projections to the flocculus are of considerable importance.

We have examined these afferents in 3 rhesus monkeys following injections of horseradish peroxidase into their flocculi. After three days,the monkeys were perfused with 1% paraformaldehyde:2% glutaraldehyde in 0.1M phosphate buffer and the labelled neurons were demonstrated with the TMB or BDH methods.

The projections to the flocculus were largely from six sources: 1.The contralateral dorsal cap of Kooy in the inferior olive, 2. parts of the vestibular complex, bilaterally, largely medial vestibular nucleus,but also regions of most of the other nuclei,3. the perihypoglossal complex,bilaterally,and the raphe nuclei at the same level,4.parts of the abducens nucleus and adjacent perigenu regions,bilaterally,5.midline and lateral parts of the nucleus reticularis tegmenti pontis,bilaterally,6.the pontine nuclei particularly the contralateral dorsolateral and ventrolateral rostral nuclei. Virtually every neuron in the dorsal cap of Kooy was labelled. Also a large number of the neurons in the nucleus intercalatus,nucleus Roller,nucleus praepositus hypoglossi and nucleus raphe compacta were labelled. The medial vestibular nucleus projection was patchy and involved a small fraction of the neurons mostly dorsomedial and caudal in the nucleus. There were small projections from the superior,inferior,supravestibular,interstitial vestibular and infracerebellar(y-group)nuclei. Several groups of labelled neurons were located about the genu of the facial nerve. Dorsal to the genu,the supragenu nucleus was heavily labelled. Medial to abducens nucleus and in its dorsal and rostral borders there were large numbers of labelled neurons comparable to the motor neurons. The pontine nuclei represented by far the largest projection. Small numbers of labelled cells were scattered through the medial and ventrolateral reticular formation,particularly the lateral reticular nucleus,and in the lateral cuneate and facial nuclei.

The sources of input to the flocculus are largely nuclei associated with vestibular and oculomotor activity,but their diversity and broad distribution suggest a complex interaction of a multiplicity of vestibular and oculomotor signals. One input that has been largely ignored is the massive projection from the pontine nuclei which may be a dominating influence on flocculus function.

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158.21 SOME AFFERENT CONNECTIONS TO THE OCULOMOTOR COMPLEX IN THE MACAQUE MONKEY. D.J. Magnuson, M. Rezak and L.A. Benevento. Dept. of Anat., Univ. Ill. Med. Ctr., Chicago, IL 60612

Although the oculomotor complex is known to control the musculature of the eye, relatively little is known about the monosynaptic inputs to the various subdivisions of this complex. It was the aim of this study to identify these inputs and thereby assess the relative contributions that these structures make in the control of oculomotor functions.

Discrete injections of horseradish peroxidase (HRP) were made in specific portions of the oculomotor complex through a stereotaxically implanted cannula which was used in order to avoid any uptake by structures other than our desired target. Control injections of HRP were made at points lateral, anterior, posterior, dorsal and ventral to the oculomotor complex.

We found that the oculomotor complex receives input from a large number of structures including the ipsilateral nucleus of the posterior commissure, the trochlear and perihypoglossal nucleus as well as the contralateral pretectal olivary and abducens nucleus. Bilateral projections from the intermediate layers of the superior colliculus and both the superior and medial vestibular nuclei as well as from the midbrain reticular formation were observed.

More specifically, it was found that the non-retinorecipient pretectal nucleus of the posterior commissure has a relatively sparse ipsilateral input to the nucleus of Darkschweitz and the interstitial nucleus of Cajal, while the retinorecipient pretectal olivary nucleus had a strong contralateral projection to the Edinger-Westphal nucleus (EW). Since EW receives a heavy direct foveal input via the pretectal olivary nucleus (Soc. Neurosci. Abs., 5:794, 1979) the present findings provide for a quick route whereby information from the fovea may effect the intrinsic musculature of the eye.

It is also important to note that the intermediate layers of the superior colliculus receive convergent input from visual cortices and have bilateral projections to the rostral portions of EW as well as the anterior median nucleus which function in bulging of the lens and pupil constriction during the accomodative reflex.

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158.22 THE ARBORIZATION AND SYNAPTIC PATTERNS OF PREMOTOR AXONS RELATED TO HORIZONTAL EYE MOVEMENT IN OCULOMOTOR NUCLEUS IDENTIFIED BY HORSERADISH PEROXIDASE. N. Furuya* and C. H. Markham. UCLA School of Medicine, Department of Neurology, Los Angeles, CA 90024.

Two different cell groups are known to send ascending excitatory influences from the horizontal canals to the medial rectus (MR) motoneurons. One group consists of internuclear interneurons in abducens nucleus (AB interneurons). The other is composed of secondary vestibular neurons which pass in the ascending tract of Deiters (ATD) (Baker and Highstein, 1978). It is not clear if this pathway carries canal or otolith information or both. Further, there is disagreement whether the ATD terminates ipsilaterally or possibly contralaterally also.

In the present experiment, cats were anesthetized with 1% procaine and Ketamine, and the stimulating electrodes were implanted in both labyrinths, both abducens nuclei and the individual extraocular nerves in one orbit. A few days prior to the acute experiment, horseradish peroxidase (HRP) was applied to the MR muscle and nerve in one orbit. Premotor axons were recorded intracellularly and were characterized by their responses to horizontal rotation and stimulation of the labyrinths, abducens nuclei and extraocular nerves. HRP was then injected into the axons through the recording electrode. After sacrifice, the midbrain was cut in 100 μm coronal serial sections and treated with diaminobenzidine. Each single axon was traced using camera lucida technique. The terminal arborizations and synaptic connections of HRP-injected premotor axons were observed.

At the time of writing, there are several findings: 1) AB interneurons showed arborization and termination only in the contralateral oculomotor nucleus. 2) Two types of secondary vestibular axons responding to horizontal rotation were identified. One type showed crossing fibers and terminated in both oculomotor nuclei. Another type revealed arborization only in the ipsilateral oculomotor nucleus. 3) Some of the secondary vestibular axons ran clearly lateral to the medial longitudinal fasciculus, presumably in the ATD. 4) Horizontal canal information is carried in the ATD to both oculomotor nuclei. 5) Those fibers which terminated ipsilaterally could be seen to have terminal boutons very close to MR motoneurons.

158.23 ACCESSORY ABDUCENS NUCLEUS INNERVATION OF RABBIT RETRACTOR BULBI MOTORNEURONS LOCALIZED WITH HRP RETROGRADE TRANSPORT. J.F. Disterhoft and M.T. Shipley, Dept. of Anatomy, Northwestern Univ. Med. Sch., Chicago, IL., 60611.

Analysis of the conditioned reflex arc established during tone-signalled nictitating membrane (NM) conditioning in rabbit requires an understanding of the neuroanatomical circuitry involved. NM extension in rabbit is a passive result of retractor bulbi (RB) contraction (Cegavske et al., J. Comp. Physiol. Psychol., 1976, 90, 411). As a first step in delineating this circuitry with neuroanatomical methods, we have localized the brainstem motorneurons innervating RB using HRP pathway tracing.

Thirteen male Dutch rabbits were used. The extraocular mm. were visualized by retracting the eyelids and incising the conjunctiva in the field of an operating microscope. RB injections were made after the scleral insertions of the other extraocular mm. were cut and the eyeball collapsed. 20 mg of HRP dissolved in 40 μl of 2% DMSO in H_2O was injected into all 4 RB slips. The animals were sacrificed after 48 hours. Perfusions and TMB reactions were as described by Mesulam (Neurosci. Short Course, '78).

Acc. VIth motorneurons were heavily labeled after all RB injections. This nucleus is ventral and slightly caudal to the VIth nucleus and just dorsal to lateral superior olive. Axons from Acc. VI coursed dorsal and rostral through the main VIth nuc., to join its axons and form the VIth nerve root. Additional labeled neurons were seen: (1) between Acc. VI and VI nuc. along the trajectory of the Acc. VI axons; (2) in VI nuc.; (3) in the dorsal, intermediate region of the VIIth nuc. shown to innervate orbicularis oculi in rabbit (Van Gehuchten, 1906); (4) in the oculomotor complex; and (5) in the Vth motor nuc. All labeled cells were ipsilateral to the injection except in the oculomotor complex where there was heavy, bilateral labeling.

Our data indicate that the tracing technique used is exquisitely sensitive to HRP that leaks from the injection site. It is possible that labeling in the main VIth nuc. after RB injections may be attributed to this effect. To minimize this possibility we cauterized the lat. rectus prior to injecting RB in 3 experiments. Uptake in VI was drastically reduced with heavy, apparently complete, Acc. VI labeling. These experiments are being continued.

Knowledge of the inputs to and the neurophysiology of the neurons controlling RB should advance our understanding of the cellular basis of NM classical conditioning. Since our data show that most (if not all) of these motorneurons are located in Acc. VI nucleus in rabbit, the analysis of this final common path should be feasible.

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158.24 SINGLE MUSCLE UNITS OF THE CAT MEDIAL RECTUS MUSCLE. M.A. Meredith* and S.J. Goldberg. Dept. of Anatomy, Med. Coll. of Va.-VCU, Richmond, VA 23298

Conjugate eye movements in the horizontal plane require the synchronous activation of the lateral rectus (LR) muscle of one eye and the medial rectus (MR) muscle of the other eye. While the characteristics of LR muscle units have been reported, the present experiments were conducted to examine the mechanical properties of MR muscle units.

Cats were anesthetized and the oculomotor (OC) nerve was stimulated in the brainstem. In the orbit, the MR muscle and the 4 slips of the retractor bulbi (RB) muscle were freed from their insertions and attached to individual sensitive strain gauges. The cortex above the superior colliculus was aspirated. Cells in the OC nucleus were identified by their antidromic response to OC nerve stimulation. Single OC cells were penetrated with a micropipette and those which elicited mechanical responses in the MR muscle when intracellularly activated were identified as MR motoneurons. A variety of stimulus patterns were presented to the MR motoneurons and the subsequent muscle unit responses were recorded.

Although every mechanical parameter of each muscle unit could not always be examined, MR muscle units appear to be faster and somewhat stronger than reported for those of the LR. MR motor units present a wide range of twitch tensions (9-130mg) and maximum tetanic tensions (18-590mg). The average twitch contraction time was 5.7 msec., and the average fusion frequency was 212 pulses/sec with a range of 75-250 pulses/sec. Further investigation is needed to examine the possible presence of functionally different muscle units within the MR muscle as well as to understand the significance of the differences in the responses of LR and MR muscle units.

Although no individual motoneurons were encountered which caused contractions of the RB muscle, stimulation of the whole OC nerve always caused contraction of the RB muscle. Experimental controls including extirpation of all extraocular muscles other than the 4 RB slips, lesions of the brainstem isolating the abducens nucleus and the mechanical response characteristics to single pulse stimulation of the OC nerve all indicate that RB axons present in the OC nerve were responsible for the contraction of the RB muscle. However, the recorded twitch tension of the RB muscle to OC nerve stimulation indicates that the number of RB motoneuron axons in the OC nerve is probably quite small and their functional significance is a matter for future investigations.

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158.25 THE AVIAN ACCESSORY OCULOMTOR NUCLEUS. D. Lyman* and E. Mugnaini. Dept. of Biobehav. Sci., Univ. of Connecticut, Storrs. CT 06268.

Studies were undertaken on adult chickens to define the accessory oculomotor nucleus (AOMN), the avian homologue of the Edinger-Westphal nucleus. Reconstruction of serial sections demonstrate each AOMN is a discrete bean-shaped nucleus with a medioventral hilus of efferent fibers. Located dorsal and lateral to the main oculomotor nucleus, AOMN (1070x770x410μm; average volume 1.48×10^{-1} mm^3; 2370 neurons; 1.6×10^4 neurons/mm^3) is surrounded by a thin shell (~50μm) of fine myelinated fibers in which a few small neurons lie. In our material AOMN is not divisible into distinct medial and lateral compartments as claimed by others. AOMN neurons have round, fusiform or polygonal perikarya measuring 10-23μm in diameter; the nuclei are 6.9-10.6μm. A plot of cell perimeter vs. area suggests at least two populations: large, and medium to small size cells. Nearly all cells exhibit a varyingly prominent ring of Nissl substance adjacent to the nuclear envelope. Most of the large and medium sized cells contain, in addition, uniformly distributed Nissl bodies similar to those of the larger oculomotoneurons. The small and some medium size cells have few large, irregular chromatophilic masses. A small percentage of the large and medium sized cells have only a sparse, granular array of chromatophilic bodies. Throughout the rostrocaudal extent of AOMN the larger cells tend to be located laterally. Retrograde transport of HRP injected into the ciliary ganglion demonstrates AOMN is the sole origin of preganglionic fibers in the III nerve. Large and medium diameter HRP-labeled axons fasciculate in the rostral portion of the III nerve root while large and medium size cells throughout the ipsilateral AOMN demonstrate reaction product. Even after multiple injection of peroxidase, some AOMN neurons, predominantly of small diameter, remain unlabeled. In Golgi sections of adult chickens the shell contains fine varicose axons, apparently continuous with fibers in the adjacent lateral central gray, which arch dorsally and ventrally around AOMN, becomming progessively scarce medially. Many of these fibers enter the nucleus to come into close apposition with dendritic shafts and cell bodies in AOMN. AOMN neurons are multipolar with 4-8 primary dendrites that branch scarcely beyond third order and taper only moderately. Infrequent pleiomorphic spinous evaginations occur on the dendrites; a few somatic spines are present. Most dendritic branches remain in the nuclear domain; however, dendrites are often seen extending across the shell up to 150μm into adjacent regions. Dendrites of neurons in adjacent nuclei do not enter AOMN. Electron microscopic investigations are in progress. Preliminary observations show simple axo-somatic and axo-dendritic contacts without complex synaptic fields such as glomeruli and serial synapses. Supported by NIH grant 09904.

158.26 THE DISTRIBUTION OF HORSERADISH PEROXIDASE-LABELED PROPRIOCEPTIVE NEURONS FROM INDIVIDUAL EXTRAOCULAR MUSCLES IN THE ADULT PIGEON. Avrim R. Eden, Manning J. Correia, and Paul G. Steinkuller*. Departments of Otolaryng., Physiol. & Biophys., and Ophthalmol., University of Texas Medical Branch, Galveston, TX 77550.

Horseradish peroxidase (HRP) was injected in one of the six extraocular muscles in each of six adult pigeons. The experiment was repeated three times for each muscle for a total of 18 animals. The HRP injection (0.3-0.5 microliter; 50% solution) was divided into smaller aliquots deposited along the length of the muscle belly. Although infrequently experienced, any excess HRP was immediately suctioned. Following a 16-20 hour post-injection survival, the brain was fixed by bilateral transcardiac intracarotid catheterization and perfusion. The brains were serially sectioned (40 micron intervals) in either the coronal or sagittal planes, and the HRP reacted by the tetramethylbenzidine (TMB) blue reaction process.

Heavily labeled proprioceptive neurons were consistently clustered in a small segment (0.8 mm x 0.3 mm x 0.2 mm) of the ipsilateral nucleus descendens nervi trigemini (TTD) caudal to the superior olive for all the extraocular muscle injections except the lateral rectus. The confinement of labeled proprioceptive neurons to precisely the same small segment of the TTD for all the five extraocular muscles was such that tissue sections from different muscle injections in different animals could be superimposed without overlap. In the lateral rectus injections approximately one-third of the labeled proprioceptive neurons were located more rostrally in the ipsilateral nucleus motorius nervi trigemini (Mv), the remaining two-thirds located in the same portion of the ipsilateral TTD as the other extraocular muscle proprioceptive neurons. There were no contralateral labeled proprioceptive neurons.

The labeled proprioceptive neurons in the TTD were remarkedly uniform in shape and size. These multipolar neurons had a mean axial measurement of 23 microns (S.D. ± 3, n = 44). Those of the lateral rectus in Mv were somewhat smaller and more varied in shape.

While the absolute number of labeled proprioceptive neurons varied from animal to animal, in each experiment the number of labeled proprioceptive cells relative to the number of labeled motoneurons ranged between 5-13%, e.g. Inferior oblique injection: 35 labeled proprioceptive neurons, 711 labeled motoneurons; medial rectus injection: 273 labeled proprioceptive neurons, 2149 labeled motoneurons.

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159.1 CONNECTIONS OF THE C LAMINAE OF THE DORSAL LATERAL GENICULATE NUCLEUS WITH THE VISUAL CORTEX IN THE CAT. Alan C. Rosenquist and Denis Raczkowski. Dept. Anat., Sch. of Med., Univ. Pennsylvania, Phila., Pa. 19104.

In the course of investigating the thalamocortical connections of the several retinotopically defined visual cortical areas in the cat, we have made the discovery that the ventral C laminae (C1-3) of the dorsal lateral geniculate nucleus (LGNd) project to nearly the entire visual cortex. Our method was to inject a solution of horseradish peroxidase (HRP) and tritiated leucine into cortical areas at retinotopically defined sites, identified by recording through the pipette containing the injection mixture. Two days later, the animal was sacrificed and the tissue processed for HRP histochemistry using o-dianisidine, or prepared for autoradiography. We have injected ten visual cortical areas: 17, 18, 19, 20a, 20b, 21a, ALLS, AMLS, PLLS and PMLS. Except for those injections involving areas ALLS and AMLS, HRP labeled neurons were always present in the parvocellular C laminae. It is likely that layers C1-3 all contribute to the projection since labeled neurons were seen distributed throughout the width of the region intercalated between the magnocellular C lamina and the optic tract. This projection is retinotopically organized. An injection made into area PLLS on the horizontal meridian (HM), 5^{o} removed from the vertical meridian (VM), led to labeling of cells in the medial aspect of the LGNd, the region containing a representation of central vision. In another experiment, an injection was placed in area 20a, at a retinotopic locus adjacent to the HM some 70^{o} removed from the VM. Reflecting the shift in retinotopic locus of the injection, all of the labeled cells were confined to the lateral portion of the parvocellular C laminae, where the representation of the visual periphery is located. Finally, a cortical descending projection which reciprocated the geniculocortical projection was demonstrated autoradiographically.

The fact that the dorsal layers of the LGNd (A, A1 and C) project to cortical visual areas beyond the striate cortex has prompted the suggestion that the geniculocortical system in the cat is unique. Our finding that the ventral layers (C1-3) project to nearly the entire visual cortex also serves to underscore this conclusion. Funded by EY-05342 & 02654

159.2 GOLGI LIKE STAINING OF THALAMIC RELAY NEURONS FOLLOWING INJECTION OF HORSERADISH PEROXIDASE (HRP) INTO CATS VISUAL CORTICAL AREAS. K.Albus and G.Meyer*, MPI Biophys.Chem., 34Göttingen, Fed.Rep.Germ.

In the course of an experimental series of HRP injections into visual cortexwe have found neurons in the thalamus whose somata, dendrites and sometimes also axons were completely filled with diffuse HRP reaction product. This Golgi like staining has provided new informations on the typology of geniculate and extrageniculate relay neurons; it allows also to relate directly morphologically identified neurons in the thalamus with their respective cortical projection areas. The following neuronal types have been found when HRP was injected at the 17/18 border (classification of neurons is according to Guillery, R.W., J.comp.Neur. 128:21-50, 1966). Dorsal lateral geniculate nucleus (LGNd): 1) Large radiate neurons (classI). 2) Medium size neurons with dendritic appendages (classII). 3) A few small cells. The soma size is like that of small classII neurons, or smaller. Mostly at the terminal parts of the dendrites short, spine like appendages or clusters of spines are found. Some cells included in this group have dendritic appendages, but their dendrites are fine, with only few ramifications not following the tufted branching pattern typical for classII cells. The latter type may represent an intermediate form between classII and III, the former probably classIV. LGNd, C-laminae: 1) Large radiate cells (classI), located mainly in C but also more ventrally. 2) Few medium size neurons with dendritic appendages (classII). 3) Small to medium size neurons (classIV). One type of this group has a spindle shaped or triangular soma; 4-6 dendrites of wavy appearance are covered with a variable number of spine like protrusions; cells of this type with extremely flattened fusiform soma are seen more ventrally. Another type has a round or polygonal soma like the small cells in the A-laminae. Medial interlaminar nucleus (MIN): 1) Large to medium size neurons (classI). 2) Few medium size neurons with dendritic appendages (classII). 3) Small neurons with fusiform soma (classIV ?); located mainly in ventral and medial parts of the nucleus. The dendrites of all cell types in MIN typically branch in a bipolar direction following the principal axis of the nucleus. Posterior, and lateroposterior nucleus: 1) Medium size neuronswith a few spine like or club shaped specialisations on beaded dendrites, tufted branching pattern; in comparison with classI andII neurons of LGNd the axons of these cells are finer. 2) Medium size neuron with dendritic appendages (similar to classII neurons in LGNd).

HRP has been injected in a number of cases into either area18 or into area 17. A preliminary analysis of the data shows that with the exception of classII neurons all cells described above project to area 18. ClassII cells become stained when HRP is injected into the callosal zone at the 17/18 border, or into area 17. Further analysis will possibly allow to estimate the proportion between the various neuronal types projecting to the cats visual cortex.

159.3 AN EM-AUTORADIOGRAPHIC ANALYSIS OF THE CORTICOCOLLICULAR PROJECTION IN THE CAT. Mary Behan, Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

The projections of visual cortical areas 17, 18 and 19 to the superior colliculus have been studied using the EM-autoradiographic method. Following injection of ^{3}H-proline into the region of area 17 which contains the representation of the central visual field, labeled terminals were found throughout the stratum zonale and all three sublaminae of the stratum griseum superficiale, but were most numerous in SGS2. This laminar distribution of area 17 terminals in the superior colliculus is similar to that established by light microscopic autoradiography and degeneration studies, i.e., in regions of the colliculus which contain the central representation of the visual field, SGS1 contains a moderate number of area 17 terminals (Updyke, JCN, 173:81, '75).

Our EM-autoradiographic data show that terminals of area 17 neurons are remarkably uniform in size, measuring approximately 1.3μm mean diameter. Like retinal terminals, area 17 terminals contain round vesicles, mitochondria and synapse asymmetrically upon dendrites and dendritic spines. Occasionally dense-core vesicles, coated vesicles or multivesicular bodies are found in either pre- or postsynaptic profiles. Most terminals synapse upon a single profile, but two or more contacts may occur. However, these multiple contacts are formed less frequently by terminals of area 17 neurons than by retinal terminals. In the stratum zonale and SGS1, SGS2 and SGS3 the number of labeled terminals synapsing upon two or more postsynaptic profiles is 9%, 16%, 9% and 8% respectively.

Some of the postsynaptic processes of area 17 terminals contain scattered round vesicles and form the intermediate elements of serial synapses. The percentage of these vesicle-containing postsynaptic profiles varies considerably: 28% in the stratum zonale and 15%, 10% and 21% in SGS1, SGS2 and SGS3 respectively.

Preliminary EM-autoradiographic data indicate that terminals of cortical neurons within areas 18 and 19 are very similar to those of area 17, i.e., they contain round vesicles, mitochondria and synapse asymmetrically upon one or more postsynaptic profiles, some of which contain scattered round vesicles.

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159.4 FUNCTIONAL PROPERTIES OF THE CORTICOTECTAL PROJECTION IN THE RABBIT. L.H. Ostrach, J.W. Crabtree*, and K.L. Chow, Dept. Neurol., Stanford Univ. Sch. Med., Stanford, CA 94305.

Extracellular single unit activity in rabbit superior colliculus (SC) was recorded during electrical stimulation of the visual cortex (VC). Two types of electrodes were used to deliver 0.1 msec square wave pulses of 10-10,000 μA to the VC. Three silver ball electrodes in a triangular array were placed on the dura over the VC. The pair that elicited the largest evoked response in each SC penetration was used to drive single units. Low impedance tungsten-in-glass microelectrodes were driven through the dura 1 mm into the VC after matching cortical and collicular receptive field (RF) locations. Receptive fields for each SC neuron were defined (Masland et al., J. Neurophysiol. 34: 148, 1971) and their locations mapped on a tangent screen. Upon identification of a cortically activated unit, the response threshold was determined and 10 successive responses were photographed to calculate the average response latency. The location of units within laminae of the SC was determined using micrometer readings and histological reconstruction of each penetration.

In the silver ball electrode preparation twelve penetrations yielded recordings from 116 SC units. Thirty three units (28%) were driven by stimulation of the ipsilateral VC. In 11 penetrations in the microelectrode preparation 104 SC units were recorded; 32 (31%) were driven by the ipsilateral VC. Stimulation of the contralateral VC failed to activate SC units. The frequency distribution of RF types that were driven was not significantly different ($\chi^2 = 5.06$; $df = 6$; $p > .50$) between the two kinds of stimulation.

Units of all RF types were driven by VC stimulation. Only the motion (MOT) type of RF cells showed a significant difference between the proportion of normally encountered MOT cells and the proportion of this type that were driven by VC stimulation ($Z = -2.11$; $p < .05$). There was a greater proportion of MOT cells driven than that proportion found in the normal population.

The laminar distribution of VC activated SC cells was: 70.8% in stratum griseum superficiale, 24.6% in stratum opticum, 4.6% in stratum griseum intermediate. Both response thresholds and latencies were extremely variable and were unrelated to depth in SC.

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159.5 REACHING TO THE PERIPHERY ABOLISHES DEFICITS IN PERIPHERAL VISION OF MONKEYS WITH SUPERIOR COLLICULUS LESIONS. D. Kurtz, C. Leiby* and C.M. Butter. New England College of Optometry, Boston, MA and Neuroscience Lab., Univ. of Mich., Ann Arbor, MI 48109.

Kurtz and Butter reported that monkeys with selective lesions of the superior colliculus (SC) are impaired in discriminating between stimuli when they are presented in the periphery of the visual field but not when they are presented centrally. They attributed this impairment to the lesioned monkeys' deficiency in orienting their eyes to the periphery. We evaluated this interpretation by testing monkeys with SC lesions in a discrimination task in which stimuli too brief to fixate were presented peripherally or centrally. We also evaluated the effects on discrimination performance of reaching to the periphery vs. the center to make the choice response.

In this task, a pair of stimuli differing in color was presented for 2 sec. or 100 msec. on a screen facing the monkey immediately after it fixated a panel centrally located on the screen ('fixation panel'). The stimuli were presented at varying degrees of eccentricity (8^{o}, 20^{o}, 32^{o}) from the fixation panel. On half the sessions, the monkey's choice response was made by pressing one of two panels located centrally, 8^{o} from the fixation panel; on alternate sessions the choice response panels were located peripherally, 32^{o} from the fixation panel. Preoperatively, the monkeys' performance was worse the greater the spatial separation between the response sites and the stimuli: their performance with stimuli presented at 32^{o} eccentricity was worse when the response panels were central than when the response panels were peripheral. The reverse was true when the stimuli were presented at 8^{o} eccentricity. After SC lesions, the monkeys were not impaired in discriminating between centrally-presented stimuli. They were impaired in discriminating between peripherally-presented stimuli when they reached to response panels located centrally but not when they reached to response panels located peripherally. The impairment was independent of stimulus duration.

Since the impairment was abolished when the monkeys reached to the periphery to make the choice response, it cannot be due to reduced sensory capacities in peripheral vision. Neither can the impairment be due to a deficiency in fixating the peripheral stimuli, since the impairment was present when the stimuli were too brief to fixate. The impairment may be due to a deficiency in shifting attention from central to peripheral vision, a process that may normally accompany eye movements. This attentional loss may be compensated by an attentional mechanism linked to arm movements; this mechanism presumably involves extra-collicular structures, perhaps posterior parietal cortex.

159.6 VISUAL INPUT TO NUCLEUS RETICULARIS THALAMI AND ITS MODULATION BY MESENCEPHALIC RETICULAR AND CORTICOFUGAL INFLUENCES. F.Schmielau* (SPON:E.Pöppel). Institute for Medical Psychology, Ludwig-Maximilians University of Munich, D-8000 Munich 2 , Federal Republic of Germany.

Extracellular recordings were obtained from neurons of the feline nucleus reticularis thalami (NRT)dorsal of the lateral geniculate nucleus (LGN). The responses of these cells to light stimuli and to electrical stimuli,applied to the optic chiasm (OX),visual cortex (VC), superior colliculus (SC), and to the mesencephalic reticular formation (MRF),were investigated.Reversible inactivation of VC by cooling was used to demonstrate corticofugal influences upon NRT neurons. 45% of the cells responded to light stimulation. Binocular driven cells were preferentially found in a lamina close to LGN, in the perigeniculate nucleus (PGN) of the NRT. Those neurons responded to OX-stimulation mainly with short latencies (0.9-1.5 msec) indicating a monosynaptic retinal innervation.In contrast, more dorsal cells of the NRT responded with much longer latencies (up to 6.0 msec). 92% of the NRT cells were driven by VC-stimulation with a latency between 0.8 and 4.0 msec. As no NRT neuron could be driven antidromically, their axons apparantly do not project to VC. 43% of the cells were activated by stimulation of SC. The typical response pattern to all electrical stimuli was a grouped discharge (Primary excitation), which was followed by a peroid of inhibition (Postexcitatory i.) Stimulation of the MRF caused a pronounced inhibition in 63% of the neurons, throughout the nucleus.However two laminae were found where MRF stimulation evoked activation. Cortical cooling reduced the spontaneous activity in NRT cells, and caused a prolongation of the primary excitation as well as an enhancement of the responses to light stimulation. On the basis of these findings, and derived from recent anatomical data, a model is proposed, showing the NRT as an interface between retina,visual cortex and the mesencephalic reticular formation on one side, and the lateral geniculate nucleus on the other side. Via the NRT,inhibitory influences are mediated to LGN. Thus, various structures may exert a powerful control of the geniculocortical transmission of visual information.

159.7 RELATIONSHIP OF THE SUBDIVISIONS OF THE PULVINAR COMPLEX TO THE VARIOUS VISUAL AREAS WITHIN THE OCCIPITAL LOBE OF MACAQUE MONKEYS. G. P. Standage and L. A. Benevento. Dept. of Anatomy, Univ. of Illinois, Col. of Medicine, Chicago, IL 60612.

Our past neuroanatomical studies have described the projections of the inferior pulvinar (PI) and lateral pulvinar (PL) to the occipital cortex (areas 17, 18 and 19 of Brodmann) in the macaque monkey (e.g., Brain Res. 108, 1976; 167, 1979). Recent physiological and anatomical studies of visual topography in old world monkeys have demonstrated that cortical areas 18 and 19 contain a number of visual representations (e.g., Zeki, Nature 274, 1978). It was of interest then to refine our understanding of pulvino-cortical projections by relating their organization to these multiple visual areas within occipital cortex. Combined electrophysiological, histological, horseradish peroxidase (HRP) and autoradiographic techniques were used. Injections of HRP and tritiated amino acids were effectively restricted to 1 mm^3 or less of the cellular layers of cortical tissue. Standard extracellular recording techniques were employed as an aid for identification of the regions (as defined by Zeki) to be injected with the tracers.

It was found that PI projects preferentially to primary visual cortex (V1) and the cortex of the lunate and superior temporal sulci. Areas receiving preferential input from PI were also found to be the primary projection targets of V1, i.e., V2 and the area within the superior temporal sulcus (STS of Zeki, analogus to MT in new world monkeys, Ungerleider, 1979). Projections from the portion of PL which is adjacent to PI (PLα) parallel those of PI, thus providing in addition to the tectal input via PI, cortico-thalamocortical interconnections for these cortical regions. The remainder of PL projects to V4 and inferotemporal cortex, while the immediately adjacent portions of the medial and lateral pulvinar nuclei project to area PGa of Seltzer and Pandya (1978). With the exception of MT, prestriate areas injected with HRP show that their strongest inputs are from adjacent cortical areas. Although pulvinar projections overlap, it may be that specialized visual cortical subregions receive unique patterns of input from specific subnuclei of the pulvinar complex each of which has its own particular input from the tectum, pretectum and various cortical regions. (Supported by NIH Grant EY 2940)

159.8 VISUOTOPIC ORGANIZATION OF STRIATE PROJECTIONS TO INFERIOR AND LATERAL PULVINAR IN RHESUS MONKEY. Leslie G. Ungerleider, Thelma W. Galkin* and Mortimer Mishkin. Lab. Sensorimotor Research, NEI and Lab. Neuropsychology, NIMH, Bethesda, MD 20205.

Anatomical material from two series of monkeys (Macaca mulatta) was used to determine the full extent and visuotopic organization of striate projections to the pulvinar. One series had been processed for degeneration by the Fink-Heimer procedure following unilateral lesions of lateral, posterior, or medial striate cortex, areas representing the central, peripheral, and far peripheral hemifield, respectively; collectively, the lesions included all of area 17. The second series had been processed for autoradiography following tritiated amino-acid injections into striate sites representing the center of gaze and eccentricities ranging from 0.5^{o} to 45^{o} in either the upper or lower hemifield.

The results were as follows. (1) Within the rostral third of the inferior pulvinar (PI), striate projections representing a progression from central to far peripheral vision terminate in a progression from dorsolateral to ventromedial PI, with central vision represented at the dorsolateral margin of the nucleus, adjacent to the caudal tip of the dorsal lateral geniculate nucleus. The vertical meridian is represented at the lateral and dorsal borders of PI, while the horizontal meridian runs obliquely across the nucleus in a dorsolateral to ventromedial direction, with the lower hemifield represented dorsomedially and the upper hemifield, ventrolaterally. Missing from the rostral third of PI, however, are striate projections representing the fovea itself. (2) These latter projections add a rostral-caudal dimension to the topography. Thus, whereas eccentricities greater than 5^{o} are represented entirely within the rostral third of PI, those of 5^{o} to about 2^{o} extend into the middle third as well, and those of less than 2^{o} occupy the middle and caudal third, with the center of gaze located at the caudal pole of the nucleus. (3) At the level of the caudal two-thirds of PI, where striate projections representing eccentricities of 5^{o} or less terminate, the lateral pulvinar (PL) also receives a striate projection. This second projection originates only in the parts of striate cortex representing central vision, and appears to be a mirror image of the projection to adjacent PI, with the representation of the vertical meridian forming the common border. (4) Whereas the striate projection zone occupies the entire rostral half of PI, it occupies only a dorsal segment in the caudal half of this nucleus, and only a limited, adjacent segment in PL.

These results, coupled with the known visuotopic arrangement of striate and pulvinar projections to the prestriate cortical area OB, indicate the existence of two sources of striate input to area OB that are in perfect register: one, direct, i.e., corticocortical; and the other, indirect, via PI and PL.

159.9 CROSSED CORTICOFUGAL PROJECTIONS IN THE CAT. N. Berman and B. R. Payne. Depts. Anatomy and Physiology, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Multiple injections of a mixture of tritiated leucine and proline were made into the lateral, postlateral, suprasylvian and ectosylvian gyri of 3 adult cats and the brains were processed for autoradiography. Survival times were 2 to 7 days; exposure times were 10 to 14 weeks. Transported label was found in many ipsilateral and in several contralateral subcortical regions. These contralateral regions included the claustrum, caudate-putamen, thalamic intralaminar nuclei, pretectum, superior and inferior colliculi.

In the claustrum, label was found in the dorsal part of the caudal claustrum but did not extend to its most dorsal margin. Label in the putamen was found in its most dorsolateral part just medial to the external capsule. In the head of the caudate, the label formed several dorsomedially to ventrolaterally oriented strips. Label in the dorsal part of the body of the caudate was found as far caudal as the level of the rostral pole of the lateral geniculate nucleus. These labeled axons from the cortex to the contralateral claustrum, caudate and putamen cross in the corpus callosum. In the thalamus light label was present throughout the contralateral central medial nucleus, the most medial nucleus of the intralaminar group. The axons cross the midline within the nucleus. The pretectal region contralateral to the injected side contained three labeled zones: a dorsoventrally oriented strip in the medial part of the anterior pretectal nucleus, a patch within the posterior pretectal nucleus and a band just beneath the pia within the nucleus of the optic tract. The axons from the cortex to the contralateral pretectal region cross in the posterior commissure. In the contralateral superior colliculus label was present at the lateral margin of the stratum griseum superficiale and in the lateral brachium throughout its rostrocaudal extent. Label was also present just beneath the pia along the entire mediolateral extent of the superior colliculus at its rostral and caudal borders. The projection from the cortex to the contralateral superior colliculus crosses in the tectal commissure. This finding confirms a similar report by Powell (1976). Label was present in the inferior colliculus contralateral to the injected side in the dorsomedial division of the central nucleus, in the pericentral nucleus and in the dorsal part of the external nucleus. The labeled axons cross in the commissure of the inferior colliculus. A similar projection has been described by Rockel and Jones (1973).

Thus, the crossed corticofugal projections in the cat are more extensive than has been previously reported.

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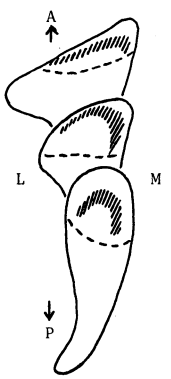
159.10

Withdrawn by Author

159.11 VISUAL AREA IN CAT CLAUSTRUM. 1: INPUTS AND TOPOGRAPHY. H. Sherk* and S. LeVay (SPON: U. Dräger). Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115.

The visual regions of the cat's cortex, including areas 17,18, 19, and the Clare-Bishop areas, send inputs to the posterior part of the ipsilateral claustrum. Do they form a map of the visual field there? To answer this question, we made small injections of ^{3}H-proline at physiologically defined sites in these areas and studied the claustral projection zones autoradiographically. In addition we did extensive physiological mapping of receptive fields in 3 cats, and made more limited observations in another 9 animals.

Both anatomical and physiological methods demonstrated a single, orderly map of the contralateral visual hemifield, occupying the posterior 3 mm of the claustrum. The lower visual field lies rostrally, the upper field caudally. The far periphery is represented at the dorsal surface and the vertical meridian (VM) more ventrally. Rostrally the visual map forms a thin slab occupying only the dorsal claustrum. The cells below it are visually unresponsive: their input is from the splenial gyrus. A series of vertical electrode penetrations made across the mediolateral extent of the claustrum gives a series of very similar receptive field progressions, moving rapidly from the far periphery to the VM at the same elevation. Thus one visual field point maps onto a line that traverses the width of the claustrum. This is clearest rostrally where the claustrum is broad and flat-topped. Caudally the lines representing visual field points wrap around a central core representing the VM. The 3 coronal sections at left, taken at different rostrocaudal levels, show labeling from ^{3}H-proline injections in area 17 at 3 different visual field elevations, all about 15° from the VM. The dashed lines show the approximate boundaries of the visual region.



Claustral injections of HRP retrogradely labeled a tier of pyramidal cells in the middle of layer VI of the visual cortical areas, and smaller numbers of cells at the III/IV laminar border. In addition, labeled cells were found in the n. centralis thalami, the medial geniculate n., and the lateral posterior n. The latter two nuclei, however, were shown by ^{3}H-proline autoradiography to project not to the claustrum but to adjacent cortex. The visual input to the claustrum appears therefore to derive solely from the cortex.

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159.12 VISUAL AREA IN CAT CLAUSTRUM. 2: STRUCTURE, RECEPTIVE FIELDS AND OUTPUTS. S. LeVay and H. Sherk* Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

The internal structure of the claustral visual area was studied by the Golgi method and electron microscopy. The principal cell type is a large spiny-dendrite cell whose axon leaves the claustrum after issuing collaterals. A second type is smaller, and has beaded spinefree dendrites and a local axon. After lesions of visual cortex, degenerating terminals in the claustrum were all of Gray's type 1; 80% of them synapsed on spines, 20% on beaded dendrites. Thus both cell types receive a direct cortical input. No complex or dendrodendritic synapses were seen. In general the structure of the visual claustrum resembles that of cortex, aside from the absence of layers.

The receptive field properties of 231 single units were examined using tungsten electrodes in 12 paralyzed, anesthetized cats. 30 cells were studied quantitatively, the rest with hand-projected stimuli. Most cells responded vigorously to a long slit or dark bar, appropriately oriented and moved slowly across their receptive fields. 209 of 231 cells were orientation-selective, with tuning curve half-widths at half height as narrow as 17°. Sequences of similar or systematically shifting preferred orientations were often encountered on vertical penetrations. Very few cells showed strong directional preferences. All cells except those with fields in the far periphery were binocular, the majority being equally influenced by the two eyes. Velocity tuning was broad. The most striking property of claustral units was their summation with increasing slit length: most cells responded poorly to 2.5° or 5° slits and increasingly well as the slit was increased to 40°, yet stimulation of the central few degrees was required for a response. Stationary slits elicited poor or no responses. Spontaneous activity was very low (0-2 spikes/sec). No auditory or somatosensory responses were obtained within the visual claustral region. The response properties of claustral cells might be explained by a convergence of inputs from a number of layer VI cortical cells (Gilbert, '77) with a common orientation preference but varying ocular dominance and directionality.

Claustral efferents were studied using ^{3}H-proline and HRP transport. There is a retinotopic projection to all ipsilateral visual cortical areas, and to at least one cortical representation of the vertical meridian (the 17-18 border) on the contralateral side. The projection to area 17 ends diffusely in all layers, but most lightly in layer V. No subcortical projections were seen. Since the majority of cells in area 17 have smaller receptive fields and tend to be more monocular and direction-selective than claustral cells, it is unlikely that the claustrum provides a major excitatory input, at least to this area.

(Supported by NIH EYRO-1960 and 1 F32 EY 05296)

159.13 CORTICO-CORTICAL AND THALAMIC CONNECTIONS OF STRIATE AND EXTRASTRIATE VISUAL REGIONS IN THE CORTEX OF THE MOUSE. Vance Lemmon and Alan L. Pearlman. Dept. of Physiology and Biophysics, and Dept. of Neurology, Washington University School of Medicine, St. Louis, MO 63110.

The neocortex of the occipital region of the mouse, like that of other mammals, contains several anatomically and physiologically distinct visual regions. We have analyzed the thalamic and cortico-cortical connections of the striate (Area 17) and extrastriate (Areas 18a and 18b) visual regions in the mouse to provide a framework for our studies of the reeler mutant mouse. Horseradish peroxidase (0.01µl) was pressure-injected through a micro-pipette into the superficial aspect of these areas; after 20-24 hours the animals were fixed and the brains sectioned and processed with the DAB-cobalt chloride procedure.

Area 17 receives thalamic afferents from the dorsal lateral geniculate nucleus (LGd). Areas 18a and 18b do not receive afferents from the LGd; the thalamic input to 18a is provided by n. lateralis posterior and to 18b by n. lateralis. Areas 17 and 18a are reciprocally connected, as are areas 18a and 18b. Area 18b projects to area 17, but we have not found definite evidence for a projection from 17 to 18b. In each area the cells of layers II, III and V provide these association connections. In addition, area 29 in the posterior-medial aspect of the hemisphere projects to both 17 and 18a.

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159.14 SUBCORTICAL PROJECTIONS OF THE INFERIOR PARIETAL LOBULE (AREA 7) OF THE STUMPTAIL MACAQUE MONKEY. Joseph T. Weber and Tom C.T. Yin. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, Wis. 53706.

The anterograde transport of ^{3}H-proline and ^{3}H-leucine was used to investigate the subcortical projections of the inferior parietal lobule in the stumptail monkey. Following placement of large multiple injections (.4 µl total, at 50 µCi/µl) of a combination of the two amino acids within area 7, transported label was observed over a number of nuclei within the basal ganglia, thalamus and brainstem.

With regards to the basal ganglia, transported label lies ipsilaterally over the putamen, claustrum and caudate nuclei. The label over the putamen and the claustrum is found over their mediolateral extent, but tends to stay within the more ventral aspects of these nuclei. Label over the caudate nucleus is observed within the head and body regions.

Within the ipsilateral thalamus, dense aggregates of silver grains are seen to overlie the oral, lateral and medial divisions of the pulvinar nucleus and over the lateral posterior nucleus. The transported label over these above nuclei is usually "patchy" in appearance. These regions have been shown by others to be the recipient zones of ascending projections from the superior colliculus and pretectal nuclei. Less dense patches of label are evident over the supragenicular nucleus and single loci of silver grains are seen at the ventral border of the ventral posterior medial nucleus and over the ventral lateral nucleus (pars postrema). Sparse label also overlies the region at the border of the central lateral and medial dorsal nuclei and over the centromedian and reticular nuclei of the thalamus.

The densest concentration of transported label within the brainstem is located ipsilaterally over the dorso-lateral and dorso-medial pontine grey. This label is segregated into small dense islands and overlies the regions between the fascicles of the corticospinal tract. Sparse label is also found over the superior colliculus at the border of the pretectal nuclei and is restricted to lamina IV.

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159.15 BINOCULAR DISTANCE ESTIMATION IN THE FLY, CALLIPHORA. Hendrik E. Eckert and Kurt Hamdorf. Dept. Animal Physiol., University of Bochum, P.O. Box 102148, D-463 Bochum, FRG.

Stereoscopic distance estimation in flies was investigated by employing the landing response (LR) which can be elicited by approaching objects. The LR consists of a series of leg movements of which the most conspicuous is the sudden upward throw of both foreleg tibiae. The foreleg response to 20 stimulus presentations was measured and a standard response was defined in which 50% of the stimuli elicited a response (P_{50}).

Two vertical stripes, 1 cm to 6 cm apart were moved from a starting position of 20 cm away in 1 cm steps towards the animal; a P_{50}-response was elicited at a distance depending on the angular separation of the stripes: if they were 3 cm or more apart, a P_{50}-response was elicited at a distance corresponding to 30 deg angular stripe separation. However, for stripe separations 2 cm or less apart, this angle changes to 14 deg. In these experiments, the starting position of stripes, e.g., 2 cm and 4 cm apart, was the same and thus, the change in angular separation between starting and P_{50}-position is different. Therefore, the starting position of the stripes was changed so as to keep the increase in angular separation of the stripes constant for a 2 cm and a 4 cm pattern. Under these experimental conditions in which only 30% of stimulus presentations with the stripes 4 cm apart elicit a reaction, a 100% response is elicited with a pattern of stripes 2 cm apart. This indicates that flies are capable of distance estimation for distances of up to 65 mm away from the animal. This distance agrees well with theoretical calculations (Eckert and Hamdorf, J. Comp. Physiol., in press).

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159.16 FREQUENCY DOMAIN ANALYSIS OF HUMAN STEREOPSIS. A. Norcia, Stanford University, Stanford, CA 94305 and C.W. Tyler*, Smith-Kettlewell Institute of Visual Sciences, San Francisco, CA 94115.

The activity of cortical neurons selectively tuned for retinal disparity was studied under binocularly correlated noise modulation. The amplitude and phase of the steady-state evoked response was measured as a function of the amplitude and frequency of the input disparity modulation. The display consisted of a 14x20 deg noise plane that was square-wave modulated between a range of small crossed and uncrossed disparities.

The display was generated using feed-back shift register noise passed through push-pull digital delay lines input to the red and blue color guns of a modified broadcast TV.

Responses were recorded on the mid-line using a bipolar derivation of 3 and 6 cm nasalward from the inion referenced to the earlobe. The 2nd harmonic (2f) response was synchronously demodulated using a digital sin/cos-phase switch. The resulting filter characteristic is a sinc-function with a nominal band width of .08 hz at 3 db down. Filter output was integrated over 10 sec periods with and without temporal modulation. Sessions were run at either a constant disparity or a constant temporal frequency.

At $\pm$15 arcmin disparity, the amplitude spectrum obtained from 2 subjects consists of a multiple peaked function with an upper limit of 12-13 hz. This range can be extended by decreasing the disparity, suggesting that stereopsis is velocity limited.

The highest amplitude peak occurs near 3 hz in a region where the subject sees a plane moving in depth. The other peaks occur in a region where the subject sees depth but no coherent movement in depth. This suggests that neurons responsible for local disparity detection may be represented in the response to high frequencies, whereas neurons responsible for global stereopsis may be represented in the response to frequencies below 5 Hz.

In the low frequency region, the response amplitude is linearly proportional to log disparity amplitude for values less than $\pm$30 arcmin. Psychophysical threshold can be predicted from an extrapolation to zero volts.

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159.17 PATTERN PROCESSING AND SLOW WAVES IN VISUAL CORTEX. J.D. Glass and R.W. Hall*, Department of Pharmacology, Sch. of Med., University of Pittsburgh, Pgh., PA 15261.

Within the past two decades, vision research has seen enormous strides in our understanding of the cortical mechanisms for encoding the physical features of a visual stimulus. Work on the adult visual system has led to further studies providing new insights into the postnatal development of the encoding process. These studies are of great potential value to our understanding of developmental visual disorders in humans. Since the animal work has been performed with single-unit recording while investigations of the human visual system have been performed with slow-wave recordings, extrapolation from animals to humans must be approached with caution. We have therefore initiated a series of experiments to determine how pattern processing mechanisms of the visual cortex in animals are reflected in the slow-wave response. With such knowledge one would be able to make more direct comparisons between electrophysiological studies in animals and humans.

In four cats, bipolar nichrome wire electrodes were implanted into areas 17, 18 and 19 (visual cortices) and 4 (motor cortex). Recordings were taken with the cat alert and restrained, with its head in a fixed position. Stimuli were presented at approximately 0.3 Hz. Three stimuli, equated for luminance, were used, diffuse and two checkerboard patterns with the checks subtending 1.2° and 3.5° of visual angle. The stimulus panel subtended 42° of visual angle. Three intensities, 17.6, 11.7, and 7.8 ft. lamberts were studied. The contrast ratio between the white and black checks was 124:1. Twenty-five trials were summed with a CAT averager. The responses were separately analyzed for amplitude and variance with a general purpose computer.

Repeated recordings over several months showed the response to be extremely reliable. The response to the diffuse stimulus was most different from the response evoked by the 1.2° checks. Differences between the diffuse stimulus and the 3.5° checks were present, but were not as large as for the 1.2° checks. The differences in these waveforms occurred primarily between 55 to 200 msec following the stimulus. The evoked potential components occurring prior to 55 msec were identical, regardless of the stimulus. Minor differences could be seen in the "late waves"; but these were not as substantial as those occurring prior to 200 msec. Changing the intensity of the stimulus altered the amplitude of the response. However, intensity changes altered the pattern-dependent differences in the waveform only for the highest intensity evaluated. The responses recorded from motor cortex were similar across stimuli.

159.18 HOMOLOGY OF MONKEY AND HUMAN VEP. K. Nakayama, M. Mackeben and E. Sutter. The Smith-Kettlewell Institute of Visual Sciences, San Francisco, CA 94115.

The primary visual cortex in the human is a multifolded surface, comprising the calcarine fissure and the mesial walls of the occipital lobes. This is bordered by multiple secondary projection fields extending outward to the surface of the occipital lobe. Because of this complexity and the lack of anatomic detail on specific VEP subjects, a consensus as to the origins of human VEP components is lacking. In the monkey striate cortex, the central 10-15° of the visual field are represented on the unconvoluted outer surface of the occipital lobe. Secondary projection fields extend forward and are in part buried in the lunate and superior temporal sulci.

As an alternative approach in the investigation of human VEP origins, we are examining the pattern VEP of the alert monkey (Macaca fascicularis), using sinusoidal gratings. Despite the complete lack of correspondence regarding the anatomical lay-out of monkey and human projections, the steady-state VEP in both species shows striking similarities in the following aspects: (1) extremely narrow spatial and temporal frequency tuning, especially at higher contrasts (shown in humans by Tyler, C.W., Apkarian, P., and Nakayama, K., Exp. Brain Res. 33:535, 1978), (2) a match between VEP thresholds and psychophysics as in humans (Campbell, F.W. and Maffei, L., J. Physiol. 207:635, 1970), (3) a predominance of 1st harmonic over second harmonic responses for appearance/disappearance sequences and its dependence on spatial frequency (Bodis-Wollner, J. and Hendley, C.D., Soc. for Neurosci. Abstract Vol. 4, #1971, St. Louis, 1978).

Because of the large differences in gross cortical anatomy, we suggest that these VEP similarities represent functional properties of homologous cortical areas. Thus, further physiological investigation of the origins of the monkey VEP could greatly aid in determining the origin of human VEP components.

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159.19 TRIETHYLTIN: VISUAL SYSTEM TOXICITY IS AMBIENT-TEMPERATURE DEPENDENT R.S. Dyer and W.E. Howell. Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711

Carbon monoxide (CO) hypoxia produces toxicity which is ambient-temperature dependent, and is probably related to the influence of ambient temperature on core temperature in hypoxic rats (Annau & Dyer, Fed Proc., 36: 1624, 1977). CO at an ambient temperature of 20°C produces a dose-related decrease in core temperature. At an ambient temperature of 30°C the same exposure level of CO is more toxic but does not reduce core temperature. Acute exposure to triethyltin (TET) is known to produce edema and vacuolization of myelin. In addition, TET is a metabolic poison, inhibiting oxidative phosphorylation and coupled respiration in mitochondria, thereby producing a cytotoxic anoxia. In the present investigation, TET also produced a dose-related decrease in core temperature which, following a single injection of 9 mg/ kg TET bromide in saline, reached a level of 33.3°C (ambient temp 20°C). Animals given 9 mg/kg TET and maintained at ambient temperatures of 30°C only decreased their core temperatures by 1.5°C compared to control. To investigate whether these toxicant by temperature interactions have consequences for CNS function, 40 male Long-Evans hooded rats were surgically implanted with screw electrodes for recording flash evoked cortical potentials and were divided into 4 groups of 10. Each group was exposed to either 0 or 6 mg/kg TET and maintained for 7 hrs at either 20° or 30°C. After 7 hrs all animals were maintained at 20°C for the next 5 days. Flash evoked potentials were recorded immediately before exposure, and at 1.5, 4, 7, 24, 120 and 324 hrs post exposure. There was a significant increase in N1 latency of the cortically recorded response at all points up to 7 hrs for both warm (maximum increase mean ± SEM, = 12.5% ± 3%) and cold (maximum increase = 15 ± 3%) exposure conditions. At 24 hr the animals exposed at 20°C had returned to baseline, but the animals exposed at 30°C retained elevated N1 latencies at 24, 120 and 324 hrs post exposure. P3 latencies showed a similar pattern, but the warm exposure group returned to baseline at 324 hrs. It is hypothesized that metabolic demand placed upon the CNS by maintaining normal core temperature is responsible for the apparently permanent increase in N1 latency in the animals exposed to TET and maintained in a warm environment for 7 hrs.

160.1 THE SURVIVAL AND DEGENERATION OF MONKEY RETINA AND ITS PATHWAYS AFTER STRIATE CORTEX REMOVAL. J. Dineen*, A. Hendrickson, and E.G. Keating. Dept. Ophthalmology, Univ. Washington, Seattle WA 98195 and Dept. Anatomy, SUNY Upstate Med.Ctr., Syracuse NY 13210.

The morphology of the retina and its projections was studied in two adult Macaca fascicularis monkeys that had received a complete bilateral removal of striate cortex at 5+ years of age. These animals had subsequently undergone extensive behavioral testing (Dineen and Keating 1979) for 2 years. One day before sacrifice, the retinal projections of the right eye were labeled by an intravitreal injection of 500uci tritiated proline, and then the brain was processed for autoradiography. The retina of the left eye was flat mounted and the right eye was serially sectioned in the horizontal plane.

Light microscopy of the retina and dorsal lateral geniculate nucleus (dLGN) revealed that the dLGN had undergone complete retrograde neuronal degeneration, indicating that the striate cortex lesions were complete. There was no evidence of lamination remaining in the dLGN. Although sections of the retina looked quite normal, counts of retinal ganglion cells in the whole mounts showed a loss of 30% of the ganglion cells near the fovea and 10% of the peripheral ganglion cells. Dark field microscopy of the autoradiographs from degenerated dLGN demonstrated a surprisingly normal distribution of retinal input which was sharply laminated into S, magnocellular and parvocellular laminae; however, the parvocellular layers did have fewer grains than the magnocellular and S layers, and showed marked disruption at some levels.

Dark-field examination of extra-geniculate pathways labeled by autoradiography revealed other alterations in these retinal pathways: 1. the ventral geniculate nucleus showed exaggerated and enlarged terminal fields; 2. the olivary, pretectal and posterior pretectal nuclei all showed a marked reduction in retinal input; and 3. the contralateral superior colliculus projection was thinner and had lost its normally patchy configuration. The accessory optic and suprachiasmatic nuclei appeared normal.

These results suggest that: 1. the major period of retrograde transneuronal retinal ganglion cell degeneration occurs later than 2 years after striate cortex removal in adulthood; 2. the marked loss of parvocellular retinal input which occurs after long survival in monkeys lesioned as infants (Weller, Kaas and Wetzel 1978) was not found in our animals, but some changes which could be interpreted as the onset of this process were observed; and 3. retinal ganglion cell terminations in nuclei other than dLGN are also affected by striate cortex removal. This occurs in nuclei which receive direct striate input like superior colliculus as well as in those like pretectum which do not. (Supported by EY-01208, EY-01730, EY-07013 and EY-02941)

160.2 QUANTITATIVE ANALYSIS OF RETROGRADE TRANS-SYNAPTIC DEGENERATION IN THE CAT'S RETINA. D. Labar[1], H. Pearson[1], B. Payne[2], P. Cornwell[3]* and N. Aggarwal.[3]* Depts. of Anat.[1] and Physiol./Biochem.[2], The Medical College of Penna., Philadelphia, PA 19129, and Dept. of Psych.[3], Penna. State Univ., University Park, PA 16802.

The retrograde transneuronal effects on the ganglion cells of the cat's retina caused by neonatal visual cortex ablation were studied with Nissl-stained whole-mounted retinae. From retinae of three cats with bilateral lesions of areas 17, 18 and 19 and three normal cats, 4,380 cells were drawn and their mean diameters measured in samples from the central retina and from 2mm dorsal to area centralis. In the peripheral retina of normal cats, ganglion cell size was distributed tri-modally (α (Y) mode, centered at 34-36 μm diam.; β (X) mode, 18-20 μm diam.; γ (W) mode, 10-12 μm diam.). In cats with neonatal cortical lesions, ganglion cell size in the periphery was distributed bi-modally, with a "large" cell mode, similar to α (Y) mode in normals, and a "small" cell mode, similar γ (W) mode. The small cell peak in lesioned cats, however, was broader and centered on a larger diameter (12-14 μm) than the γ mode in normal cats. In central retina, the unimodal ganglion cell size distributions had slightly smaller cells in the lesioned cats ($\bar{x}$ diam. ± S.D.: normals: 10.87 ± .66 μm; lesioned: 10.05 ± .65 μm). Ganglion cell density was calculated to see if absence of the β (X) mode in lesioned cats was reflected in a decreased overall density of ganglion cells. In peripheral retina, density was 32% lower in lesioned than in normal cats ($\bar{x}$ density ± S.D.: normals: 1129 ± 225/mm^2; lesioned: 758 ± 134/mm^2), and in central retina was 16% lower in lesioned cats ($\bar{x}$ density ± S.D.: normals: 7448 ± 115/mm; lesioned: 6261 ± 621/mm^2).

Severe retrograde transneuronal degeneration, reported to produce loss of up to 75% of ganglion cells and a complete loss of "X" cells, occurs in the monkey retina after neonatal visual cortex lesions (Cowey, Percep. 3:257; Weller et al., Brain Res. 160:134). We conclude that although the cat is like the monkey in that medium sized ganglion cells are most susceptible to retrograde transneuronal degeneration, cell loss is far from complete in the cat, and a sizable population of ganglion cells remains after neonatal visual cortex ablation. Our results also indicate that retrograde transneuronal degeneration is more severe in peripheral retina than in central retina.

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160.3 PRENATAL DEVELOPMENT OF THE RETINOGENICULATE PATHWAY IN THE CAT. C.J. Shatz and A.C. DiBerardino. Dept. Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

The prenatal development of connections between retina and lateral geniculate nucleus (LGN) was studied by means of orthograde axonal transport of ^{3}H-proline or HRP injected intraocularly in 23 fetuses of known gestational age (Gestation is 65 days.) By embryonic day 32 (E32), the youngest age at which unilateral eye injections have been successful, a clear bilateral projection to the thalamus is present: the optic tracts are densely labeled and label is seen within the anlage of the LGN. By E39 the LGN has increased in size and substantial label is found throughout both ipsilateral and contralateral LGNs. Little difference is seen in the pattern of labeling on the two sides and there is no indication that input is segregated into right- and left-eye territories. Segregation is found, however, by E46, and is more evident on the ipsilateral side. Inspection of cresyl violet (CV) stained sections indicates a complete absence of cellular lamination at this age. The segregation of eye input was verified in another E46 fetus by injecting one eye with ^{3}H-proline and the other with HRP. Within each LGN regions containing exclusively HRP or silver grains were found, indicating the onset of segregation. Nevertheless, overlap of eye input as indicated by dually labeled regions is still extensive.

Segregation, as determined by double-label experiments, is much further advanced by E54 and the first suggestion of cellular lamination can be seen in CV stained frontal sections cut through the LGN. However, the pattern seen in this plane of section is far from adult-like and the characteristic A- and C-laminae could not be identified. To aid in identification embryos were injected with ^{3}H-thymidine on E30, when it is known that neurons primarily comprising the C-laminae are generated (Hickey,1979). In autoradiographs of an embryo killed at E54, thymidine labeled cells were found largely within posterior and lateral LGN regions, suggesting that at this age the C-layers lie posterolateral to the A-layers, and that the nucleus might appear more familiar if viewed in horizontal section. This was confirmed in another double-label experiment at E54 in which the LGN was cut horizontally. The pattern of segregation is remarkably adult-like with C-layer input clearly positioned posterolateral to that to the A-layers. Even viewed horizontally however, almost no cellular lamination could be seen in CV stained sections (see also Kalil, 1978). Thus, the onset of segregation precedes that of cellular lamination by at least 10 days. Further, as in the monkey (Rakic, 1977), in the cat the characteristic laminar segregation of eye input present in the adult is only achieved secondarily, due to the refinement of an initially diffuse set of connections.

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160.4 POSTNATAL DEVELOPMENT OF CORTICOGENICULATE AND CORTICOTECTAL CONNECTIONS IN PIGMENTED RABBITS. A. S. Kelly* and D. A. Schwartz (SPON: A. Gordon). Dept. Physiol., Univ. Calif. Sch. Med., San Francisco, CA 94143.

We have studied the normal postnatal development of connections from striate cortex to two subcortical centers, the lateral geniculate nucleus (LGN) and the superior colliculus (SC), in dutch belted rabbit pups. In order to visualize the rather sparse initial innervation of these nuclei we have used two anterograde transport methods. Horseradish peroxidase (HRP) (Sigma Type VI, 20-40% in saline) or a mixture of HRP and tritiated amino acids ((L 3,4(n)) proline, 20-40 uCi and (L 4,5) leucine, 10-20 uCi) was injected into the striate cortex at 4 - 8 separate sites. Total injected volume was 0.16-0.3 ul. Following survival times of 22-28 hrs, pups were sacrificed and alternate frozen sections were reacted by the Mesulam method using tetramethyl benzidine for demonstration of the HRP (J. Histochem. Cytochem. 26, 106, 1978), and/or were processed for autoradiography. The results indicate that by 36 postnatal hrs corticofugal fibers have penetrated parts of both the LGN and SC. Relatively dense label was found in the anterolateral portion of the LGN. Label was much sparser or absent in more medial and posterior portions however. Label was rather diffusely spread throughout the anterior part of the SC. More caudally it was restricted to the middle of the SC, being sparse or absent medially and laterally. Some indication of segregation of the label to stratum opticum was seen at this time in the middle of the SC. This became more pronounced in the next few days, and innervation also spread to the caudal borders of the LGN and SC. By the time of eye opening (postnatal day 9-10) both nuclei were completely innervated and label in the SC was primarily concentrated in stratum opticum.

Mustari and Lund (Brain Res. 112, 37, 1976) demonstrated that in the rat, unilateral visual cortical lesions up to postnatal day 15 resulted in expansion of the remaining contralateral corticotectal pathway to innervate the entire stratum opticum of both superior colliculi. We have attempted to demonstrate this sort of plasticity in the rabbit. Pups underwent unilateral ablation of visual cortex 12-36 hrs after birth. Six weeks or three months later the remaining visual cortex was injected as above with a cocktail of 40% HRP, 40 uCi proline and 20 uCi leucine in 0.3 ul saline. Animals survived 24-28 hrs, and again alternate sections were processed for autoradiography or reacted for HRP. In 6 animals studied to date, no evidence of a crossed corticotectal pathway has been found. Although it is possible that earlier or prenatal lesions might result in the formation of such a crossed pathway, we feel it is noteworthy that no crossed connections are seen with ablations done at a time when the corticotectal pathway is known not to be complete.

160.5 MATURATION OF CORTICOTECTAL INFLUENCE IN CAT SUPERIOR COLLICULUS. B.E. Stein and H. Gallagher* Dept. of Physiology, Medical College of Virginia, Richmond, VA 23298.

The maturation of visual cells in cat superior colliculus (SC) requires approximately two months. Toward the end of the 2nd week of post-natal life, the specialized properties of directional selectivity and binocularity begin to appear. While the appearance of these properties is believed to reflect the development of corticotectal influences, corticotectal projections already exist in 2-day old kittens. In the present investigation, we sought to assess the functional state of corticotectal projections at different developmental stages.

Single units (n=188) were recorded in 63 kittens 7-54 days of age. Corticotectal activity was blocked by placing crushed saline ice onto the brain and later reinstated by rewarming the tissue with warm saline. In adults, cortical cooling depresses the activity of visual cells receiving projections from cortex. In the present study, a marked correlation between the maturation of specific receptive field properties and cooling-induced depression was observed.

Prior to 13 days of age, SC cells were predominantly monocular, best activated by stationary pulsed light, and cortical cooling had no significant effect upon their activity. The increased effectiveness of movement and the development of directional selectivity and binocularity coincided with the appearance of cooling-induced depression. Regardless of age, directional selectivity and binocularity in a given cell was a powerful predictor of that cell being affected by cortical cooling. Thus, 67% of the cells exhibiting either one or both of these characteristics was depressed, while only 16% of the cells which had neither property was affected by the same procedure. Since there is an increase in the proportion of directionally selective and binocular cells with age, there is a coincident increase in the incidence of cooling-induced depression in older animals. Typically, response depression consisted of a decrement in the number of evoked impulses apparent within 3 minutes of cooling and nearly complete elimination occurred after 4-5 minutes. Responsiveness was reestablished to the precooling level after 5 minutes of cortical rewarming.

These data indicate that, despite the presence of corticotectal projections in 2-day old neonates, these inputs are functionally immature. Their maturation, as indicated by the development of cooling-induced depression and the appearance of specific receptive field characteristics, is a prolonged process requiring approximately 8 weeks of post-natal life.

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160.6 INTRACORTICAL CONNECTIVITY IN THE VISUAL SYSTEM OF NORMAL AND BINOCULARLY DEPRIVED RATS. H. Jackson, A. Frankfurter, and A.B. Butler. Dept. of Neurosurgery, Univ. of Virginia Medical Center, Charlottesville, VA 22908.

We have recently devised a method for depositing horseradish peroxidase into the supragranular layers of the cerebral cortex. Variations on this procedure allow either visualization of isolated cellular morphology or gross patterns of intracortical connectivity. Injections of supragranular layers in area 17 of the rat result in labelling of layer II and III neurons as well as layer V pyramidal neurons. Although the axons of layer II-III neurons and apical dendrites of layer V cells can be seen traversing layer IV, layer IV itself is largely devoid of labelled cells and terminals. Layer III neurons of area 17 possess descending axons from which emanate a series of oblique collaterals. These collaterals apparently terminate within discrete columns in the lateral peristriate cortex (area 18). Layer V neurons also innervate these columns through horizontal axon collaterals. Within lateral peristriate columns labelled by injection of area 17, both terminal and cellular label are seen. The majority of cellular label is found in layer V with the balance scattered over more superficial layers. Terminal label is seen across layers V-II. These results indicate reciprocal connections between discrete columns in area 17 and the lateral peristriate region. These connections are formed by layer V pyramidal cells which innervate at least the supragranular layers of the associated column and by supragranular neurons which likely project to layers V-II.

We are now investigating the effects of bilateral enucleation on this cortical organization. Preliminary observations on subjects enucleated 1-3 days after birth suggest several possible effects of this treatment. At six weeks after enucleation, injections of area 17 result in diminished labelling of lateral peristriate columns (terminals and cells) which suggests a decrease in reciprocal connections between these regions. On the other hand, there seems to be an increase in the callosal projection from area 17. Finally, there is an apparent increase in terminal label within layer IV of area 17. At present it is unclear whether this reflects a true projection from layers II and III to layer IV or simply a shrinkage of layer IV following enucleation. A detailed examination of these and other possible effects is currently underway.

161.1 GROWTH CONES IN THE LONGITUDINAL CHANNEL SYSTEM OF THE DEVELOPING AMPHIBIAN SPINAL CORD. R.H. Nordlander* and M. Singer (SPON: J. Brodkey). Dept. of Anatomy, Case Western Reserve Univ. Sch. of Med., Cleveland, Ohio 44106.

Electron microscopy of the developing spinal cord of the amphibian tail has shown that earliest fiber tract development occurs by the ingrowth of axons into small but discrete longitudinally oriented spaces between neighboring cells of the neural tube (Nordlander and Singer, J. Comp. Neurol., 180:349, 1978). These spaces, which are located at the periphery of the cord, are empty at the most primitive levels but with further development are infiltrated by increasing numbers of neurites.

Growth cones were observed in both transverse and longitudinal sections of the channels and were distinguished from other channel components by their generally larger size, irregular outlines, and cytoplasmic contents. Overall cytoplasmic density of the growth cones was usually less than that of either other axons or the neuroepithelial cell processes which also occupied the channels. Most growth cones were seen as bulbous endings containing a light granular matrix and few organelles, among them assorted vesicles, mitochondria, occassional autophagic vacuoles, and agranular reticulum. Microtubules were usually lacking. A few growth cones differed from this general description by displaying focal dense accumulations of the organelles mentioned above. "Synaptoid" profiles (Nordlander and Singer, Cell Tissue Res., 166:445, 1976) were seen facing other axons and presumptive glial processes. Fine cytoplasmic processes, presumably microspikes, extended from the bulbous portions of the growth cones into spaces between adjacent cells and axons.

Growth cones always appeared in the most peripheral position within each channel. This was especially obvious in longitudinal sections of channels containing more than one growth cone. In these instances the more rostral cones were directly peripheral to trailing axons of fibers whose cones appeared more caudally. This observation suggests that new fibers are added to these fasicles at progressively more peripheral positions.

161.2 MECHANISMS OF AXONAL GUIDANCE DURING THE FORMATION OF CENTRAL NERVOUS SYSTEM COMMISSURES. Jerry Silver. Dept. of Anatomy, Case Western Reserve Univ., School of Medicine, Cleve., OH. 44106.

What are the structures within the embryonic central nervous system that guide axons across the midline during development of the commissural pathways (corpus callosum, anterior commissure)? I have investigated this question histologically with the use of serially sectioned, timed mouse embryos. During day 15 of gestation the previously separated, medial walls of the cerebral hemispheres (septal regions) begin to fuse ventrally. On day 16, fusion proceeds rostro-dorsally. For about 100μm forward of the lamina terminalis and subjacent to the interhemispheric fissure, the fusion process is augmented by an influx of presumptive glial cells which appear to migrate medially from the ventricular germinal zones of each hemisphere. This local accretion of glial precursors results in the formation of a structure which resembles a "sling" coursing beneath the interhemispheric fissure and suspended from the medial walls of each lateral ventricle. Within the hemispheres, axons of the corpus callosum grow in a nucleisparse zone between the germinal region and cortical plate. The pioneer axons approach the "sling" on day 17 and apparently are directed across the midline by this glial scaffolding. Later developing axons of the corpus callosum cross rostrally but are not supported by a glial bridge. Instead, they traverse the cerebral midline (which at this stage is unfused far rostrally) by fasciculating on the pioneering and caudally formed callosal axons.

The fibers of the anterior commissure appear to be guided across the midline by a different mechanism. Movement of these axons occurs through a preformed pathway of intercellular glial channels. The channels form within the lamina terminalis on day 14 in advance of the outgrowth of the first commissural axons(E16).

I have also examined the developmental stages of a primitive, acallosal marsupial, Didelphis virginiana (opossum). In this species, fusion of the medial walls of the cerebral hemispheres and glial sling formation do not occur. Instead of traveling dorso-medially the "callosal" axons turn ventrally and pass contralaterally by way of the anterior commissure.

These observations have demonstrated that oriented glial tissues, by forming channels or "slings" and, presumably, by assuming a variety of other configurations, may play crucial roles in the guidance of axons. However, orderly fasciculation of axons is also an important guidance mechanism during CNS development. The developmental sequence in the opossum suggests that genetic alterations may change fundamental morphogenetic events (such as midline cerebral fusion) which, in turn, can alter glial patterns and, thereby, contribute to the evolution (or malformation) of axonal pathways. To further explore this theory we are studying an acallosal mouse mutant. (Supported by NIH grant NS-15731-01)

161.3 AN ELECTRON MICROSCOPIC STUDY OF THE DEVELOPING CORPUS CALLOSUM IN FETAL AND NEONATAL RATS. Karen L. Valentino and E.G. Jones. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The development of callosal axons has been studied in rats by means of light and electron microscopy. Axons were identified by anterograde tracing techniques, and then followed as they cross the midline and grow to their targets in the cerebral cortex. The relationships between the growing axons and other cell types in the developing corpus callosum were also examined.

Material from rats aged 15 days gestational to 7 days postnatal was processed for routine electron microscopy. In other animals, prepared for both light and electron microscopy, horseradish peroxidase (HRP) was injected unilaterally into the cortex and white matter to anterogradely label the growing axons. This method also labels other cell classes via ventricular, subarachnoid, and perhaps vascular diffusion.

Some preliminary observations include the organization of the axons into bundles separated by elongated interstitial cells and associated spaces. Axonal growth cones filled with HRP can be identified and occasionally, transient synapse-like structures on as yet unidentified elements in the callosum have been observed. There is some evidence for degeneration of a proportion of the young axons.

Astrocyte and oligodendrocyte precursors, as well as earlier glial precursors can be recognized. In addition to these cells, many macrophages are found in the corpus callosum and in the associated vascular, perivascular, and meningeal tissues. When labeled by injected HRP, these cells can be seen with the light microscope to surround the callosum and extend for long distances along its fibers as they penetrate into the hemispheres. With the electron microscope in normal and HRP material, the phagocytic cells are often oriented along the callosal axons, with processes around them. The origin of the macrophages is uncertain, although they are tentatively believed to arise from non-neuroepithelial sources. Their function and fate are likewise unclear: it is uncertain to what extent they serve to guide callosal axons across the midline and towards their targets and whether they are ultimately transformed into microglial cells.

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161.4 A MECHANISM FOR THE GUIDANCE OF PERIPHERAL SPIRAL GANGLION CELL AXONS IN THE DEVELOPING MOUSE AUDITORY SYSTEM.
Paul R. Carney* and Jerry Silver (SPON: Scott Brand). Dept. of Anatomy, Case Western Reserve Univ., School of Medicine, Cleve, OH. 44106.

Cajal (1890) and others have described the earliest peripheral spiral ganglion cell axons as a wandering bundle that perforates its way into the otocyst. Upon their arrival at, but before entering, the otocyst, it was also believed that the pioneer fibers became stationary for awhile, awaiting differentiation of the cochlear canal.

Examination of timed mouse embryos at days 9.0 - 10.5 by the use of light and electron microscopy as well as quantitative histological techniques, has revealed mechanisms of axonal guidance and entrance of fibers into the otocyst that differ from previous beliefs. During embryonic days 9.0 - 10.0, a discrete region of cell death occurs in the caudo-medial portion of the basal otocyst (basal otocyst is the presumptive cochlea). This necrotic process is accompanied by a local breakdown of marginal gap junctions and basement lamina, with obvious widening of the extracellular spaces between the neuroepithelial cells. This sequence of events progresses as a wave to the lateral and rostral portions of the presumptive cochlea. During embryonic day 10.5, pioneering, peripheral fibers take a circuitous route and ascend from the spiral ganglion to innervate the neuroepithelia of the presumptive cochlea, directly behind the zone of cell death. En route to the cochlear rudiment, the fibers seem to follow a condensation of cells that appears prior to the outgrowth of the first spiral ganglion cell axons. This cell aggregation, shaped like a funnel, begins broadly at the caudal portion of the spiral ganglion, and narrows at its ending in the zone of cell separation at the caudo-medial margin of the basal otocyst. It is at this particular site of cell disunion that the fibers are first directed into the presumptive cochlea. The enviroment surrounding the remainder of the otocyst and spiral ganglion (i.e., that region devoid of auditory axons) is a loosely arranged cellular meshwork.

These observations suggest that the itinerant peripheral axons of the spiral ganglion may not wander, perforate, or ever be stationary but, instead, grow along a series of extrinsic stuctures that may guide them directly towards and allow them immediate passage into their target destination.
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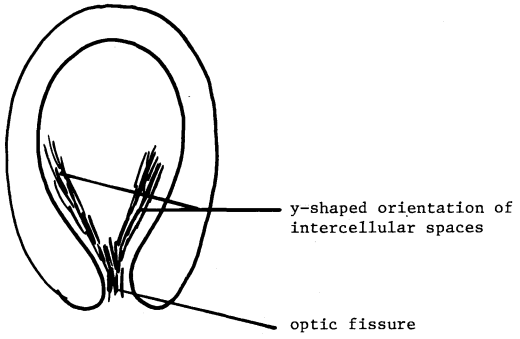
161.5 PRENEURAL PATHWAYS IN THE EMBRYONIC CHICK RETINA. Susan R. Krayanek* and Stephen Goldberg (SPON: R. Clark). Dept. of Anat., Univ. of Miami Sch. Med., Miami, Fla. 33101

Optic axons of the early chick retina grow in a directed fashion toward the optic fissure (Goldberg, S. and A.J. Coulombre, J. Comp. Neurol, 146:507, 1972). Embryonic chick retinas were examined for factors that may guide the earliest optic fibers. At stage 16, prior to the morphological appearance of optic axons, intercellular spaces were oriented in the direction of the fissure. Serial section reconstruction of lu parasagittal sections, revealed that the oriented spaces were arranged in a y-shaped pattern around the fissure (Figure). Oriented spaces were present at the entrance to the optic stalk but spaces within the stalk itself were not oriented in the direction of optic fiber outgrowth. Electron microscopy of stage 16 retinas, showed the spaces to be bounded by aligned processes of neuroepithelial cells. At stage 18, optic axons could be seen growing adjacent to and forming close contacts with the oriented neuroepithelial cells. Thus, alignment of neuroepithelial cells in the chick embryonic retina may aid in the guidance of optic axons.



Stage 16 retina (flat mount view).

161.6 ABSENCE OF DEGENERATIVE DEBRI GUIDANCE IN THE SPECIFIC GROWTH OF SEPTAL TISSUE GRAFTS IMPLANTED INTO NEONATAL HOSTS. E. Lewis, P. Kelly and C. Cotman Dept. of Psychobiology, U.C. Irvine, Irvine, CA 92717.

Pieces of embryonic septal tissue placed in the entorhinal cortex of neonatal rat hosts (P2-P3) will innervate the host hippocampal formation and send processes into appropriate laminar zones within the molecular layer of the dentate gyrus. What mechanisms guide the axons of implanted neurons to their target cells in the host brain? Results from studies of tissue grafts in adult recipients indicate that processes emanating from the graft may follow the path of degenerating axons in the host neuropil. We have assessed this role of degenerative debri in the implant paradigm by 1) characterizing the distribution and time course of removal of degeneration following implant surgery in neonatal rats, and 2) examining the growth of septal implants in a host brain devoid of observable degenerative debri.

A distinct pattern of degeneration argyrophilia (De Olmos stain) is present in rats sacrificed 24 and 48 hours following implant surgery on day 3. By 72 hours following surgery, virtually all argyrophilic material has been removed. Some areas in the hippocampal formation destined to receive innervation from the implanted septal tissue never show degeneration products at any time after surgery.

The rapid removal of degenerative debri in the immature brain makes it possible to test for the presence of debri guidance by introducing a delay between the time at which the implant cavity is made and the time of implantation. A cavity created in the entorhinal cortex of 3 day old rats was used to accommodate an embryonic septal graft (E18) implanted on day 9. Also on day 3, a transection of the septohippocampal fibers in the neonatal host was performed to eliminate native septal efferents. One month later the growth of the septal implant was examined with a stain for acetylcholinesterase (AChE). Implanted cells sent numerous AChE positive processes toward the host dentate gyrus. In the dentate molecular layer, AChE reaction product occupied the same two laminar zones that stain positively in normal rats.

Thus, axons from implanted septal neurons are guided to the hippocampal formation through a host neuropil from which degeneration argyrophilia has been removed. Furthermore, the laminar distribution of these axons in the dentate molecular layer is independent of the pattern of degenerating afferent terminals in the host immediately following the implant surgery. This suggests that axons of implanted septal neurons maneuvering through an immature host neuropil are guided by mechanisms similar to those which are active during the development of laminar specificity in vivo. Supported by grants NS 08597 and MH 19691.

170.1 BRANCHING OF AFFERENT AXONS IN THE SPINAL NERVE. Richard E. Coggeshall and Lauren A. Langford. Depts. of Anatomy and of Physiology and Biophysics and the Marine Biomedical Institute. The University of Texas Medical Branch, Galveston, Texas 77550.

An important concept in the understanding of the organization of the peripheral nervous system is that each dorsal root ganglion cell gives rise to a single central process and a single peripheral process. If this is true, and there are no extraneous axons in the roots or nerves and no extraneous neurons in the ganglion, then there should be an equal number of axons in dorsal roots, nerves and ganglion cells in the dorsal root ganglion. In a previous study, however, we demonstrated that there were no extraneous axons or neurons but the numbers of dorsal root axons and dorsal root ganglion cells were not equal. Thus we concluded that dorsal root axons branch in the dorsal root.

In the present study we are determining the number of dorsal root ganglion cells and axons in the spinal nerve for segments L6-S3 in the rat. The axons in the spinal nerve and dorsal root are counted with the electron microscope. The rats have had unilateral ventral rhizotomies of segments L6-S3 and sympathectomies to remove the motor fibers and postganglionic fibers from the spinal nerve. Our results are presented in the following table.

RAT	SEG	GANGLION CELLS TOTAL	SPINAL NERVE MY + UN = TOTAL	DORSAL ROOT MY + UN =TOTAL
1	S3	1249	1156 + 3340 = 4496	1035 + 2607 = 3642
2	S3	907	802 + 2705 = 3507	929 + 1225 = 2154

Note that there are many more axons than ganglion cells for these nerves. If these preliminary results are confirmed, then there is a great deal of branching of peripheral processes of dorsal root ganglia in the ganglion or proximal peripheral nerve. Possible consequences of this arrangement will be discussed.
Supported by grants NS 10161, NS 11255 and NS 07377.

170.2 PATTERNS OF CUTANEOUS INNERVATION IN NORMAL AND REINNERVATED HAIRY SKIN. J.K. Terzis, P.A. Ballard* and B.G. Turnbull*. Depts. of Surgery & Physiology, McGill University, Montreal, P.Q., Canada.

Restoration of sensation following nerve transection and repair is seldom normal. Whether factors affecting reinnervation are restricted to the repair site, or whether additional factors operate distal to the lesion is not known.

Innervation patterns of normal and reinnervated skin subsequent to section and repair of the great auricular nerve in rabbits were studied. Proximal to the repair, single fascicles were placed onto a recording electrode, and the cutaneous area innervated was determined by brushing the dorsal surface of the ear. The fascicular map and areas of highest activity were transcribed onto photographs of the ear, and the location of the fascicle in the nerve noted. This procedure was then repeated distal to the nerve lesion. Single fiber recordings were obtained using microdissection techniques. Patterns of reinnervation were assessed at 3, 6, and 9 months post-injury. Normal fascicular maps had smooth contours and exhibited moderate overlap; the area of highest activity was centrally located and the transition from low to high activity areas was uniform. A topographical relationship existed between the location of the fascicle within the nerve and the location of the cutaneous territory on the dorsal surface of the ear. Distal fascicular recordings revealed the same patterns, but the areas innervated were smaller. The normal pattern of innervation was greatly distorted in the repair groups. Proximal fascicular maps in test animals were huge with excessive overlap. The areas of highest activity were ectopic, and the transition among areas of different activity was abrupt. The topographical correspondence between fascicle and skin area innervated was lost in the early groups. Fascicular recordings distal to the repair showed minimal distortion, although some parameters remained abnormal.

All single fiber receptive fields (RF's) lay within mapped fascicular territories, and the density and overlap among RF's corresponded well to the relative activities as determined by the fascicular maps. Normal RF's had smooth contours, and showed uniform activity. Reinnervated RF's showed two basic patterns: most were smaller than normal RF's, but some fibers innervated large skin regions, with diffuse or multiple areas of activity. Such multiple RF's were found both with proximal and distal recordings, indicating that such fields probably arose from peripheral sprouting.

Despite the fact that various parameters improved with time, distortions in cutaneous innervation patterns persisted throughout the study. These findings are useful in understanding clinical observations of altered sensibility and false localization subsequent to nerve injury and repair.

170.3 DORSAL HORN OF NEONATE RATS "IN VITRO": NEURONAL RESPONSE TO AFFERENT VOLLEYS AND AFFERENT TERMINALS' RESPONSE TO SUSPECTED TRANSMITTERS. Ian Hentall and Howard Fields, Dept. of Neurology, UCSF, San Francisco, CA 94143.

The hemisected spinal cord from neonate rats has been used for "in vitro" pharmacological studies of compound dorsal and ventral root potentials. We describe here its use for study of single elements in dorsal horn. Approximately 8 thoraco-lumbar segments are dissected from 1-10 day old rats and perfused with oxygenated, artificial, cerebrospinal fluid at 28°C.

Second Order Neurons: A medial-to-lateral approach through the dorsal horn of the hemisected cord using 30MΩ KCl-filled microelectrodes revealed 11 extracellular units with properties stable for at least 3-4 hours. These properties were: (1) spontaneous activity of 5-10Hz., (2) a response to electrical stimulation of dorsal rootlets consisting of 1-3 spikes after an inhibitory period (seen in multiple sweeps) of about 50msec., (3) abolition of both spontaneous and evoked activity by 10^{-6}M tetrodotoxin and 10mM Mg^{++}. Of adult mammalian dorsal horn units, these most strongly resemble a type found in the substantia gelatinosa. We find the neonatal dorsal horn in Nissl-stained sections to be undifferentiated,with few dendrites. However, the existence of this activity in 2nd order neurons makes the preparation useful for assessing connectivity and transmitter actions in the dorsal horn.

Primary Afferent Terminals: Single units were recorded from attached dorsal root ganglia with ventral roots cut, and their threshold for antidromic activation from the dorsal root entry zone was measured by computer-controlled search (0.3Hz. pulses). The following drugs were applied; 10^{-6}M (D-ala^2) met-enkephalinamide (ENK), 10^{-6}M morphine sulfate (MS), 10^{-7}M naloxone (NAL), 10^{-5}-10^{-6}M substance P (SP), and 10^{-6}M serotonin (5HT). Eighty units were looked at, all in the slowest range of conduction velocities of 0.12-0.75 m/sec (at 28°C); their velocities increased by about 70% at 37°C, into the range typical for mammalian C-fibers. ENK increased thresholds in 5 of 16 units and MS in 7 of 11. This strengthens similar findings on mammalian C-fibers "in vivo", since blood pressure effects and other artefacts can be ruled out. NAL reversed the opiate-induced increase in threshold. SP decreased thresholds in 4 of 7 units, including one opiate-responsive one. Thus the primary effect of SP is probably not to release enkephalin from interneurons. 5HT raised thresholds in 5 of 15 neurons, and in 2 other cases increased the effect of MS without itself having any influence. Separation of direct and indirect actions upon terminals by use of high Mg^{++} conc. has not yet been achieved, since the antidromic response often disappears in this circumstance (5 of 9 units); this could be due to blocking of Ca^{++} spikes or a direct effect on Na^{+}-spike threshold.

170.4 ANATOMICAL CHARACTERIZATION OF ULTRAFINE PRIMARY AXONS IN LAYER I OF THE DORSAL HORN OF ADULT CATS. Stephen Gobel, William Falls and Emma Humphrey*. Neurocytology and Experimental Anatomy Section, Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

Virtually all of the primary axonal endings that have been described in layers I and II, as well as those in the deeper layers of the dorsal horn are relatively large elliptical endings, measuring 0.5 x 1.0 μm or larger. This study identifies a morphologically distinct group of ultrafine primary axons in layer I whose endings and parent branches are much finer than those of primary axons which terminate throughout the dorsal and ventral horns. The terminal arborizations of primary axons in adult cats were visualized either by filling them with horseradish peroxidase (HRP) in experiments in which HRP was applied to the cut central ends of cervical and lumbar dorsal roots or in Golgi analyses of layer I in medullary, cervical and lumbar dorsal horns. Ultrafine primary axons consist of long, unmyelinated strands (0.3 μm or less in diameter) which extend for several hundred μm in the rostrocaudal axis of layer I. Each strand gives rise to enormous numbers of very closely spaced fine endings, most of which are less than 0.5 μm in diameter. For example, as many as 14 endings have been found along a 10 μm length of strand whereas primary axonal strands in layer II rarely have even three endings in a 10 μm length of strand. At least two different kinds of ultrafine primary axons exist; one has only fine rounded endings, while the other has highly elongated, fusiform endings.

Electron microscopical analysis of the axonal endings of HRP-filled ultrafine primary axons shows that they contain a few dense core vesicles and form synapses in layer I on dendritic spine heads, and on dendritic shafts. The postsynaptic densities of these axodendritic synapses vary. Some contain appreciable postsynaptic densities and resemble Gray type I synapses. Some contain subsynaptic Taxi bodies while many others contain only scant amounts of postsynaptic dense material. Ultrafine primary axons also receive synapses on their endings and on their strands from processes which contain a mixture of small oval and highly flattened synaptic vesicles.

Ultrafine primary strands in layer I, unlike some primary axonal strands in layers IIa and IIb (Gobel and Falls, Brain Res. 175: 335-340, 1979), have not been found originating from thicker parent branches. A few have been traced from layer I directly to 0.3 μm parent branches in Lissauer's tract in the spinal cord. Such observations suggest that ultrafine primary axonal strands in layer I originate from primary neurons with either unmyelinated or extremely fine myelinated central axonal branches.

170.5 SPINAL TERMINATIONS OF LOW THRESHOLD MECHANORECEPTIVE AFFERENT FIBERS. M. David Egger, Natalie C. G. Freeman*, Patricia Masarachia*, Sasha Malamed* and Eric Proshansky*. Dept. of Anatomy, CMDNJ-Rutgers Med. Sch., Piscataway, N.J. 08854.

Using the method of intra-axonal injection of horseradish peroxidase, we stained functionally identified afferent fibers from slowly adapting (Type I), rapidly adapting and Pacinian corpuscle receptors in the glabrous skin of the hind paw of the cat. At the light microscopic level, we were able to observe the distribution of terminal collaterals of single afferents over a rostrocaudal extent greater than 3.5 mm. Swellings in the terminal collaterals, presumably corresponding to synaptic contacts, occurred predominantly in Rexed's laminae IV and V for both the slowly adapting and rapidly adapting terminal fibers. The Pacinian corpuscle afferents, on the other hand, sent a major projection more dorsally, concentrated in lamina III and dorsal lamina IV. The swellings in the terminal collaterals of the slowly adapting afferent fibers approached about 2.0 μm in longest dimension; those of the rapidly adapting and Pacinian corpuscle afferent fibers approached 4.0 μm or more in longest dimension. Many collaterals of the Pacinian corpuscle afferents formed characteristic grape-like arbors of terminal swellings. In addition to light microscopy, electron microscopic observations, similar to those of Ralston and co-workers (Neuroscience Abstracts, 1978, 4: 570; Anatomical Record, 1980, 196: 152A), were made of terminals of three of the slowly adapting afferent fibers and of one Pacinian corpuscle afferent. The predominant mode of termination in these labelled synaptic contacts was axodendritic, including what appeared to be series of boutons en passant strung along single dendrites. In some cases, it appeared that more than one dendrite was related postsynaptically to a single terminal swelling. The vesicles in the labelled terminals were round, clear and about 30-50 nm in maximum diameter. Consistent with the light microscopic appearance, the vesicle-containing terminal regions of the Pacinian corpuscle afferent appeared to be larger than those of the slowly adapting afferent fibers.

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170.6 THE INTERMEDIOMEDIAL ZONE OF THE LUMBOSACRAL SPINAL CORD IN THE CAT: AFFERENTS AND PEPTIDERGIC ELEMENTS. J.C. Bresnahan, M.S. Beattie, G.M. Mawe and R.H. Ho. Dept. Anat., Coll. Med., The Ohio State University, Columbus, Ohio, 43210.

The intermediomedial zone (IMZ) of the spinal gray has been implicated in autonomic processing, and more recently has been shown to be a major source of spinothalamic tract (STT) axons in the cat at lumbosacral levels (Carstens and Trevino, '78). We have examined this region to determine the nature of primary afferent input and its overlap with axons traveling in the dorsal portion of the lateral funiculus (DLF), and the relationship of these afferents to substance-P (SP) and methionine-enkephalin (ENK) immunoreactive elements.

Primary afferent input to the IMZ was assessed by light and electron microscopic observations of terminations in this region after anterograde, diffuse, injury-filling of lumbar and sacral dorsal roots with horseradish peroxidase (HRP), and after transganglionic HRP labelling of sciatic nerve. The projection to the IMZ from axons in the DLF was similarly analyzed by applying HRP to cut axons in the DLF. SP and ENK elements were labelled by either Sternberger's PAP method or the indirect immunofluorescence technique of Coons.

Dorsal root labelling reveals a dense, discrete projection of axons emanating from the dorsal columns. The terminal distribution of these axons forms a continuous column in the lumbosacral cord roughly corresponding to the position of the intermediomedial nucleus described by Petras and Cummings ('72). The synaptic contacts of these axons occasionally form relatively complex glomerular-like structures similar to those seen in the substantia gelatinosa (SG). Unlike in the SG, synapses were often seen on proximal dendrites or cell bodies, sometimes associated with sessile spines. These synapses contained small, clear, oval vesicles with an occasional small dense core vesicle (DCV).

The major projection zone of the dorsal roots overlaps, but does not coincide with the dense labelling of SP and ENK elements seen in lamina X. Axons of DLF origin contribute a medial projection to this region.

The correspondence between the primary afferent termination pattern and the location of spinothalamic tract cells seen in previous studies is striking. In conjunction with our observations of descending input of possible brainstem origin, and the proximity of this region to SP and ENK immunoreactive elements this pattern suggests that the IMZ may be an important processing center for nociceptive, cutaneous, and perhaps visceral information. (Supported by N.I.H. Grants NS-10165 and NS-14457.)

170.7 SUBSTANCE P ANALOGUE REDUCES TAIL FLICK LATENCY WHEN GIVEN INTRATHECALLY TO THE RAT. K. Yashpal*, D.M. Wright* and J.L. Henry. (SPON: G.E. Lucier). Dept. Research in Anaesthesia and Dept. Psychiatry, McGill University, Montreal, PQ.

Substance P has been found in small diameter afferents and has an excitatory effect specifically on nociceptive neurons in both dorsal horn and trigeminal nucleus caudalis. To test the hypothesis that substance P has an excitatory role in transmission in spinal pain pathways the present study was done. Its point of genesis was the assumption that any such agent should produce hyperalgesia, manifested at least as a reduction in pain threshold. Male Sprague-Dawley rats were implanted chronically with intrathecal polyethylene catheters by a method similar to that of Yaksh (Physiol. Behav. 17: 1031, 1976). After recovery times of 5 or more days each animal was tested for nociceptive threshold by measuring the reaction time to tail flick from a noxious radiant heat stimulus. Trials consisted of a number of such stimuli delivered at 5 min intervals. The substance P analogue, eledoisin related peptide (ERP), was dissolved in artificial CSF and administered in doses of 1.25 to 10 μg in a volume of 10 μℓ. When given one min prior to a test for tail flick latency ERP caused a dose-dependent reduction from pre-administration control times: the first tail flick response after ERP administration was reduced to a mean of 22% of the control latency at the highest dose, and to a mean of 90% of the control latency at the lowest dose. The second response after ERP administration had almost returned to control levels. A similar volume of vehicle administered to the same rats under identical conditions failed to alter tail flick latency. Thus, the results of the present study, showing that the substance P analogue ERP produces a transient hyperalgesia in the tail flick test, support the earlier suggestion that substance P has an excitatory role in transmission in spinal pain pathways.

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170.8 RESPONSES OF THORACIC DORSAL HORN UNITS TO VISCERAL INPUTS AND TO AMINO ACIDS IN THE CAT. S.B. Backman* and J.L. Henry (SPON: P. Anninos). Dept. Physiology, McGill Univ., Montreal, PQ.

Responses of dorsal horn neurones to visceral inputs were studied because some of these neurones may be involved in spinal cardiovascular reflex pathways. In chloralosed cats single unit extracellular spikes were recorded from the dorsal horn in segments T1-T4 using multibarrelled micropipettes. The centre recording barrel was filled with 2.7 M NaCl. Barrels for iontophoresis were filled with Na-L-glutamate (1 M, pH 7.4), γ-aminobutyric acid (GABA, 1 M, pH 4.3), glycine (1 M, pH 3.5) and NaCl (165 mM acidified to pH 5.5) used as a control against artifacts due to changes in current or in pH. The ipsilateral inferior cardiac nerve and upper thoracic sympathetic chain were stimulated electrically to activate visceral afferents. Units responding to these visceral inputs were also tested for somatic inputs by electrical stimuli to the skin of the forelimb or by natural stimuli. Units responding to visceral inputs were recorded between 1.47 and 3.37 mm from the surface. Only 4 of 17 such units had ongoing activity. Latencies of responses to visceral afferent stimulation ranged from 1.58-19.26 msec. Responses of 2 of the 17 units occurred at about 1.5 msec; the remaining were greater than 3.2 msec, with two modes at 5 and 18 msec. Evoked responses had burst sizes of 1-6 spikes with a mean of 2.2. About half of the viscerosensitive units tested for somatic input could also be excited by skin stimuli. All units were excited by glutamate (10-25 nA). Evoked responses could be inhibited by GABA and glycine (5-40 nA); these had about equipotent effects on longer latency responses but shorter latency responses tended to be less inhibited by glycine. These results confirm earlier reports of visceral inputs to thoracic dorsal horn neurones but in addition demonstrate that not all of these neurones also receive somatic input. In addition, viscerosensitive dorsal horn units are excited by glutamate and inhibited by GABA and glycine. The differential effects of GABA and glycine suggest the possible existence of different functional groups within the population of viscerosensitive neurones.

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170.9 HYPOPHYSECTOMY PREVENTS EXCITATORY ACTIONS OF NALOXONE ON NOCICEPTIVE UNITS IN THE DORSAL HORN OF THE SPINAL CAT.
J.L. Henry, Dept. Research in Anaesthesia, McGill University, Montreal, PQ.

The finding that naloxone excitation of dorsal horn units in the spinal cat (Neuroscience 4: 1485-1491, 1979) shows a diurnal variability (The Physiologist, 22: 71, 1979) suggested the possibility that the pituitary might participate in this excitation, for example by secreting on a diurnal rhythm an agent which inhibits spinal nociceptive neurones. Six cats were hypophysectomized under nembutal anaesthesia by suction via a trans-buccal approach. After recovery times of 5 to 26 days they were anaesthetized with chloralose. Spinal cords were transected at L_1, and segments L_5-L_7 were exposed for recording. Extracellular unit spikes were recorded with glass micropipettes filled with 2.7 M NaCl. Single units responding in a reproducible way to periodic applications of noxious radiant heat were tested. Naloxone, in doses of 0.1-0.5 mg/kg, failed to alter either the on-going discharge rate or the response to noxious radiant heat. On the other hand, in two sham operated cats and four intact cats, the same doses of naloxone induced elevations in both on-going activity and the nociceptive response. These results support the earlier suggestion that a circulating factor is exerting an inhibitory effect on transmission in spinal nociceptive pathways and furthermore implicates the pituitary in the regulation of circulating levels of this factor.

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170.10 INHIBITORY EFFECTS OF MEDIAL AND LATERAL MIDBRAIN STIMULATION ON ENCODING BY SPINAL DORSAL HORN NEURONS OF NON-NOXIOUS AND NOXIOUS SKIN STIMULI.
E. Carstens, H. Bihl*, D.R.F. Irvine, M. Zimmermann* II. Physiol. Inst., Univ. Heidelberg, Heidelberg (W. Germany).

Electrical stimulation in the midbrain periaqueductal gray (PAG) and lateral reticular formation (LRF) strongly inhibits spinal dorsal horn neuronal responses to noxious skin heating. To determine if non-noxious input is also under descending control, we examined the effects of midbrain stimulation on dorsal horn neuronal responses to controlled brushing of hairs in the cutaneous receptive field. Single lumbar dorsal horn neurons with hair follicle input were recorded in cats anesthetized with Nembutal and N_2O. Controlled brush stimuli were applied with a motor-driven toothbrush. The averaged response to 10 identical brush stimuli (excursion 8 mm, 1 per 6 sec, velocity 3.2- >27 mm/sec) was established without and again during midbrain stimulation (mean 30 Hz, 450-600 μA).

Two classes of units were studied: those receiving only A-fiber input from hindlimb nerves and responding only to brush and light touch (class 1), and those receiving A- and C-fiber input and responding in a graded fashion to brush, pressure and noxious heating (class 2). Brush-evoked responses were reduced during PAG stimulation in 18/26 class 2 (range: to 32-103% of control) and 11/19 class 1 units (range: 42-113%). LRF stimulation reduced the brush-evoked responses of 25/27 class 2 (range:6-103%) and 11/15 class 1 units (range:37-103%). More powerful inhibitory effects were induced from LRF than PAG in units in which both were tested (mean inhibition by LRF: 72%; by PAG: 81%, for 15 class 1 and 21 class 2 units).

Unit responses increased monotonically with velocity of hair movement up to a certain level. When the velocity series was repeated during PAG or LRF stimulation, various changes were observed in the linear portion of the velocity response curve including reduction in slope and/or increase in intercept with velocity axis ("threshold" velocity). The averaged effects of PAG and LRF stimulation on velocity coding by class 1 and 2 units were identical (slope reduction to 77% of control; threshold increase of 1.5 mm/sec; N= 18 units). This contrasts with findings on graded noxious heat intensity coding, whereby PAG stimulation reduces the slope, while LRF stimulation increases the threshold, of the temperature-response curve (Carstens et al., J. Neurophysiol. 43:332-442, 1980).

Class 2 units also responded to noxious radiant skin heating (50°C, 10 sec). On the average, heat-evoked responses were more powerfully inhibited (PAG: 55%; LRF: 54%) than brush-evoked responses (PAG: 81%; LRF: 72%) by midbrain stimulation in 15 units tested.

The results indicate that complex mechanisms are involved in the descending control of input to spinal dorsal horn neurons from different cutaneous receptor classes.

170.11 PROPERTIES OF NEURONS IN THE CAUDAL RAPHE NUCLEI OF THE CAT WITH AXONS THAT PROJECT TO THE SPINAL CORD.
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The physiologic properties were investigated of neurons in the caudal raphe nuclei (CRN) of the medulla with axons that project to the spinal cord (raphe spinal neurons). Adult cats were anesthetized with alpha-chloralose (60 mg/kg) and paralyzed with gallamine. The activity of single neurons was recorded using standard extracellular techniques, and raphe spinal neurons were identified by the presence of an antidromic response after stimulation of the spinal cord at C2. In 35 raphe spinal neurons, the location of the axon of the unit within the white matter of the spinal cord at C5 was determined by microstimulation techniques. Thirteen units had axons located in the dorsolateral funiculus (DL units), 12 units had axons in the ventrolateral funiculus (VL units), and 10 units had axons located in the ventromedial funiculus (VM units). Although there was considerable overlap, the perikaryon of DL units were found primarily in the most ventral portion of the CRN, VM units were found in the most dorsal portion of the CRN, and VL units were found in an intermediate position. The conduction velocity and percentage of neurons that responded to different modalities of sensory stimuli of these units are summarized in Table I. Conduction velocities of the VM fibers were significantly faster than the DL fibers. Almost all the units responded to gentle tapping at sites throughout the body surface. Compared to VM units, the DL units appeared to have less sensory convergence from hair, visual, or auditory stimuli. The VL units had an intermediate profile. This is in agreement with the properties of a separate population of 241 CRN units in which a higher percentage of units in the dorsal region responded to cutaneous, visual, and auditory stimuli compared to units in the ventral region. These data indicate that there are functional differences between neurons located in the dorsal portion and ventral portion of the CRN in terms of position and diameter of spinal-projecting axon, and response to sensory stimuli.

TABLE I

Unit Type	N	Cond. Vel.	Sound #test	Sound %resp	Hair-sen. #test	Hair-sen. %resp	Light #test	Light %resp
DL	13	27.2 ± 3.3	9	22	12	8	9	0
VL	12	38.4 ± 5.5	10	0	10	30	10	10
VM	10	61.1 ± 12	4	50	6	50	5	60

170.12 LOCUS COERULEUS PROJECTIONS TO THE SPINAL CORD: ANATOMY & PHYSIOLOGY.
C. J. Hodge, A. V. Apkarian†, R. T. Stevens†, G. D. Vogelsang* and H. J. Wisnicki† Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

The locations of cells in the brain stem of the cat containing catecholamines (CA) were determined using glyoxylic acid induced histofluorescence. Retrograde transport of HRP, conjugated with wheat germ agglutinin, injected into the lumbar spinal cord was used to determine which of the CA containing areas in the pons projected to the lumbar cord. The areas which were labeled by both these techniques included the caudal locus coeruleus (LC), the subcoeruleus (SC) and the parabrachial nuclei.

The effects of LC and SC stimulation on dorsal horn cell responses to cutaneous stimulation were then determined. Extracellular recordings were made from lumbar DHC in cats anesthetized with chloralose. Bipolar stimulating electrodes were placed in LC bilaterally. The effects of repetitive stimulation of LC, either ipsilaterally or contralaterally, on DHC responses to innocuous hair displacement and noxious thermal stimuli were determined. All LC stimulation sites were evaluated histologically. DHCs were divided into four groups on the basis of their response characteristics to cutaneous stimuli. Group I cells responded only to innocuous stimuli. Group II cells responded only to noxious stimuli. Group III cells responded to both innocuous and noxious stimuli. Group IV cells had characteristics of "novelty detection cells."

Of thirty-two group I cells encountered, twenty-four had responses that were inhibited and three had responses that were facilitated by LC stimulation. Sixteen group II cells were studied. Thirteen of these cells were strongly inhibited and one was facilitated by LC stimulation. Eighty-two percent (9/11) of group III cells had their responses to noxious cutaneous stimuli inhibited by LC stimulation. When the effects of LC stimulation on responses of cells of this group to innocuous skin stimulation was tested (4 cells), two showed inhibition and two facilitation. Both group IV cells were strongly facilitated following LC stimulation. The effects of stimulating the ipsilateral or the contralateral LC were always the same. The inhibitory effects were not abolished by pretreatment with the serotonin depletor parachlorophenylalanine, but were abolished by pretreatment with reserpine. The anatomic and physiologic results indicate that the projections from LC to the spinal cord are functionally bilateral and primarily inhibitory. The coeruleo-spinal system is one of the mechanisms by which alterations in noradrenergic activity might affect behavioral responses to pain.

171.1 PROJECTIONS OF RETINAL GANGLION CELLS IN THE FETAL CAT. Robert W. Williams and Leo M. Chalupa. Department of Psychology and Physiology Graduate Group, University of California, Davis, CA 95616.

The prenatal organization of retinofugal projections was studied in fetal cats with anterograde transport methods. Laparotomies were performed on timed-pregnant cats, and following the exposure of a fetal head horseradish peroxidase (HRP) was injected into the right eye, while the left eye was injected with tritiated leucine. After a twenty-four hour survival period the fetuses were removed by Caesarian section and perfused through the heart with saline followed by a glutaraldehyde and paraformaldehyde mixture. Alternate sections through the thalamus and midbrain were processed for HRP or autoradiographically using conventional procedures. Retinal whole mounts were examined to check for possible damage which could have resulted from the injections. To date our results indicate that by ten days before birth all subcortical structures which are known to receive direct retinal projections showed some sign of label. In general, on embryonic day 57 the ipsilateral projection is less developed than the contralateral input. This is especially clear in the dorsal lateral geniculate nucelus (dLGN). In the dLGN there is no indication of laminar organization in neutral red counterstained sections. However, the contralateral and ipsilateral inputs were clearly segregated. In frontal sections the bands of crossed and uncrossed label were oriented approximately vertically rather than in the horizontal plane as is found in the adult cat. In the thalamus label was also seen at the medial border of dLGN and most likely, this represents the retino-pulvinar projection. In the fetuses 10 days before birth the densest label was in the ventral lateral geniculate nucleus (VLGN) and in the contralateral superior colliculus. The collicular label was most dense just ventral to the stratum zonale, that is, in the dorsal portion of the superficial grey layer. Since this region of the superior colliculus (e.g., McIlwain, J. Neurophysiol., 1978) and the VLGN (Spear et al., J. Neurophysiol., 1977) receive predominantly W-type ganglion cell projections, our findings imply that the projections of W-cells may develop earlier than those of other retinal ganglion cells. On going studies of earlier fetal material should provide more information regarding the validity of this suggestion.

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171.2 DISRUPTION OF CORTICAL BINOCULARITY DUE TO EARLY EXPERIENCE WITH 32° OF ROTATIONAL DISPARITY BETWEEN THE LEFT AND RIGHT EYES' VISUAL FIELDS. Michael R. Isley*, Diane C. Rogers*, Michael Podell*, and Paul G. Shinkman. Univ. North Carolina, Chapel Hill, NC 27514.

Binocular visual cortical cells in normally reared kittens have a mean interocular disparity (IOD) of 0° between preferred stimulus orientations in the left and right eyes. In previously reported experiments, we showed that when kittens' early visual experience consisted of left- and right-eye visual fields optically rotated in opposite directions about the visual axes (16° of disparity between the two eyes), the IOD distribution was found subsequently to be centered about the rotation experienced during early development. Except for the IOD distribution, the organization of visual cortex resembled that found in normally reared kittens, in terms of ocular dominance, orderly arrangements of orientation columns, and topographic representation of the visual field.

The present experiment was part of a series designed to assess the parametric limits of this plasticity in the development of visual cortex. Kittens were reared as before, wearing goggles fitted with small prisms that introduced equal and opposite rotations of the visual fields about the visual axes. For these kittens, however, the prisms were arranged so as to introduce 32° of rotational disparity between the two eyes' visual fields (16° counterclockwise in the left eye and 16° clockwise in the right eye for some animals, and the opposite for others). Kittens experienced 75-100 hr of visual experience starting at 4 weeks of age and continuing for 1-2 months, and remained otherwise in the dark. Control kittens wore goggles with prisms that introduced 0° disparity. The results differed in three principal ways from the earlier (16°) experiments. First, the ocular dominance distribution was U-shaped rather than normal, with some increase in the number of visually unresponsive cells. Second, the distribution of preferred interocular orientation disparities was not centered at 32°; it was nearly rectangular, with significantly greater variance than that of normally reared kittens or control kittens. Third, there was a significant overrepresentation of cells with preferred stimulus orientations in the dominant eye near horizontal or vertical (± 22.5°). We conclude that under these rearing conditions a rotational disparity of 32° (with concomitant interocular positional disparity) disrupts the development of cortical binocular connections in terms of both ocular dominance and interocular matching of preferred stimulus orientations.

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171.3 REORGANIZATION IN THE CORTICOTECTAL PROJECTION OF THE CAT FOLLOWING NEONATAL DAMAGE OF VISUAL CORTEX. Leo M. Chalupa, Robert W. Williams, Robert W. Rhoades and Stephen E. Fish. Department of Psychology and Physiology Graduate Group, University of California, Davis, CA 95616.

Unilateral damage of visual cortex in infant rats (Mustari and Lund, Brain Res., 112, 37-44, 1976) and hamsters (Rhoades and Chalupa, J. Neurophysiol., 41, 1466-1494, 1978) results in an aberrant crossed corticotectal projection from the intact visual cortex. Therefore we sought to determine whether or not a similar reorganization occurs in the cortico-collicular projection of the cat. For this purpose unilateral ablations of visual cortex were made in three cats on the second day after birth. When these animals reached adulthood a series of injections of tritiated amino acids were made into the visual cortex contralateral to the damaged side. Similar cortical injections were also made into the visual cortex of two adult normal animals. The brains were sectioned and processed autoradiographically using standard procedures. It was found that in the control animals collicular label was seen only on the side ipsilateral to the injected cortex. In contrast, in the neonatally brain damaged cats label was found in both the contralateral and ipsilateral superior colliculus. On both sides it was confined to the superficial grey layer and homotopic portions of the tectum were labeled. However, the contralateral label was much less dense and also more restricted than that on the ipsilateral side. The main focus was in the rostral portion of the contralateral tectum. Furthermore, unlike in rodents the crossed projection was not limited to the medial portion of the contralateral colliculus. These autoradiographic data indicate that reorganization in the cortico-collicular projection of the cat does occur as a consequence of neonatal, unilateral ablation of visual cortex. Thus, our findings suggest that reorganization in the corticotectal projection may be a general response of the developing mammalian visual system to early damage of the visual cortex.

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171.4 SYNAPTIC CHANGES IN THE VISUAL CORTEX OF MONOCULARLY DEPRIVED HOODED RATS. S. Boyce* and Eva Fifkova, Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

Monocular lid-suture of 2 months (P75) causes a significant decrease in the synaptic density of the deprived visual cortex and an increase in density of the visual cortex connected with the functional eye. In these experiments the lid-suture was performed at the time of the physiological eye opening (P15) when synaptogenesis of the visual cortex is not yet completed. In the present experiments, therefore, the postdeprivation changes were studied at P15 under conditions of unobstructed vision (4 pups) and at P21 under conditions of unobstructed vision (4) and monocular deprivation (5). The first period of axodendritic synaptogenesis appears to be completed by P15, since there is no increase in synaptic density of axodendritic contacts between P15 and P21. Monocular lid-suture of 7 days duration (from P15 to P21) had no impact on the density of these synapses. Between P21 and P75 a second period of axodendritic synaptogenesis occurs, which seems to be affected by deprivation: by P75 a loss of axodendritic synapses has been noted. The density of axospinal synapses increases dramatically from P15 to P21. Deprivation of 7 days results in a significant increase in the density of axospinal synapses in the functional cortex, leaving the density of the deprived cortex unchanged as compared to controls. Between P21 and P75 in controls the axospinal synaptogenesis continues at a lower pace in layers I-II, while in layers III-IV a loss of these contacts occurs. In the functional cortex in layers I-II of deprived rats, the axospinal synaptogenesis continues; it, however, ceases in these layers in the deprived cortex. This results in the, by P75 observed, higher axospinal density in functional layers I-II and lower density in the deprived ones, indicating a halt of synaptogenesis in the latter. In layers III-IV of the functional cortex, a loss of synapses similar to that of controls occurs leaving, however, the density here still significantly higher than in controls. Contrary to that in the deprived layers III-IV, the loss of synapses was twice as large as that of controls between P21-P75 indicating disuse as a likely cause of this change. From the comparison of our previous results on the synaptic development in the visual cortex (Juraska and Fifkova: J.Comp.Neur.183:257,1979) and the present data, it is obvious that the density of axodendritic synapses between P10 and P15 does not change, whereas, the density of axospinal synapses is considerably increased in this interval and even more so between P15 and P21. Also, qualitative changes occur in spines between P10 and P21. At P10 the spine apparatus is not yet developed; at P15 it has a very immature configuration and in some spines it appears to gain its adult form by P21. (Supported by NIH Grant EY 01500-06.)

171.5 BRIEF PERIODS OF VISUAL EXPERIENCE CAN MODIFY OCULAR DOMINANCE OF CORTICAL NEURONS IN KITTENS. V. S. Ramachandran*, Marylouise Ary* (SPON: M. Konishi). Div. of Biology, Calif. Inst. of Technology, Pasadena, CA 91125.

Kittens 5-6 weeks of age were used to study the effects of brief visual experience on the ocular dominance of cortical neurons. Kittens were deprived monocularly for 2-3 days, and as in earlier studies, the ocular dominance shifted almost completely towards the experienced eye. Four of these kittens had their deprived eye stimulated by rotating high contrast gratings for 3-4 hr while still anesthetized and paralyzed. Recordings soon after stimulation showed a small but significant increase in the extent to which neurons could be driven by the deprived eye. Essentially identical results were obtained in a 5th animal which was not anesthetized or paralyzed during stimulation but was fixed in a head holder. Two control kittens had their deprived eyes exposed to a white screen for 4 hr instead of rotating gratings and no change in ocular dominance was observed. However, if 4 days of darkness was allowed to elapse after grating stimulation, the degree of recovery seen was greater than if the recording was done immediately after stimulation.

Another two kittens were revived from anesthesia and their deprived eye was stimulated on 4 successive days for 1 hr each day. They were kept in total darkness whenever thay were not being stimulated so that the deprived eye received only a total of 4 hr of experience as in the previous animals. In these animals, recovery of functional connections through the deprived eye was even more striking and in fact the two eyes became almost equally dominant. Thus, distributed stimulation may be more effective than massed stimulation. A third control kitten was kept in total darkness for 4 days (i.e., without grating stimulation) and no significant recovery was seen.

We conclude that: a) very brief periods of visual experience (3-4 hr) can modify the eye preference or ocular dominance of visual cortical neurons, and b) even after active synaptic stimulation has ceased, some time dependent process akin to "consolidation" may continue to occur in the dark.

171.6 INTERHEMISPHERIC COMPETITION DURING POSTNATAL DEVELOPMENT. M. Cynader, F. Lepore and J. P. Guillemot. Dept. of Psychol., Dalhousie Univ., Halifax, N. S. and Dept. of Psychol., Université de Montréal, P. Q., Canada.

The corpus callosum is a bidirectional pathway interconnecting the visual cortices on the two sides of the brain. This report shows that it is possible to induce a functional asymmetry in the connections of the corpus callosum by arranging for one hemisphere to receive visual input during postnatal development only via a projection from the other hemisphere.

Four kittens were reared normally from birth until 21 days of age. Then the optic chiasm was surgically sectioned, abolishing retinal input to the opposite side of the brain, and the left eyelid was sutured shut. The kittens subsequently received normal visual exposure until 4 months of age. This procedure ensures that visual input reaches the left visual cortex only via the following pathway: right eye-right lateral geniculate nucleus (LGN)-right visual cortex-left visual cortex (via corpus callosum).

In normal cats, optic chiasm section greatly reduces, but does not abolish the influence of the contralateral eye in the visual cortex. While only 10% of the neurons are driven more strongly through the contralateral eye than the ipsilateral eye (versus 55% in normal cats), over 80% of the units encountered near the area 17/18 border receive some input via the contralateral eye. All contralateral eye input is abolished by removal or inactivation of the opposite hemisphere.

In the kittens described above, recording at the area 17/18 border on the side of the brain ipsilateral to the exposed eye (the "exposed" hemisphere) reveals no input at all from the contralateral (deprived) eye. This finding indicates a loss of functional input from the other side of the brain to the exposed hemisphere. Recordings on the side of the brain ipsilateral to the deprived eye (deprived hemisphere) in the same kittens showed that most neurons (over 60%) receive their dominant input from the contralateral (exposed) eye despite the chiasm section. This indicates a substantial increase in the effectiveness of callosal input from the "exposed" hemisphere to the "deprived" hemisphere.

The results show that the functional effectiveness of callosal connections can be markedly enhanced or diminished on different sides of the same brain. In effect, our manipulation has transformed the corpus callosum from a bidirectional pathway to a one-way route. Information originating in the exposed hemisphere flows to the deprived hemisphere but not in the reverse direction. (This research supported by USPHS Grant EY02248, and grants from the MRC (MT5201) and NSERC (A9939 and A9902) of Canada.

171.7 ORIENTATION COLUMNS IN CATS REARED IN STRIPED CYLINDERS SHOWN WITH ^{14}C 2-DEOXYGLUCOSE. Dorothy G. Flood*, Paul D. Coleman, Robert C. Emerson, and Mary E. Bahn* (SPON: V. Laties). Dept. of Anatomy and Center for Visual Science, Univ. of Rochester, Rochester, NY 14642.

We have demonstrated variable degrees of modification in the preferred orientation of visual cortical cells with single unit recording and also with ^{14}C 2-deoxyglucose (2-DG) following rearing in striped cylinders. Cats were exposed to either horizontal or vertical stripes for 2 or 6 hrs/day for 10 wk. Orientation selectivity was determined physiologically for single cells from the medial bank of the postlateral gyrus. The cells of one cat showed a strong bias for the rearing orientation while those of all others showed no clear bias. Cats reared in striped cylinders and control cats then received 100-150 μCi/kg of 2-DG (NEC-495) while the right visual field (left hemisphere) was stimulated with horizontal stripes and the left visual field (right hemisphere) with vertical stripes (0.3 cycles/deg, moving at 6°/sec, and covering 35° x 45° in each hemifield). After 45 min the hemispheres were removed, frozen at -40°C, sectioned at 20 μm, and exposed to x-ray film. In the control cats, both hemispheres showed a similar amount of labeling. In the stripe-reared cat with a strong physiological bias, the hemisphere seeing the orientation of stripes viewed during rearing was more heavily labeled (Brain Res., 173 (1979) 538-542). In the stripe-reared cats that showed no physiological bias, the hemisphere seeing the orientation of stripes viewed during rearing was either similar to labeling in the controls or slightly more heavily labeled than in the controls. The 2-DG method is a more sensitive indicator of small alterations due to rearing in striped environments. Supported by NSF grant BNS-7912273 and PHS grants AG-1121 and EY-01440.

171.8 VISUAL FIELD DEFICITS OF CATS REARED WITH ROTATION OF ONE OR BOTH EYES. Carol K. Peck, Grayson Barber*, Cindy Pilsecker* and Robert Wark*. Department of Psychology, Pomona College, Claremont, CA 91711.

Following surgical rotation of one or both eyes, cats show substantial evidence of visual function in the rotated eye(s). On most observations of visuomotor behavior, they are difficult to distinguish from normal. The present experiments measured the visual fields of cats which had undergone surgical rotation of one or both eyes at 3 d, 16 d, 3 mos, or 6 mos of age. The horizontal visual field was tested using the procedures previously described by Sprague and by Sherman. Procedures for hemispheric perimetry were also developed to test both vertical and horizontal fields.

All animals showed excellent localization of visual stimuli and responded to the actual location of targets in space rather than to the retinal locus normally associated with that location. This is additional evidence in support of proposals that the cat's nervous system remains plastic to at least 6 mos.

In cats with rotation of only one eye, the visual fields of the unoperated eye were normal, extending from 90° ipsilateral to 30° contralateral. Cats with surgery at 3 or 6 mos had essentially normal fields in the rotated eye as well, while cats with surgery at 3 or 16 d had restricted horizontal fields. Specifically, they responded only to stimuli in the ipsilateral hemifield; they were blind in the contralateral visual field. Their superior and inferior visual fields were normal. The field deficits related consistently to visual field coordinates, could not be explained by simple physical displacement of the retina or other peripheral factors, and were independent of other visual abilities (e.g., acuity). Cats with bilateral rotations could see across the midline with at least one eye. Thus, the extent of the field depends on the sensorimotor experiences of the cat both before and after surgery, but the smaller fields of cats with rotations of one eye cannot be attributed to a competitive disadvantage, as usually understood, because non-corresponding retinal loci are involved.

When tested with both eyes open, half of the experimental cats did not respond throughout the visual field seen by each eye alone. The binocular field was less than the sum of the two monocular fields; greatest losses were found at the temporal margin of the field ipsilateral to the rotated eye. Changes in eye position (e.g., convergence during binocular viewing) were not involved. We suggest that they indicate a suppression of the deviated eye which must have a central origin.

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171.9 PERMANENT DEFICIT IN VISUAL ACUITY FOLLOWING NEONATAL SURGICAL SECTION OF THE POSTERIOR CORPUS CALLOSUM IN CATS. Andrea J. Elberger. Dept. of Anatomy, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104, and Dept. of Neurobiology and Anatomy, Univ. of Texas Med. Sch. at Houston, Houston TX 77025.

Visual acuity in cats was measured by testing the threshold for discrimination of square wave gratings. Using a modified Lashley jumping stand (Mitchell et al, Vision Res. 16:363, 1976), kittens were trained to jump to the projected image of a square wave grating when compared with the projected image of uniform grey of the same mean luminance. Jumping distance was 10, 12, 15 or 18 inches, depending on the age and motor ability of each animal. If 7 out of 10 trials were correct the spatial frequency of the grating was increased by changing the projected image, not by increasing the jumping distance. Images were back projected onto hair-trigger trap doors; an incorrect response caused the trap door to open. No food or water deprivation was used; the cat's desire to avoid a trap door opening was sufficient to induce adequate performance on the behavioral task.

Three groups of kittens were tested: Normal, unoperated cats (3 cats); Anterior Corpus Callosum sectioned cats, with less than 50% of the C.C. length sectioned (4 cats); Posterior Corpus Callosum sectioned cats, with a minimum of the Posteriormost 30% of the C.C. sectioned (7 cats). Surgeries were performed from the day of birth to 22 days. Testing was begun as early as the cat's 40th day of age and continued once or twice per week until at least 175 days of age. Results for Normal and Anterior C.C. cats did not differ significantly, and their data were combined to form a Control group of 7 cats.

Compared with the Control group, the Posterior C.C. cats showed a deficit in visual acuity. From the beginning of testing the Control group improved in spatial acuity gradually until 125-155 days of age, when the stable adult level was achieved (5-7.5 cycles per degree), after which the visual acuity apparently remained unchanged. The Posterior C.C. cats also improved in acuity gradually over time, but they improved at a slower rate than the Controls, and the stable adult level of spatial acuity (2.5-3.5 c.p.d.) remained statistically significantly lower than that of the Control cats. The lowered visual acuity following neonatal Posterior Corpus Callosum section may be related to the disruption in depth perception in kittens tested on the visual cliff (Elberger, Vision Research 20:177-187, 1980), and may be affected by the alteration in binocular vision seen both behaviorally (Elberger, Experimental Brain Res. 36:71-85, 1979) and electrophysiologically (Elberger, Neuroscience Abstract #2630, 1979) in this same type of cat.

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171.10 A POSSIBLE ROLE FOR CYCLIC NUCLEOTIDES IN PLASTICITY OF VISUAL CORTEX. Takuji Kasamatsu. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

We have proposed that catecholamine (CA)-containing terminals in the cerebral cortex may play an important role in the changes in neural connectivity that can take place in the postnatal critical period in response to alterations of sensory experience. The local availability of norepinephrine correlates well with the level of modifiability as determined in experimental paradigms which lead to either an increase or a decrease in the number of normal binocularly driven cells in kitten visual cortex (J. Comp. Neurol., 1979; ARVO Abst., 1978). A later study showed that if the visual cortex was perfused with a β-adrenergic blocker, propranolol, at the same time as monocular lid suture, the usual ocular dominance shift was prevented (ARVO Abst., 1979). These previous results suggested that the level of cyclic nucleotides in the postsynaptic visual cells may be involved in changes in ocular dominance.

Six- to seven-week-old kittens were bilaterally implanted at the postlateral gyrus with a cannula-minipump system which contained 4 mM 6-hydroxydopamine (6-OHDA). After one week the minipump on the left side was replaced with one which was filled with 1-10 mM dibutyryl cyclic AMP (dbcAMP) in 0.4% ascorbate saline (pH 3). The implanted cannulae were left in place at this time, and the right eyelid was sutured. Single unit recordings were made following a week of monocular deprivation. Tungsten-in-glass microelectrodes were placed 2 mm anterior to the site of perfusion.

Three observations have been made. First, the overwhelming majority of sampled cells had normal receptive field properties. Second, the usual shift of ocular dominance was observed in the hemisphere perfused with dbcAMP, although the other hemisphere contained many binocular cells, as expected from our previous results. Since exogenous dbcAMP was perfused into cortex whose CA-terminals had been destroyed by prior 6-OHDA treatment, the present results strongly suggest that the cyclic nucleotide worked directly on the postsynaptic visual cells. Third, under the current paradigm the proportion of binocular cells was lower than normal in the control, nonperfused hemisphere which had been pretreated with only 6-OHDA. This may indicate that at the concentration of cyclic nucleotide employed, some spread of dbcAMP took place from the perfusion site to the other hemisphere. By varying the concentration of cyclic nucleotides stored in the minipump, the concentration-effect relationship is currently being studied.

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172.1 CELL LINEAGE ANALYSIS IN LEECH NEUROGENESIS BY TRACER INJECTION AND ABLATION OF IDENTIFIED EMBRYONIC CELLS. D. A. Weisblat, S. S. Blair* and G. S. Stent. Dept. Mol. Biol. UCB, Berkeley, CA 94720.

Glossiphoniid leeches are well-suited for neurodevelopmental studies because both the early embryo and the segmental ganglia of the adult nerve cord contain large identifiable cells, accessible to observation and experimental manipulation. Five bilateral pairs of teloblasts, consisting of one pair of mesodermal progenitors (M) and four pairs of ectodermal progenitors (N,O,P and Q), produce columns of stem cells that ultimately give rise to the segmental tissues of the animal, including the ventral nerve cord.

By injecting horseradish peroxidase (HRP) into identified blastomeres, we have previously shown that a spatially stereotyped fraction of the neurons of the hemilateral segmental ganglia derives from the ipsilateral N teloblast and that the remainder derive from the ipsilateral OPQ teloblast precursor. Similar HRP injection experiments to be described here have revealed that some descendants of the OP teloblast precursor also appear to occupy stereotyped sites within the developing ganglion, whereas others give rise to non-neural ectoderm. Moreover, some descendants of the Q teloblast migrate medially to the ventral midline from their original position at the extreme lateral edge of the embryo.

Labeling the progeny of one teloblast with HRP in an embryo in which a second teloblast, or teloblast precursor, has been ablated by injection of pronase or deoxyribonuclease reveals an altered spatial distribution of the progeny of surviving teloblasts. If an N teloblast is ablated, some progeny of the surviving N teloblast are found on the ablated, contralateral side. By contrast, the distribution of the progeny of the OPQ teloblast precursor appears unaffected. However, if the OPQ teloblast precursor is ablated, the surviving OPQ teloblast precursor does contribute progeny to the ablated side.

172.2 DEVELOPMENT OF AN IDENTIFIED NEURON AFTER REMOVAL OF ITS PERIPHERAL TARGETS IN GRASSHOPPER EMBRYOS CULTURED IN VITRO. Corey S. Goodman and Kimberly A. Ridge*. Dept. of Biol. Sci., Stanford University, Stanford, CA 94305

We previously reported on the differentiation and cell lineage of the first three progeny of the median neuroblast (MNB) in the metathoracic segment during grasshopper embryogenesis (Goodman and Spitzer, Nature, 1979). The third cell division of the MNB gives rise to a cell which develops into the identified neuron DUM 5 (DUMETi) whose peripheral axons innervate the extensor tibiae (ETi) muscles in each of the two metathoracic legs.

Here we report on the embryonic development of DUM 5 after removal of one or both of its peripheral targets prior to its arrival. This type of experimental manipulation requires that the embryo continue its normal development outside of the egg case and thus in vitro. We have modified the methods of Mueller (Dev. Biol., 1963) such that grasshopper embryos develop in culture from day 7 (35%) to the day of hatching (day 20); this includes having the embryos proceed through katatrepsis in culture.

Our first experimental use of this culture system has been to remove DUM 5's targets. Normally, the axons of DUM 5 arrive at the ETi muscles on day 12 and extend over the muscle by day 13. DUM 5 becomes electrically uncoupled and electrically excitable shortly before this time (days 11-12). Although it initially has many extra peripheral branches, it loses these incorrect branches by day 15. The cell body rapidly enlarges in diameter after day 12 and becomes the largest amongst the MNB progeny.

We removed one or both of the metathoracic legs on days 10-11, and examined the morphology and physiology of DUM 5 on days 13-15. DUM 5 becomes electrically uncoupled and electrically excitable, even when deprived of both targets. However, its cell body does not gain its distinctive size, and it retains some of its multiple peripheral branches. When only one leg is removed, it retains multiple branches on the operated side but loses them on the normal side. In such cases, the cell body develops its distinctive size.

Thus, for the identified neuron DUM 5, we conclude that (i) the development of uncoupling and excitability are independent of direct contact with its normal targets, and (ii) the development of its normal cell body size and peripheral morphology are dependent on interactions with its targets. We are currently using other experimental manipulations in an attempt to understand the interactions of lineage and environment in the survival and differentiation of this identified neuron.

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172.3 EMBRYONIC MORPHOGENESIS OF AN IDENTIFIED SENSORY NEURON. R. David Heathcote* (SPON: David Bentley). Zoology Department, University of California, Berkeley, CA. 94720.

In insect appendages, a class of cells termed peripheral pioneer neurons have been shown to establish the first connection between the peripheral and the central nervous systems (Bate, C.M. Nature 260: 54-56, 1976). Whether pioneer neurons establish such connections for all sensory organs is unknown. To investigate this, I have examined the embryogenesis of the wing Stretch Receptor (SR) sensory complex in the locust, Schistocerca nitens.

SR is a sensory neuron that provides feedback from wing position to flight motor neurons in adult locusts. The morphology of SR, its synaptic connections to identified motor neurons, and its firing pattern in adult flight have been described. The monosynaptic excitation of the First Basalar flight motor neuron by SR throughout postembryonic development shows that this synapse, although mediating an adult behavior, is already present early in development (Heathcote, R.D., J. Comp. Neurol. 191: in press). This suggested that SR has an embryonic origin.

SR was located in living embryonic locusts using Nomarski optics and intracellular injection of Lucifer yellow dye. At approximately 40% of embryonic development, a group of cells located at the junction of the pleuro-tergal border is associated with a folding of the epithelium. Following this, a cell appears which can be distinguished from other cells by its large size, multipolar shape and its association with the epithelium. Dye fills of this cell show; 1) many somal branches interdigitating among the epithelial cells, 2) a single long axon extending toward the CNS and making a Y-shaped branch to two different ganglia and 3) many fine branches from this main axon growing into the epithelium. Dye fills of many other cells in the vicinity of SR show that no other cells have axons at this stage. The tips of the two axonal branches end in expanded and filamentous growth cones. The progress of the growth cones into the CNS was followed by injecting dye into SR at different embryonic stages. Most of the identified central branches of SR were found to be present by 60%. The fine processes branching off of the axon of SR disappear by 55% and are not visible in the oldest animals injected (90%).

The cell studied is unequivocally identified as SR by 1) its unique peripheral branching pattern (it is the only cell to make a Y-shaped branch to two different ganglia), 2) its size, multipolar shape and the location of its soma, and 3) the configuration of its central branching. Therefore, SR is present and reaches the CNS by 45% of embryogenesis (this is much earlier than any bipolar sensory neuron has been shown to reach the CNS). Moreover, since SR is the first neuron from its region to send a process to the CNS, it appears to establish nerve 1D2.

172.4 EMBRYONIC DEVELOPMENT OF GRASSHOPPER GIANT INTERNEURONS AND THE CERCAL SENSORY AXONS WHICH INNERVATE THEM. S. Martin Shankland Neurobiology Group, Univ. Calif., Berkeley, Ca. 94720

The grasshopper has large interneurons (the so-called Giant Interneurons or GIs) in its terminal abdominal ganglion which receive sensory input from the cercus. I have characterized these neurons by axonal diffusion cobalt fills. The four largest axons in the abdominal connective derive from cells whose dendrites associate with the cercal sensory afferents, and will be defined as GIs. Three of these cells appear to be segmental homologues, and send their grouped axons into the lateral region of the connective. The other GI has a quite distinct branching pattern and an axon in the medial connective, and is believed to be homologous to the Medial Giant Interneuron (MGI) of crickets.

In adult grasshoppers, MGI has a contralateral cell body located at the ganglion equator, and a neurite which crosses the neuropil in the horizontal plane. A small dendrite arises at the ganglion midplane, but the major dendrite extends from the junction of neurite and axon. This dendrite forms an egg-shaped cluster of branches. Heavily ramified and interwoven cercal sensory axons terminate over the surface of this dendrite, thus forming a hollow shell of sensory arbor which contacts the tips of the dendritic branches. These two structures coordinately form the cercal sensory glomerulus. The major dendrite has two fine, unbranched processes which continue from the glomerulus ipsilateral to the axon into the contralateral glomerulus.

I have examined the embryonic development of both the sensory axons (Shankland; Neurosci. Abstr. 5:178) and the GIs. Cobalt fills show that the sensory axons first enter the CNS at 65% of embryogenesis. The formation of branching patterns was followed for several different types of sensory afferent. Silver stains of the embryonic neuropil demonstrate that the GIs appear at the same stage. No GI branching patterns were discernable from 35% to 60%, but at 65% a large neuron was identified as MGI on the basis of its branching structure and association of its dendrite with the future site of the glomerulus. The sensory axon terminals and MGI dendrite then mature morphologically during subsequent embryonic stages. Therefore, presynaptic and postsynaptic processes appear simultaneously within the neuropil, and undertake a concurrent arborization during embryonic life which leads to their adult form.

172.5 TOPOLOGY OF AN ADULT INSECT SENSORY NERVE IS ESTABLISHED BY THE PIONEER AXON PATHWAY. Haig Keshishian. Neurobiology Group, Univ. Calif., Berkeley, Calif. 94720.

The development of nerve branch 5b1 of the metathoracic leg of the grasshopper Schistocerca nitens was examined in cultured embryonic preparations, using both time-lapse microphotography and intracellular Lucifer Yellow dye-fills. In adults, 5b1 arises from the subgenual organ on the anterior-proximal region of the tibia, runs along the anterior-ventral side of the femur, and ultimately crosses over to the posterior-ventral side of the femur to fuse with branch 5b2 at the femoral-coxal border (Heitler & Burrows, '77, J.E.B.,66: 221). This branch anatomy is conserved throughout post-embryonic development, and is evident in the embryonic metathoracic leg.

The earliest event in the organization of the nerve branch is the genesis of a pair of pioneer neurons (Bate, '76, Nature, 260: 54), hereafter termed the PN1 neurons. This cell-pair is the sole progeny of a mother cell that appears at the tip of the limb-bud ectodermal epithelium at the 29% stage of embryonic development (Keshishian, Dev. Biol., in press). The mother cell divides within the epithelium, and its progeny migrate out of it to occupy a characteristic locus near the tip, between the ectoderm and the inner squamous mesoderm of the limb-bud. From there the cell-pair initiate axonogenesis, projecting their axons proximally between the two epithelia. Initially running along the anterior side of the limb-bud, the axon pair cross to the posterior side of the limb at the mid-femoral region, growing along the ventral face. The axons continue proximally on the posterior side, and reach the CNS by the 35% stage.

The path traced out by the PN1 axons establishes the topology of the adult sensory nerve branch. This was determined by serially dye-filling the PN1 cell-pair after their initial differentiation, and examining their relationship to the genesis of the nerve branch, using Nomarski optics and timed embryos. As the limb-bud elongates the somata of the PN1 cells become displaced from the tip. Ultimately they come to lie on the anterior-proximal region of the tibia. By the 40% stage the previously naked PN1 axons become ensheathed by glia. As a result, the axons and associated glia become a distinct chain of cells retaining the original topology of the PN1 axons. Dye-fills up to the 55% stage reveal that the most distal elements of this primitive branch are the PN1 somata. By the 45% stage the somata lie immediately proximal to the subgenual organ. Tracing the nerve branch to the coxal border, it fuses with another posterior branch, possibly 5b2. Thus a nerve branch possessing the adult topology is generated around the PN1 cells, providing a substrate for the subsequent fasciculation by metathoracic leg afferents.

172.6 EXPERIMENTAL INFLUENCES ON THE DEVELOPMENT OF CLAW LATERALITY IN LOBSTER. K. S. Kent* and C. K. Govind (SPON: J. G. Hildebrand). Boston University Marine Program, MBL, Woods Hole, MA 02543

The claws of the adult lobster, Homarus americanus, are dimorphic. The cutter claw is long and slender while the crusher claw is large and stout. Initially, both claws resemble the adult cutter. Differentiation of the claws and claw closer muscles occurs over a series of juvenile molt stages. In the fourth stage, the closer muscles are composed of both fast and slow muscle fibers. The proportion of fast muscle increases in the presumptive cutter claw until the closer muscle is predominantly composed of fast muscle fibers. In the presumptive crusher claw, the proportion of fast muscle decreases until the closer muscle is entirely composed of slow muscle fibers. There is an equal chance that the crusher will develop on the right side as on the left side. However, claw laterality can be influenced if one claw is removed prior to the fourth and fifth stages. The remaining claw will become a crusher while the regenerating claw will become the cutter. Earlier results by Lang and co-workers (Science, 201:1037-1038, 1978) suggested that claw use might influence crusher claw determination. Lobsters reared in smooth plastic chambers which lacked added substrate often did not develop a crusher at all.

We have repeated these experiments and further tested the hypothesis that claw use is a factor in crusher claw determination. Lobsters raised from the fourth stage in the presence of mud, gravel, crushed plastic or crushed oyster shells developed dimorphic claws. However, virtually 100% of the lobsters raised in substrate-free chambers did not develop a crusher claw at all. These results suggested that claw use, stimulated by an appropriate substrate, might indeed be a requirement for crusher claw determination. This hypothesis was further tested by manipulating the right claw during the fourth and fifth stages. Banding the right claw closed and cutting off the dactyl of the right claw did not affect claw laterality. As in control conditions, the number of lobsters developing right crushers equalled the number developing left crushers. However, tenotomy of the right claw closer muscle greatly reduced the number of lobsters developing right crusher claws. This treatment prevented isometric tension development in the closer muscle whereas banding or dactylotomy did not. These results indicate that there is some minimum requirement for tension development in the closer muscle at early critical stages in order for crusher claw determination and transformation to occur. The development of a certain level of tension may, in turn, depend upon the presence of an appropriate substrate. The role played by the innervating axons in mediating this tension requirement remains to be resolved. (Supported by NIH and NSF grants awarded to Fred Lang.)

172.7 COLLATERAL SPROUTING OF IDENTIFIED MOTONEURON FOLLOWING MICROLESION IN THE LARVAL PRAWN *MACROBRACHIUM ROSENBERGII*. D.R. Friedlander and C. Levinthal. Department of Biological Sciences, Columbia University, New York, N.Y. 10027.

Our experiments in the CNS of the larval prawn showed that the removal of a motoneuron (the giant motoneuron, or GMN) frequently results in anomalous connections involving interneurons that were deprived of a target cell which has been deleted (Friedlander and Levinthal, Soc. Neurosci. Abstr. 5:245,1979; Friedlander, Ph.D. Thesis, 1980). This paper deals with the effect of the removal of a GMN on the anatomy of the neuromuscular connections in the larval prawn. The GMN contributes the largest axon innervating the deep flexor muscles responsible for the tail flip response. These muscles are homologous to those described in the adult crayfish. Microlesions were produced by focusing a beam of light which has a significant percentage of its energy in the ultraviolet, on a region of the ganglion containing the GMN. We followed the innervation in film strips of aligned light micrographs of 1 μm thick cross sections, stained with toluidine blue. Finer details of the arborizations were analyzed by observation of 20 μm thick horizontal sections of larvae that had been stained in block with silver.

The normal innervation of the flexor muscles is provided by axons of the ipsilateral 4th root (3rd in crayfish). By removing a GMN, the target deep flexor muscles on one side of the abdomen are partially deprived of afferents. The deletion invariably elicited vigorous sprouting of the remaining GMN, which sent collaterals over the midplane invading the partially denervated muscles. Two muscles (Ai and P) normally innervated by the GMN are in contact with their contralateral homologues. The sprouting collaterals innervating the partially deprived Ai and P muscles originate from branches of the axon supplying in a normal way their contralateral homologous muscles.

In agreement with the results of our experiments in the CNS, these observations in the periphery indicate that the normal ipsilateral connections are not due to strict developmental rules that preclude contralateral connections. The results which demonstrate collateral sprouting in the periphery in the larval prawn are in contrast with those of lesions denervating muscles of adult Crustacea Decapoda, in which no collateral sprouting was observed (Bittner and Johnson, J. Comp. Physiol. 89:1-21,1974).

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172.8 NORMAL AND REGENERATED CNS SYNAPSES IN THE CRAYFISH DO NOT DIFFER. R.J. Strandburg and F.B. Krasne, Dept. Psychology, UCLA, Los Angeles, CA. 90024.

In contrast to the situation in mammals, a number of examples of regeneration of central connections can be found in invertebrates. The availability of identified central interneurons to which regeneration can occur in these preparations presents an opportunity for detailed analysis of such synapses which has, heretofore, been only partially exploited. Hence, in this study, the ability of regenerating sensory axons to establish normal connections with an identified CNS target cell in the crayfish was assessed intracellularly. Comparisons were made between normal crayfish (N) and regenerate preparations (R) in which root 4 (R4) had been cut and directed back to an extensively denervated (roots 2-5 severed) sixth abdominal ganglion. Eight to fifteen weeks postoperatively, an identified interneuron (A) in this ganglion was impaled, and its response to water currents, electrical stimulation of R4, and stimulation of individual sensory hairs supplying axons to R4 was studied along with several other properties of the pre- and post-synaptic neurons.

Overall, few differences were observed between N and R. In particular, unitary EPSP amplitude distributions were comparable across groups, and axons from each of three subregions of R4's receptive field that were examined had similar probabilities of reconnection with A in both groups. While the resting membrane potential and the critical firing level did not vary between N and R, input impedance was significantly lower in R. Coupled with the unitary EPSP amplitude data, this suggests that individual axons in R may provide greater synaptic input to A than their N counterparts, consistent with the possibility that regenerating afferents (which are growing to an extensively denervated target) are sprouting collaterally.

Compound electrically elicited EPSPs were similar in amplitude, rise time and half amplitude width, but differed slightly in latency to onset (R>N). This was accounted for by differences in the conduction time to the ganglion for regenerated axons. Central delay estimates further suggest that connections in both groups were monosynaptic.

The response to giant interneuron activation demonstrated that recurrent presynaptic inhibitory inputs as well as postsynaptic excitatory and inhibitory inputs were normal in R. Synaptic depression, which is normally responsible for behavioral habituation in this system, was comparable across groups, and protection from habituation was observed in both N and R if stimulation was preceded by giant interneuron activation.

172.9 EFFECT OF A LIMITED TARGET AREA ON THE REGENERATION OF SPECIFIC NEUROMUSCULAR CONNECTIONS IN THE CRAYFISH. Samuel J. Velez and William P. Hunt*. Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755.

The superficial flexor muscle of the crayfish is innervated by six axons that distribute their innervation in a very specific pattern over the muscle surface (Velez & Wyman; J. of Neurophys. 41: 75-96). Axon 6, the largest excitor, grows first through medial muscle fibers making some synaptic connections with them (the probability of these connections increases as the nerve gets closer to the lateral muscle fibers). Axon 6 innervates all the lateral fibers of the muscle, the lateral population being its main target area. The distribution of connections made by this axon was studied after the nerve was cut, and in previous work it was shown that full innervation of the entire muscle was achieved between 5 and 10 weeks when the nerve was cut between medial fibers (Ely & Velez; Soc. Neurosci. Abstr., Vol. 5, p. 677, 1979). Experiments are being performed to study the effects of a reduced target area on the regeneration of the different neurons in this system. In one group of animals the nerve is cut as it begins to cross the medial muscle fiber population, and the lateral population of fibers is removed. In a second group of animals the medial population of muscle fibers is removed and the nerve is transplanted to the lateral muscle fibers. The animals were examined after several weeks by stimulating the nerve and recording junction potentials from the muscle fibers. Preliminary results indicate that in the group where axon 6 was growing into the lateral population only, this axon regenerated all its connections in 4 weeks; when axon 6 was growing into the medial population only, the first connections were being formed by 4 weeks and full innervation was achieved several weeks later. The time course for regeneration appears to be influenced by the nature of the target area: axon 6 regenerates at a faster rate when it is growing into its main target area when compared to regeneration rate when growth occurs outside its main target area. The type of synapses formed under these experimental conditions is currently under investigation.
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172.10 MOTOR NEURON ASYMMETRY AND ITS ALTERATION IN SNAPPING SHRIMP. DeF. Mellon and J. A. Wilson, Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901

In alpheid shrimps the major claws exhibit a basic asymmetry which includes not only external morphology and overall size, but also the fine structure and junctional physiology of fibers in the major closer muscles, Cl_1 (Wilson, E.B., Biol. Bull., 4:197, 1903; Stephens, P.J., Mellon, DeF., J. Comp. Physiol.,132:97, 1979; Mellon, DeF., Stephens, P.J., J. Exp. Zool. In Press). We now report that these differences reflect predictable asymmetries in the structure of the motor neurons which supply Cl_1. The closer muscles of pincer and snapper claws of Alpheus are each innervated by two excitor and two inhibitor axon (Mellon, DeF.,Stephens, P.J., J. Comp. Physiol., 132:109, 1979). We have used cobalt backfilling of limb nerves and intracellular injection of cobalt into physiologically identified motor neurons to determine the location of the somata and the extent of dendritic arborization of these four cells. The cell body of one of the inhibitor motor neurons (DoPI) is paired with its contralateral functional homologue at the dorsal midline of the first thoracic ganglion. In pristine individuals (those in which claw asymmetry is completely expressed) the DoPI cell body of the snapper claw is twice the diameter (80-100 μM) of the pincer claw DoPI. The size difference in this neuron pair characterizes a general but less dramatic asymmetry in cell volume of the other motor neuron pairs. Differences in electrical properties are also expected and are now being sought. Pincer claws of Alpheus can be transformed into snapper claws following loss of the original snapper or damage to its nerve supply (Wilson, E.B., op. cit.;Mellon, DeF., Stephens, P.J., Nature, 272:246, 1978). Dramatic changes in neuron size and placement occur during transformation. DoPI cell body drops down to a mid-ventral position within the ganglion and its volume increases to equal that of the original snapper DoPI. It is known that an intact nerve supply to the pincer claw is necessary for claw transformation to occur, but the nature of this determinative relationship is not understood. Our observations of neuronal asymmetries and alterations lend support to the possibility that fundamental change in claw motor neuron properties initiates and provides the primary control in the transformation process.
Supported by USPHS grant NS 15006.

172.11 ELECTRICAL PROPERTIES AND INNERVATION OF IDENTIFIED MUSCLE FIBERS IN THE CRAYFISH DURING GROWTH. Gregory Lnenicka* and DeForest Mellon, Jr. (SPON: T.R. Johns). Dept. of Biol., Univ. of Virginia, Charlottesville, VA 22903.

The electrical properties, and innervation of identified muscle fibers in the crayfish superficial flexors were studied at various stages of growth. Each of the paired, sheet-like, superficial flexors of the third abdominal segment receives innervation from six identified motor neurons in a predictable pattern (Velez and Wyman, J. Neurophysiol. 41 75 1978). By limiting our studies to the two most lateral muscle fibers in the sheet, we have examined elements with predictable electrical properties, which are reliably innervated by two identified excitatory motor neurons. This allows the detection of changes in muscle fiber electrical properties, and innervation during growth.

Experiments were conducted to determine the muscle fiber length constant, input resistance (R_o), time constant, and diameter (d). The electrical constants were then calculated on the basis of cable theory. Measurements were made on crayfish ranging in size from 1 cm. carapace length (wt. 0.2g) to 5 cm. carapace length (wt. 24g). The lateral muscle fiber diameter increased from 20μ in the small animals to 320μ in the large animals.

If muscle fiber resistivity (Rm) and internal resistivity (Ri) are constant, R_o is predicted by cable theory to be proportional to $d^{-3/2}$. Although Rm decreased slightly during growth, the decrease in R_o was accurately predicted throughout the range of diameters by $d^{-3/2}$ ($p<.001$). For the range of muscle fiber diameters studied, approximately a 60-fold change in R_o was observed. Specific membrane capacitance (Cm) increased linearly with d ($p<.001$). Cm of the largest muscle fibers was nearly 7 times greater than the smallest muscle fibers. A linear increase in Cm with d has been reported for vertebrate muscle fibers (Hodgkin and Nakajima. J. Physiol. 221 105 1972), and appears to be due to a contribution of the T-tubules to Cm.

Preliminary measurements of EJP amplitudes for one of the excitatory axons indicate that no significant change occurs as the muscle fibers grow from a 20-60μ diameter to a 160-200μ diameter. Therefore, we expect to find an increase in quantal output which compensates for the decrease in R_o, and increase in Cm, during growth. Previous experiments with lobster muscle fibers have demonstrated a compensatory increase in quantal output during a 2-2.5-fold decrease in R_o (DeRosa and Govind, Science 273 676 1978).

Experiments are currently in progress to determine the effect of retarded muscle fiber growth upon the increase in quantal output, and conversely, the effects of reduced innervation upon muscle fiber growth. (Supported by a grant from the Muscular Dystrophy Association).

172.12 SYNAPTIC DIVERSITY IN THE DIMORPHIC CLAWS OF THE SNAPPING SHRIMP. Christine E. Phillips and DeForest Mellon, Jr. Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901.

The first pair of chelipeds in alpheid shrimp are asymmetric, one a large snapper claw and the other a smaller, more slender pincer claw. Removal of the snapper claw results in the transformation of the pincer into a snapper over the next several moult cycles (Wilson, E.B., Biol. Bull. 4 (1903) 197-210). The main closer muscles in both claws are innervated in a similar pattern by 2 excitatory and 2 inhibitory motorneurons, but their synaptic physiology is different. EJPs in the snapper range up to 30mV with few failures and they show facilitation at 10 Hz. Pincer EJPs fluctuate in size (≤12mV) with frequent failures and poor facilitation (Stephens, P.J., Mellon, D., J. Comp. Physiol. 132 (1979) 97-109).

The closer muscles in the two claws are innervated by contralaterally homologous neurons. As noted above, during transformation of the pincer into a snapper, the synaptic physiology of the existing pincer innervation undergoes transformation also. Therefore we were interested to see if the differences in the synaptic physiology of the two claws were reflected in their synaptic untrastructure. Nerve terminals on muscle fibers from the two claws were serially sectioned and compared. Measurements were made of terminal size, area of the terminal devoted to synaptic density and numbers of synaptic vesicles. Nerve terminals were found on the periphery, as well as deep within the fibers, and both types were measured. In snapper fibers the size of the terminal area, as well as the area devoted to synaptic densities, exceeded those in the pincer fibers by as much as a factor of 2. The numbers of synaptic vesicles per terminal was greater in snapper fibers also. By these criteria and in good agreement with its synaptic physiology, innervation of the pincer claw appeared to be in an immature state as compared with that of the snapper.

Supported by grant NS 15006 USPHS to D. Mellon, C.E. Phillips is a University of Virginia, Department of Biology, postdoctoral fellow.

173.1 PROTEIN PHOSPHORYLATION: A POTENTIAL "ACTION-SITE" OF MEMBRANE-LOCALIZED RECEPTORS. Yigal H. Ehrlich. Depts. of Psychiatry and Biochem., Univ. of Vermont College of Med., Burlington, VT 05405

The discovery of high affinity binding-sites for various neurotransmitters and neurohormones was accompanied by the admonition that site-occupancy alone cannot account for receptor function. Thus, it has been postulated that the binding of agonists induces a conformational change, which triggers a chain of events leading to neuronal responses. Such a transducing mechanism is referred to here as an "action site" of the receptor. The cyclic phosphorylation and dephosphorylation of proteins produces rapid and reversible conformational changes which have been implicated in the regulation of numerous neuronal functions. This study was initiated to determine whether endogenous phosphorylation systems in synaptic membranes constitute a target for various neurohormones and neurotransmitters, and thus have the potential for serving as an "action-site" of their respective receptors.

Preparations containing synaptic membranes were obtained from the cerebra, cerebral cortices or neostriata of adult albino rats. Endogenous phosphorylation assays were carried out by incubating the membranes in the presence of γ-^{32}P-ATP, with addition of various agonists and antagonists to the preincubation and/or reaction medium. For analyzing calcium/calmodulin-dependent phosphorylation, membranes were prepared from synaptosomes osmotically shocked in the presence of 50μM $CaCl_2$.

Addition of the potent opiate agonist etorphine or the opioid peptide β-endorphin (1-10 nanomolar) to the reaction mixture resulted in over 200% increase of endogenous phosphorylative activity in the membranes (Ehrlich,Davis,Keen and Brunngraber, Life Sci., In Press). SDS-polyacrylamide gel electrophoresis and autoradiography revealed that the protein bands whose phosphorylation was stimulated by this addition co-migrated with proteins the phosphorylation of which is dependent on calcium ions and calmodulin (Schulman and Greengard, Nature 271:478, 1978). The specific antagonist of opiate receptors, naloxone (1μM), completely blocked the stimulatory effects of β-endorphin and etorphine on protein phosphorylation. Further studies revealed that this endogenous phosphorylation system is affected also by agonists of dopamine, norepinephrine, acetylcholine and benzodiazepine receptors, tested in the presence and absence of the appropriate antagonists. These results are compatible with the hypothesis (Ehrlich, Adv. Exptl. Med. Biol., 116:75, 1979) that conformational changes induced by altered protein phosphorylation may constitute a transducing-mechanism triggered by the interaction of ligands with membrane-localized receptors.

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173.2

Withdrawn by Author

173.3 OPIOID RECEPTORS UNDERGO AXONAL FLOW. W.S. Young III, J.K. Wamsley, M.A. Zarbin* and M.J. Kuhar. Depts. of Pharmacology and Psychiatry, The Johns Hopkins University School of Medicine, Balto., Md. 21205.

Previous studies in our laboratory indicate the presence of opiate receptors on axons of the rat vagus nerve and on other small diameter fibers. These observations led to the suggestion that all or some of these receptors might be undergoing axoplasmic flow and/or axonal or dendritic transport (Atweh et al., Neuropharmacol. 17:65, 1978). We have further examined the distribution of opioid receptors in the rat vagus and elsewhere by in vitro-labeling autoradiography, a very sensitive measure of receptors (Young and Kuhar, Brain Res. 179:255, 1979). For autoradiographic studies, 8-10u sections were preincubated with 100mM sodium and 50uM GTP to rapidly dissociate endogenous ligands. The sections were then incubated with tritiated or iodinated opioid peptides for 40 min at room temperature and given two 5-min washes. Adjacent sections were co-incubated with 1uM naloxone to produce blanks. Autoradiograms were generated by the apposition of emulsion-coated coverslips.

High densities of opiate receptors were found in association with cell bodies in the nodose ganglion and in the vagus nerve trunk in the neck. Thus, opioid receptors appear in all parts of the vagus system; perhaps, particularly, in the sensory part. Ligation of the vagus nerve in the neck led to a striking buildup of receptor binding at the proximal side of the ligation which was distal to the cell bodies in the nodose ganglion. This part of the sensory cell is analagous to the dendrite of the central neuron. The accumulation of binding sites was greater at 24 hrs than at 8 hrs. The roughly estimated flow rate was about 4mm/day although this may be an underestimate. The site appears to be a valid receptor site rather than just a peptide binding site because it binds a variety of radiolabeled agonists and antagonists which are displaced by various opiate antagonists and shows stereospecificity. Agonist binding also shows a GTP shift in the vagus.

Since, after synthesis in the cell body, receptor protein must be moved to distant parts of the cell, it would not be surprising if receptors underwent axonal or dendritic movement. Thus, such movement would be a common property of most or all receptors.

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173.4 BIOCHEMICAL AND PHARMACOLOGICAL SIMILARITIES BETWEEN HIGH AFFINITY MU, KAPPA, DELTA AND SIGMA OPIATE RECEPTOR BINDING Gavril W. Pasternak, George C. Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, N.Y., N.Y. 10021

Three irreversible opiates active both in vitro and in vivo have been synthesized: the agonist oxymorphazone and the antagonists naltrexazone and naloxazone. ^{3}H-D-ala-met-enkephalinamide binding in extensively washed brain membranes was reduced following in vitro incubations with 10 μM oxymorphazone (-63%), naltrexazone (-73%), and naloxazone (-69%; all $p<0.001$) while binding in oxymorphone, naltrexone and naloxone treated controls was unaffected. In vivo administration of these newly synthesized compounds significantly lowered receptor binding in washed membranes for over 24h with the decrease slowly disappearing over 3 days. Nonopioid hydrazines were without effect. Oxymorphone was analgesic in only 17% of mice (n=12) after 24h while oxymorphazone remained analgesic in 82% (n=11) for 24h and in up to 50% for 48h. Detailed experiments demonstrated that naloxazone's prolonged inhibition of binding resulted from a relatively selective blockade of high affinity mu, kappa, sigma and delta receptor binding, as demonstrated by Scatchard analysis of the following ^{3}H-ligands: naloxone, naltrexone, diprenorphine, morphine, dihydromorphine, ethylketocyclazocine, met-enkephalin, D-ala-met-enkephalinamide and SKF 10,047. α and β adrenergic, muscarinic acetylcholine, and benzodiazepin receptor binding were not affected by naloxazone pretreatment. Naloxazone administration in vivo markedly attenuated the analgesic potency 24h later of both morphine (ED_{50} increased 12-fold) and ketocyclazocine (ED_{50} increased 7-fold) as compared to naloxone treatment. The analgesic potency of β-endorphin (1μg,icv; $p<0.001$), D-ala-met-enkephalin (50μg, icv; $p<0.01$) and D-ala-met-enkephalinamide (30μg, icv; $p<0.005$) was also markedly reduced in naloxazone, but not naloxone, animals. Despite dramatic effects on analgesia, naloxazone had little effect on morphine lethality compared to naloxone controls (LD_{50} 436$\pm$25 mg/kg and 444$\pm$40 mg/kg respectively). These results raise the possibility that the analgesic properties of the various classes of opiates, enkephalins and endorphins are all mediated through a single population of high affinity binding sites while other pharmacological actions might be produced through different receptor mechanisms.

173.5 INTERACTION OF [^{3}H] CYCLAZOCINE WITH MULTIPLE OPIATE RECEPTOR SITES. Stephen R. Zukin* and R. Suzanne Zukin* (SPON: W. Norton) Dept. of Psychiatry, Mt. Sinai Sch. of Med., New York, N.Y. 10029

Cyclazocine is a psychotomimetic opiate of the benzomorphan group that has been postulated to interact with μ, K, and σ opiate receptors (Martin et al., J. Pharmacol. Exp. Ther. 197, 517 (1976)). In an effort to understand the molecular mechanisms involved in the neuropharmacological actions of this and closely related opiates, we have studied the binding of [^{3}H] cyclazocine to rat brain homogenates. Specific binding, defined as total binding minus binding in the presence of 1 μM nonradioactive cyclazocine, constituted approximately 92% of total binding (at 1.0 nM [^{3}H] ligand) and 67% of total binding (at 100 nM [^{3}H] ligand). Scatchard analyses reveal the interaction of this drug with three distinct binding sites characterized by affinities of 1.2 nM, 9.1 nM and 65 nM (50 mM Tris-HCl buffer, pH 7.4 at 4° C). In contrast, many radiolabelled classical opiates and opioid peptides exhibit biphasic binding, but do not exhibit binding to such a low affinity site. The high and low affinity [^{3}H] cyclazocine sites exhibit differential sensitivities to sodium and also to the selective sulfhydryl reagent N-ethyl maleimide. In addition, all three sites exhibit greater than 50% loss of specific binding following incubation with trypsin (5 μg/ml) for 15 min at room temperature, and greater than 80% loss of specific binding following incubation at 60° C for 15 min in the absence of added reagents. Together, these findings indicate that all three sites have a protein-like component. Competition analyses involving rank order determinations for a series of opiates and other drugs indicate that the cyclazocine binding sites represent, in order of decreasing affinity, the classical opiate receptor (the putative "μ" receptor), a second as yet uncharacterized opiate binding site, and the specific [^{3}H] phencyclidine binding site. Specific [^{3}H] phencyclidine binding can be displaced by cyclazocine (IC_{50} = 200 nM) and by related benzomorphans, but not by morphine or naloxone. We thus propose a common binding site in rat nervous tissue for phencyclidine and some of the benzomorphan opiates. Supported by NIH grant DA-01843.

173.6 EFFECT OF NALTREXONE ON REGIONAL OXYGEN SUPPLY AND CONSUMPTION IN CATS. E. Buchweitz, L. Grandison, and H. R. Weiss*. Dept. of Physiol. & Biophysics, Coll. Med. & Dent. NJ, Rutgers Medical School, Piscataway, NJ, 08854.

In the present study, quantitative regional O_2 consumption and supply were measured in brain during competitive opiate receptor blockade by naltrexone. Fourteen adult mongrel cats, tranquilized with ketamine and anesthetized with α-chloralose, were artificially respired. A left thoracotomy was performed. Left atrial and femoral artery catheters were inserted. ^{141}Ce microspheres (15 ± 3μ in diameter) were injected and cerebral blood flow determined. In seven cats, 1 mg/kg of naltrexone was administered and twenty minutes later ^{85}Sr labelled microspheres were injected. The cats' heads were quickly guillotined in two places and frozen in liquid N_2. Nine different regions were examined. Arterial and venous O_2 saturations were measured in each region with a Zeiss microspectrophotometer. O_2 consumption was determined as the product of flow and O_2 extraction. Blood pressure, heart rate and blood gases were within the normal range, and were not altered by treatment. Cerebral blood flow was significantly decreased to 63% of the control value. Flow to the pons, medulla and hypothalamus were decreased most by treatment. Cerebral oxygen consumption was significantly decreased to 51% of control. O_2 consumption in the pons, medulla and hypothalamus were decreased most by treatment. The effects of naltrexone on oxygen consumption and blood flow were not restricted to brain regions high in opiate receptor binding sites. The effects of naltrexone were more wide spread than the anatomical distribution of receptors would indicate. The O_2 supply/consumption ratio was not altered by treatment. Oxygen supply was adequate to meet metabolic demand throughout the brain during naltrexone treatment. (Supported by NIH grant HL21172 and DA2395).

173.7 PHENOXYBENZAMINE IRREVERSIBLY ALKYLATES OPIATE RECEPTORS IN VITRO BUT NOT IN VIVO. Vina R. Spiehler, Department of Psychobiology, University of California at Irvine, Irvine, CA 92717.

Phenoxybenzamine (PBz) irreversibly displaces ^{3}H-naloxone (Spiehler, V.R., et al., Mol. Pharmac. 14:587, 1978), ^{3}H-dihydromorphine and ^{3}H-D-Ala-enkephalin binding (Robson and Kosterlitz, in Endogenous and Exogenous Opiate Agonists and Antagonists, Pergamon 1980, 107.) from brain homogenates in vitro. This alkylation is prevented by preincubation of the homogenates with levallorphan, dihydromorphine or D-Ala-enkephalin respectively. However, the antinociceptive effect of PBz is antagonized by naloxone given two hours after PBz administration (15 min before the peak antinociceptive effect) rather than before PBz administration. Therefore, the assumption that PBz exerts its opiate-like effects by acting as an irreversible ligand of opioid receptors (Jacob, J., et al. ibid, 99) in vivo, was tested by examining the brains of animals which had received PBz for the ability to bind ^{3}H-naloxone or ^{3}H-morphine at different times after s.c. administration of the drug.

Male Swiss-Webster mice (20-30g) were given 100 mg/kg PBz or saline dilution of solvent s.c. and killed 1/2 and 2 1/2 hrs after treatment. There was no significant difference in ^{3}H-naloxone binding between control and PBz-treated animals after a single wash of the brain homogenate. Therefore, PBz did not alkylate the naloxone binding sites in vivo at the time of the peak antinociceptive effect (2-3 hrs). However, there was an increase in ^{3}H-naloxone binding in the PBz-treated animals as compared to controls ($p < 0.02$) after three washes of the homogenate at 2 1/2 but not at 1/2 hrs after PBz treatment. Similarly, ^{3}H-morphine binding was increased in PBz-treated animals ($p < 0.02$) after three washes at 2 1/2 but not 1/2 hrs after treatment.

The apparent receptor expansion or increased ligand lability after subcutaneous PBz suggests that PBz is not an irreversible alkylating agonist of the opiate receptor in vivo. The long-term effect observed by Jacob et al. (ibid. 99), may be due to PBz enhancement of learning or memory consolidation in their paradigm (Gold, P. and Sternberg, D., Sci. 201: 367, 1978).

173.8 BINDING OF ^{3}H-2-CHLOROADENOSINE (CADO) TO RAT BRAIN SYNAPTIC MEMBRANES. M. Williams and E. A. Risley*. Merck Institute for Therapeutic Research, West Point, PA 19486.

Adenosine (ADO) has a pronounced depressant effect on cell firing and is a potent modulator of tissue cyclic AMP levels in the mammalian central nervous system, actions thought to be mediated via extracellular receptors sensitive to blockade by methylxanthines such as theophylline. The binding of ^{3}H-CADO (specific activity, 12 Ci/mmol), a slowly metabolized analog of ADO, was examined in synaptic membranes prepared from rat brain. Specific binding was only 20% of total unless membranes were pretreated with adenosine deaminase (ADA) to remove endogenous ADO (Bruns, R.F., Fed. Proc., 39: 1010, 1980); following such treatment, specific binding was 90-95% of total and was found to be saturable, reversible, temperature-dependent and sensitive to theophylline (IC_{50} = 9 μM). Two binding sites were observed of Kd's 1.5 and 16 nM with corresponding Bmax's of 224 and 384 fmoles/mg protein. Highest specific binding was found in enriched synaptosomal subfractions (P_2B) of rat brain, a finding consistent with a role for ADO as a neurally active substance. While ADO itself was weakly active (IC_{50} = 20 μM) presumably due to residual ADA activity, CADO was a potent inhibitor of binding (IC_{50} = 2.6 nM). Adenine, inosine, hypoxanthine and d-ribose were without significant effect on binding at 100 μM as were diazepam, α- and β-adrenergic, dopaminergic and cholinergic blocking agents. These findings provide additional evidence for there being a specific extracellular receptor through which ADO modulates nervous tissue function.

173.9 BENZODIAZEPINE (BZ) RECEPTORS ARE INDIRECTLY COUPLED TO GABA RECEPTORS THROUGH ANION RECOGNITION SITES. R.F. Squires, Rockland Research Institute, Orangeburg, NY 10962

Specific binding of ^{3}H-flunitrazepam (^{3}H-FLU) to rat brain membranes has an absolute requirement for an anion. Using both unwashed, and extensively dialysed P2 fractions ^{3}H-FLU binding is greatly enhanced by a variety of anions, but not by GABA alone. Using dialysed membranes, GABA increases ^{3}H-FLU binding 50-60% in the presence of anions, and increases their affinities for anion recognition sites. Conversely, a group of GABA mimetics including THIP, isoguvacine (IGV) and piperidine-4-sulfonic acid (P4S) decrease the affinities for anions without greatly affecting maximum ^{3}H-FLU binding. These effects of THIP, isoguvacine and P4S are fully reversed by GABA in a competitive way. THIP, P4S and IGV are pure GABA antagonists in this system but are pure GABA agonists electrophysiologically. The affinity constants for chloride, bromide and iodide are increased from about 4 mM to near 40 mM in the presence of THIP (a maximal effect). Citrate, maleate, phosphate and bicarbonate are more potent promoters of ^{3}H-FLU binding with Kd values near 1 mM, which are increased 4 to 10-fold maximally by THIP or P4S. In calf brain chloride ion promotes ^{3}H-FLU binding with higher affinity in hippocampus and cerebellum (Kd= 2mM) than in cortex (Kd=4mM). Thip increases the Kd for chloride about 12-fold in cortex and cerebellum but only 6 fold in hippocampus. Hill coefficients for chloride are less than unity in all three calf brain regions. The potent activating effect of bicarbonate is of special interest in view of the anticonvulsant effects of carbonic anhydrase inhibitors and CO_2, and the ability of bicarbonate to substitute for chloride in the generation of inhibitory post-synaptic potentials (IPSPs). At least 25 anions can promote ^{3}H-FLU binding. However, several of these do not substitute for chloride ion in IPSP generation probably because they are too large, diffuse too slowly, or act as chloride antagonists. Several anions, including chloride, bromide and bicarbonate, exhibit "flat" concentration-response curves with Hill coefficients less than unity providing further evidence for two or more anion recognition sites giving rise to multiple BZ/ion/GABA receptor complexes. Although many GABA receptors are apparently not coupled to BZ receptors, the pervasive effects of GABA receptor active substances on ^{3}H-FLU binding, suggest that virtually all BZ receptors are coupled to GABA receptors which serve to regulate a common ionophore. Anion recognition sites seem to be "sandwiched" between GABA receptors on one side and a BZ receptor on the other, with little direct interaction between GABA and BZ receptors. I speculate that the "endogenous ligand" for the BZ receptor is an excitatory neurotransmitter mediating anxiety, some convulsive disorders, wakefulness and fine motor coordination, and is specifically counterbalanced by GABA.

173.10 [^{3}H]DIAZEPAM BINDING IN DISSOCIATED PRIMARY CORTICAL CELL CULTURE: A PHARMACOLOGICAL CHARACTERIZATION. P. Mallorga,* J. F. Tallman, R. C. Henneberry* and D. W. Gallager. Biological Psychiatry Branch, NIMH, Bethesda, MD 20205 and Molecular Biology Lab., NINCDS, Rockville, MD 20852

Binding of [^{3}H]diazepam was measured in membranes prepared from fetal rat cortical tissue grown in dissociated cell culture. [^{3}H]Diazepam binding was pharmacologically characterized by its ability to be displaced by the centrally active benzodiazepine, clonazepam, and by the centrally inactive benzodiazepine, RO5-4864. In these cultures total [^{3}H]diazepam binding increased with age in culture from 214 fmol/mg protein on Day 5 to 447 fmol/mg protein on Day 11 (measured at 5 nM [^{3}H]-diazepam). When cultures were treated with an antimitotic agent, cytosine arabinoside, a "neuronal-enriched" culture was obtained. 5 nM [^{3}H]diazepam binding on membranes from these "neuronal-enriched" cultures was maximally displaced by 10^{-7} M clonazepam (K_i = 5 x 10^{-9}), while RO5-4864 had little potency at this concentration (K_i >10^{-5} M). This is similar to pharmacology observed in adult rat cerebral cortex. In contrast, membranes obtained from peripheral tissue and non-neuronal cultures of rat C_6 glioma and NB_2A neuroblastoma cell lines also exhibit [^{3}H]diazepam binding, but this binding is displaced maximally by 10^{-7} M RO5-4864 (apparent K_i = 5 x 10^{-9} M) and only slightly displaced by 10^{-7} M clonazepam (apparent K_i = 2 x 10^{-6} M). Thus, the ratio of 10^{-7} M clonazepam displaceable binding to 10^{-7} M RO5-4864 displaceable binding was chosen to characterize the type of [^{3}H]diazepam binding. Neuronal enriched cultures had a ratio of clonazepam (10^{-7} M)/RO5-4864 (10^{-7} M) displaceable binding of 2.2 which remained constant at increasing age in culture. If primary cell cultures were not treated with antimitotic agents resulting in the apparent overgrowth of non-neuronal elements, total [^{3}H]diazepam binding at 11 days in culture was 294 fmol/mg protein (measured at 5 nM [^{3}H]diazepam) and a ratio of clonazepam (10^{-7} M) displaceable to RO5-4864 (10^{-7} M) displaceable binding of 0.88. These data suggest that clonazepam displaces primary neuronal [^{3}H]diazepam binding while RO5-4864 displaces primary non-neuronal binding.

173.11 RAPID REDUCTION OF ^{3}H-DIAZEPAM BINDING IN CULTURED HIPPOCAMPAL CELLS. C.R. Walker and J.H. Peacock. Dept. of Neurology, Stanford University Medical School, Stanford, CA 94305.

Metrazol or electrically induced epilepsy in rats increases ^{3}H-diazepam (Dz) binding in brain tissue within minutes. These changes return to normal within 1 hr. and are due to an increase in receptors. These data suggest that the Dz receptor in brain can react rapidly to physiological changes.

We have found a rapid and reversible decrease in ^{3}H-Dz binding in embryonic mouse hippocampal cells in culture after incubation in serum free media. A loss of 75-80% of initial ^{3}H-Dz binding occurs within 1 hr. Recovery of 50% is found within 1 hr. and complete recovery after 24 hr. in media containing serum. The initial 50% recovery can occur in the presence of 10 µM cycloheximide, but the requirement for protein synthesis for complete recovery has not been determined because of cycloheximide toxicity.

Metrazol, 20 mM, antagonizes the loss by 30% but does not affect ^{3}H-Dz binding in serum incubated cells. Dz, 20 mM, has no effect on the loss of binding during serum free incubation. Interestingly, sodium bicarbonate, a component of the serum containing media also antagonizes the loss of binding by approximately 10-20%.

A kinetic analysis of ^{3}H-Dz binding to serum free incubated cells suggests that both the binding affinity and number of binding sites are decreased. This may account for the two component recovery of binding. Serum free media may cause a conformational change in the binding site that results in a decrease in binding affinity and also increases the degradation of the binding site. Serum replacement may allow the binding site to change conformation and restore part of the loss. New binding sites would be required to replace those lost due to degradation.

Experiments with the glial cell line, C6, demonstrate an association between dynamic cell shape changes and ^{3}H-Dz binding. Within 30 minutes after serum removal, cells change from a flattened cuboidal shape to an ellipsoid shape with bipolar processes. This dramatic change in cell shape is accompanied by a rapid and reversible loss in ^{3}H-Dz binding within 1 hr.

In summary, the binding site for diazepam on cultured hippocampal cells and C6 can be rapidly changed by incubation in serum free media. The binding kinetics suggest both a decrease in binding affinity and number of binding sites. This phenomenon may represent membrane alterations that affect both the affinity of the binding site and its stability.

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173.12 SOLUBILIZATION OF THE NEUROLEPTIC BINDING SITE OF HUMAN BRAIN. A. Davis, B.K. Madras and P. Seeman, Psychopharmacology Section, Clarke Institute of Psychiatry, Toronto, Ontario, M5T 1R8.

Dopamine (D-2) receptors were successfully solubilized from human putamen using the non-ionic detergent, digitonin. The ligand used was ^{3}H-spiperone with 1 µM (+)-butaclamol to define specific binding. A 1% (w/v) solution was added to a membrane preparation of combined mitochondrial (P_2) and microsomal (P_3) pellets, incubated at 0°C for 30 min and then centrifuged at 105,000 x g for 60 min (according to the method of Madras et al., 1980). Electron microscopy indicated the supernatant was free of membrane fragments. Membrane-bound receptors were assayed by glass-fibre filtration and soluble receptors by Sephadex G-50 column chromatography. Total recovery of receptor protein was 10-15% with a specific activity (fmol ^{3}H-spiperone bound/mg protein) approximately 30% that of the membranes. The drug specificity of the soluble binding closely resembled that of the membrane site (see Table). The stereoselectivity of the receptor was maintained upon solubilization with (+)-butaclamol being at least 350-fold more potent than (-)-butaclamol. A close correlation was also observed between these IC_{50} values and those previously obtained on the dog striatum neuroleptic binding site.

	IC_{50} (nM)	
	Membranes	Soluble
Antagonists		
Spiperone	0.14	0.41
(+)-Butaclamol	28	6.4
(-)-Butaclamol	> 10,000	~10,000
Haloperidol	15	37
Fluphenazine	17	23
Chlorpromazine	44	160
Agonists		
Apomorphine	3,200	1,070
Dopamine	86,000	49,000
NA	> 100,000	> 100,000
5-HT	> 100,000	> 100,000

Madras, B.K., Davis, A., Kunashko, P. and Seeman, P. (1980). In: 'Psychopharmacology and Biochemistry of Neurotransmitter Receptors", eds. Olsen and Yamamura, Elsevier North Holland, N.Y. in press.

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174.1 POLYPEPTIDE COMPOSITION AND KINETICS OF SCa AND SCb IN SCIATIC NERVE MOTOR AXONS AND OPTIC AXONS OF THE RAT. I. G. McQuarrie, S. T. Brady* and R. J. Lasek. Department of Anatomy, Case Western Reserve University, Cleveland, Ohio 44106.

Previous work from this laboratory has defined the composition and kinetics of the 2 slowest waves of axonal transport in guinea pig optic axons, using SDS polyacrylamide gel electrophoresis (PAGE). Each wave contained a unique constellation of proteins. For example, the slowest wave (SCa) was characterized by the neurofilament triplet composed of polypeptides with molecular weights of 200K, 145K and 68K, whereas the faster wave (SCb) was characterized by actin (43K) and clathrin (170K). The 2 waves were sufficiently coherent and had sufficiently different rates that they could be completely separated by using long injection-sacrifice intervals.

In rat optic axons, the composition and kinetics of SCa and SCb are similar to those found in guinea pig optic axons. However, detailed study of rat sciatic nerve motoneurons has revealed a number of differences. For example, the rates of SCa and SCb are faster than in optic axons. More importantly, the 2 waves overlap and cannot be separated at long injection-sacrifice intervals. Quantitative analysis of the movements of each SCb band seen on SDS-PAGE fluorographs demonstrates that they form a trailing wave which moves with SCa and is separated from the primary SCb wave by a valley. Three polypeptides of SCb are exceptional in that they remain confined to the primary SCb wave.

When the SCb rate component of motoneurons is further analyzed by separating the individual polypeptides on 2-dimensional PAGE, the composition is very similar to that seen in optic axons: there is no evidence of the neurofilament triplet and all of the traditional SCb polypeptides are seen (except for 60K). There is a single additional major labeled spot which co-migrates with cold cycled beta tubulin from guinea pig brain and also co-migrates with the beta tubulin stained spot in rat peripheral nerve. This suggests that beta tubulin, which is confined to SCa in optic axons, has a leading edge extending into the SCb wave in motoneurons. Quantitative analysis of the movement of the 53K band supports this conclusion.

Several lines of evidence now suggest that the SCb wave represents movement of the axoplasmic matrix, characterized in particular by actin microfilaments. The present study demonstrates that a component of SCb can move distally in the axon in association with SCa over long distances. This suggests that in motoneurons there may be a particularly intimate association between the axoplasmic matrix and the microtubule-neurofilament network which is the structural correlate of SCa.

174.2 AXONAL TRANSPORT OF MICROTUBULE-ASSOCIATED PROTEINS KNOWN AS tau FACTOR. M. Tytell, S. T. Brady*, and R. J. Lasek. Dept. of Anatomy, Case Western Reserve Univ., Cleveland, OH 44106.

The ability of a neuron to extend and maintain an axon is dependent on the structural framework provided by the cytoskeletal elements, microtubules and neurofilaments. The proteins which constitute those elements, tubulin and the neurofilament triplet, are continuously synthesized in the soma and delivered to the axon exclusively as constituents of the rate component of axonal transport called Slow Component a (SCa) (Hoffman and Lasek, J. Cell Biol. 66:351, 1975; Tytell and Lasek, Soc. Neurosci. Abs. 4:37, 1978), which has a rate of 0.3-1.0 mm/day and is the slowest of the rate components. When tubulin and the neurofilament proteins are pulse-labeled, one can observe that they move within the axon as a well-defined peak of radioactivity that changes little in shape as it travels. This suggests that tubulin and the neurofilament proteins are transported as intact structures which together form the microtubule-neurofilament network of the axon (ibid.). If tubulin is transported as microtubules, then microtubule-associated proteins (MAPs) should be included in SCa. We analyzed the proteins of ^{35}S-methionine-labeled SCa in the guinea pig optic nerve using phosphocellulose column chromatography and one- and two-dimensional polyacrylamide gel electrophoresis. Proteins corresponding to the MAPs known as tau factor were identified in SCa. However, we have been unable to detect any proteins in SCa which correspond to the high molecular weight MAPs.

These observations suggest that the tau proteins are the primary MAPs transported as an integral part of axonal microtubules. If the high molecular weight MAPs are also transported as part of axonal microtubules, they must be present in exceedingly small amounts; they are clearly not present in the quantities commonly observed in preparations of whole brain microtubules. If this observation is supported by subsequent analyses, then we must question the current assumption that brain microtubules are homogeneous. Axonally transported microtubules may represent a distinct subset of the total population of microtubules prepared from whole brain. This could be explained in either of two ways: (1) neuronal microtubules may be different from glial microtubules or (2) the composition of microtubules may vary in different regions of the neuron. The first alternative has some support from the observation that only tau factor and not high molecular weight MAPs have been found in preparations of microtubules from homogeneous cell lines derived from hamster nervous system (Nagle et al., Cell 12:573, 1977; Solomon et al., Cell 18:431, 1979). It is possible that this heterogeneity of microtubules with respect to their associated proteins may represent an adaption to the specific functions of microtubules in different cell types or in different regions of the same cell.

174.3 CALMODULIN AND CALCIUM ACTIVATION OF TUBULIN ASSOCIATED CA-ATPASE. S. Ochs and Z. Iqbal. Dept. of Physiology and Medical Biophysics, Indiana University School of Medicine, Indianapolis, IN 46223 USA

In the transport filament model of axoplasmic transport in nerve, the transport filaments and the materials they carry are moved down along the microtubules by their side-arms with the required energy supplied by ATP. A sizeable amount of Ca-Mg ATPase is found in nerve (Khan and Ochs, Brain Res. 81:413, 1974) which we recently showed to be activated by calmodulin (Iqbal, Garg and Ochs, Soc. Neurosci. Abs. 5:60, 1979) at micromolar concentration levels of Ca^{2+}. Calmodulin activates a wide variety of enzymes under the control of Ca^{2+} in micromolar concentrations, a level of Ca^{2+} believed to be present in the axoplasm. We report that the Ca-ATPase associated with tubulin, which could be a part of the side-arms, is activated by calmodulin and Ca^{2+} at micromolar concentrations. Use was made of 2 or 3 cycles of cooling and warming to purify tubulin from cat brain by the method of Shelanski or Borisy, the latter giving a higher yield of Ca-ATPase associated with the tubulin. The Ca-ATPase associated with the purified tubulin, assessed by colorimetric measurement of P_i, showed a broad bell-shaped increase on addition of Ca^{2+} over the range of 10^{-9} to 10^{-4}M with the highest activity found with 10^{-6}-10^{-7}M Ca^{2+}. The addition of 10-100 ng of purified mammalian nerve calmodulin (Iqbal and Ochs, These Proceedings) augmented the activity of the Ca-ATPase a little but irregularly. There was also an inhibiting effect of the added calmodulin at the higher concentration of 10^{-4}M Ca^{2+}, a phenomenon seen for calmodulin activation of other enzymes. It is likely that variation in the activation of Ca-ATPase by added calmodulin is due to the presence of different amounts of calmodulin in different preparations. A small but significant amount of a protein band comigrating with calmodulin was observed when 2-cycle purified tubulin preparation was analyzed on 12.5% acrylamide gels containing SDS. Further evidence for the presence of calmodulin attached to the tubulin associated Ca-ATPase is that the addition of trifluorperazine (TFP) caused a marked decrease in Ca-ATPase activity, the TFP binding to calmodulin and thus preventing it from activating the enzyme. The calmodulin may be bound to the tubulin associated Ca-ATPase in the course of its isolation or it may actually be a part of the Ca-ATPase enzyme. We can consider that the transport filament with Ca^{2+} present can activate the calmodulin-Ca-ATPase complex of the side-arm in the latter case, or that calmodulin is part of the transport filament serving to locally activate the Ca-ATPase of the side-arms in the process of its movement. Supported by NIH grant RO1 8706-11, NSF BNS 79-14029 and the MDA.

174.4 DEOXYRIBONUCLEASE I AND A γ-GLOBULIN BLOCK FAST AXONAL TRANSPORT WHEN INJECTED INTO THE CELL BODY OF AN IDENTIFIED SEROTONERGIC NEURON OF APLYSIA. David A. Harris,* Daniel J. Goldberg, Beverly W. Lubit, Ariel A. Sherbany* and James H. Schwartz. Div. Neurobiol. & Behavior, Depts. Physiol. and Pharmacol., Columbia Univ., Coll. Physicians & Surgeons, New York, N. Y. 10032.

Actin is presumed to function in a variety of processes involving intracellular motility, but evidence is lacking that it plays a role in fast axonal transport. Several proteins have been described that block the polymerization of actin, and these can be administered by pressure injection directly into the giant cerebral neuron (GCN). Since we have previously established that 5-HT is packaged into vesicles which move rapidly down the axons of this identified serotonergic cell, we have used movement of the transmitter to test for inhibition of fast transport.

When we injected DNase I intrasomatically into GCN and two hours later injected ^{3}H-5-HT, the subsequent movement of neurotransmitter into axons was inhibited. Maximum inhibition occurred with an amount of DNase equivalent to the molar amount of actin estimated to be present in the neuron. We presume that DNase interferes with transport by disrupting microfilaments within the injected neuron, since the enzyme has been shown to depolymerize F-actin by combining specifically and stoichiometrically with actin monomers (Hitchcock & Lindberg, Cell, 7, 531, 1976). We found DNase to be a much less effective inhibitor when it had been incubated before injection with G-actin purified from rabbit skeletal muscle.

Norberg, Thorstensson, Utter & Fagraeus (Eur. J. Biochem., 100, 575, 1979) have reported that normal serum from rabbits and other species contains an actin-depolymerizing factor (ADF). We have confirmed the presence of this heat-labile activity in γ-globulin fractionated by ion-exchange chromatography or gel filtration, and have found by viscometry that these preparations profoundly reduced the extent to which actin polymerizes in the presence of salt. When injected into GCN, these preparations of γ-globulin also blocked transport of 5-HT. Because the protein fractions used are still impure, we cannot be certain that the inhibition of transport obtained is caused by ADF, rather than by some other as yet unrecognized activity.

Although intracellular injection of macromolecules as specific pharmacological agents represents a novel probe of the biochemistry of fast axonal transport, these experiments do not yet permit the conclusion that actin functions directly in translocation of organelles along the axon. Just as studies with colchicine indicate that microtubules are necessary for transport, but do not specify a molecular role, experiments with agents that depolymerize microfilaments do not specify whether actin's role is dynamic or only cytoskeletal.

174.5 SPECIFIC AXONAL TRANSPORT OF ^{3}H-HISTAMINE AFTER INTRASOMATIC INJECTION OF C2, AN IDENTIFIED APLYSIA NEURON. Hideki Gotoh* and James H. Schwartz. SPON. Klaudiusz Weiss. Div. Neurobiol. & Behavior, Depts. of Physiol. and Neurol., Columbia Univ. Coll. Physicians & Surg., New York, N. Y. 10032.

Previous work with identified cholinergic and serotonergic neurons has indicated that one determinant of neurotransmitter specificity is a characteristic vesicle that serves to package the transmitter and to mediate its rapid axonal transport. We have now begun to examine this idea with C2, a putative histaminergic neuron in the cerebral ganglion of Aplysia californica (see Weinreich in Osborne, Biochemistry of Characterised Neurons, 1978).

We first injected the fluorescent dye, Lucifer yellow, to confirm that C2 has three axon branches. Next we injected ^{3}H-histamine into C2's cell body. Primarily using the axon in the posterior lip nerve for analysis, we found that the distribution of radioactivity in sequential one millimeter segments of the nerve at 1, 2, 3, 4 and 6 hours after injection showed the characteristics of fast transport, not of diffusion. ^{3}H-histamine was also found to accumulate rapidly behind a ligature tied in the lip nerve. The movement of ^{3}H-histamine, estimated at about 50 mm/day at 22^{0} C, was inhibited by colchicine and was slower at lower temperatures. We also injected ^{3}H-choline and ^{3}H-serotonin in order to test the specificity of transport: both substances appeared to move along the axon only by diffusion. A further indication for the specificity of transport was provided by a comparison of the amounts of ^{3}H-histamine and its degradation product (which Weinreich, 1979, has identified as γ-glutamylhistamine) in axons and cell body 6 hours after injection. Essentially all of the radioactivity in axons was shown to be in the form of histamine, whereas equal amounts of the labeled amine and its metabolite remained in the cell body, an indication that ^{3}H-histamine but not γ-glutamylhistamine is selectively exported.

We propose that in C2, as in other identified giant neurons, the transmitter is transported selectively because it is packaged in specific vesicles. Electron microscopy has revealed that C2 has characteristic large dense-cored vesicles. We are now studying the distribution of silver grains over electron microscopic radioautographs of C2's axons and cell body after intrasomatic injection in order to identify the organelles that transport ^{3}H-histamine.

174.6 REGULATION OF FAST AXONAL TRANSPORT OF SEROTONERGIC VESICLES IN AN IDENTIFIED INVERTEBRATE NEURON. Daniel J. Goldberg and John M. Aletta*, Dept. Pharm. and Div. Neurobiol. & Behav., Columbia U. Coll. P.& S., New York, N.Y. 10032 U.S.A.

Little is known of how the amount and composition of material delivered to the synapse by fast axonal transport is regulated. Modulation of fast transport in mature neurons has been demonstrated, other than in pathological conditions, mainly during axonal regeneration. The transection is usually close enough to the soma to disconnect most, or all, of the synapses, and there is typically a decrease in the transport of the transmitter or its synthetic enzymes.

We are studying the regulation of fast transport in GCN, a giant identified serotonergic neuron in the sea hare, Aplysia californica. Transport of the serotonergic storage vesicle is measured by labeling it with ^{3}H-serotonin that is micro-injected by pressure into the soma. GCN has only 1 axon which, just before exiting the ganglion, bifurcates into branches of similar diameter which run in different nerves and terminate on different targets. Normally, one of the nerves (connective) transports about twice as much ^{3}H-serotonin as the other (lip nerve). We had found that in vitro (synapses detached from nerves), when the connective was was transected close to the ganglion, within a few hours the ^{3}H-serotonin that would normally be transported in the connective was diverted into the lip nerve. Thus, the lip nerve was shown to be capable of transporting 2-3 times its normal amount of ^{3}H-serotonin (Goldberg, et al., J. Physiol. 259:473, 1976). As a first step in studying the regulation of transport, we have now been transecting the connective in vivo, so that the lip nerve synapses remain intact and receive much more than their normal complement of transported material. We find that, within a few days, the cell reduces the export of ^{3}H-serotonin so that the lip nerve is transporting close to its normal amount of transmitter.

One day after transection of the connective, total export of ^{3}H-serotonin from the soma was slightly decreased from normal but the lip nerve was still transporting twice its normal amount. By 3 days post-operation, ^{3}H-serotonin export was reduced to the point that transport in the lip nerve was only 19% greater than normal. No further change was seen at 7 days. The reduction in ^{3}H-serotonin transport was not the result of a general decrease in synthetic activity of the cell, since protein synthesis at 3 days post-operation, measured by incorporation of ^{3}H-leucine, was not different from normal. These data indicate that the cell may regulate its output of transmitter storage vesicles so as to maintain the amount received by synapses within narrow limits. Since the axon is capable of transporting an amount of material far in excess of these limits, the modulation of vesicle output may be elicited by events at the synapse.

174.7 DIFFERENTIAL TRANSPORT OF PEPTIDES IN PEPTIDERGIC NEURONS. S. Arch and T. Smock.* Biology, Reed College, Portland, OR 97202, and Physiology, Univ. of Calif. School of Medicine, San Francisco, CA 94143.

The bag cell organs of the central nervous system of Aplysia californica devote a considerable proportion of their protein synthetic activity to the production of two peptide species. After short-term incubation in radiolabeled leucine, extraction in acetic acid, and separation by isoelectric focusing PAGE, the two peptides together comprise about 40% of the labeled protein. One of these peptides is the alkaline egg-laying hormone (ELH), the other is an acid molecule (AP) with no presently known function. Both peptides are associated with particulate structures (presumably vesicles), transported by a colchicine-sensitive process, and are secreted (Stuart et al., J. Neurophysiol. 43:488, 1980) upon bag cell depolarization. The bag cells would appear, therefore, to be useful for determining if there are regulatory processes that differentially affect the disposition of two secretory peptides. To begin examining this question, the stoichiometries between the peptides in the cell somata and in transport were compared. In the absence of differential affects, the stoichiometry in the neurites should be the same as the stoichiometry in the cell somata. This is not the case. While the ELH/AP ratio is approximately 1.1 in the cell somata following a 6 hr pulse of labeling, the ratio in the neurites, reflecting the earliest evidence of labeled peptide transport, is approximately 0.6. At subsequent times (6, 12, 18, and 24 hr) the ELH/AP ratio in the neurites approaches the initial soma value. These observations indicate that AP enters transport earlier than does ELH. As a consequence, it should be that the labeled ELH/AP ratio will vary along the length of neurites. When this is examined by analyzing sequential segments of neurites, the most distal segment shows a low ELH/AP ratio while increasingly proximal segments show values increasing toward unity. The implication from these studies is that the two peptides are not disposed coordinately following their synthesis. Further study of this phenomenon will require evaluation of at least 3 possible explanations: 1) Molecular queuing at the time of vesicle filling favors insertion of newly synthesized AP over that of ELH, 2) the peptides are enclosed in different vesicle types and the type containing AP enters transport earlier than that containing ELH, or 3) the peptides are characteristic products of different subpopulations of bag cells.

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174.8 INTRAOCULAR INJECTION OF KAINIC ACID INDUCES MODIFICATIONS OF FAST AXOPLASMIC TRANSPORT IN RAT OPTIC NERVE. P. Gomez-Ramos, J.A. Donoso and F.E. Samson. Ralph L. Smith Research Center, Univ. of Kansas Med. Ctr., Kansas City, Ks. 66103.

Kainic acid (KA), an analogue of glutamate, is an extremely potent excitant of mammalian CNS and produces a selective destruction of neurons receiving glutaminergic innervation due to excessive depolarization. Intraocular injections of KA affect amacrine and bipolar cells of the retina and produce blindness in a few minutes. Since KA induces dramatic changes in the activity of the cells that innervate retinal ganglion cells, we decided to examine if under these conditions, fast axoplasmic transport (FAXT) in the optic nerve was affected.

The rate of FAXT in the optic nerve of young adult rats is 8.86 + 1.66 mm/hr determined by means of ^{3}H-proline (10 μCi) injections in the vitreous humor. To characterize the action of KA on FAXT we injected 250 nmols of KA in the rat eye at the same time with ^{3}H-proline and no FAXT of labeled material was observed in the optic nerve and optic tract two hours later. As little as 5 nmols of KA produces the same effect. When ^{3}H-proline was injected in the eye 1 hour prior to KA, the labeled material already present in the optic nerve at the time of the KA injection was observed to move at a normal rate along the optic nerve and optic tract, but no more labeled material moved out of the retinal ganglion cells perikarya during at least 24 hours. One week after the injection of 5 nmols of KA, the profile of labeled material 2 hours after ^{3}H-proline injection is similar to controls, thus indicating a recovery from the effects of KA

We conclude that KA inhibits the initiation of FAXT in retinal ganglion cells for a period of time. This inhibition appears not to be caused by the actual destruction of some retinal cells since it is transitory and probably results from the excitatory action of KA on glutamate innervated retinal cells.

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174.9 COMPUTER GENERATION OF TYPICAL AXOPLASMIC TRANSPORT ISOTOPE DISTRIBUTIONS USING THEORETICAL PRINCIPLES OF LIQUID CHROMATOGRAPHY. G. W. Gross,* G. H. Stewart,* B. Horwitz, (SPON. J. F. Hines), Departments of Biology, Chemistry and Physics, Texas Woman's University, Denton, TX 76204

We have used simple mass flux equations from liquid chromatography to generate the characteristic isotope distribution profiles created by rapid axoplasmic transport in C-fibers (1,2). Our purpose is to test the compatibility of the empirical data with chromatographic principles and to systematically investigate the validity of various assumptions about the molecular events underlying the transport phenomenon. We have assumed that all rapid transport takes place as free molecules and molecular complexes in a liquid phase from which material is lost by deposition into stationary, slowly moving or retrograde phases. Diffusion constants control mass transfer between these phases. The quantitative profile data obtained in the garfish olfactory nerve (1,2) have provided accurate information on peak spreading, peak assymetry, tailing and transport velocities. Longitudinal diffusion, which is reflected in the gaussian spread of the peak, is a sum of eddie diffusion, molecular diffusion and velocity profile effects. By plotting the peak variance at $h/\sqrt{e}$ against time, the overall diffusion constant can be obtained from the slope ($\Delta\sigma^2=2D\Delta t$). We find that $D_{(fast)}=3\times10^{-6}$ cm^2/sec and $D_{(slow)}=10^{-8}$ cm^2/sec indicating that they seem to occur in different environments. If we assume molecular diffusion to be the dominant spreading term, we can regenerate the empirical data with a surprisingly small set of specific variables. However, the assumption of diffusion in an aqueous phase appears to be necessary.

The agreement of computer generated profiles with the actual data is encouraging and supports our contention that the chromatographic model is a valuable tool for investigating the mechanism of axoplasmic transport.

Gross and Beidler (1975) J. Neurobiol. 6:213-232.
Cancalon (1978) J. Neurochem. 32:997-1007

174.10 Anterograde transport of wheat germ agglutinin: Direction of transport dependent on selective binding. T.P. Margolis*, C.M.-F. Marchand, H.B. Kistler, Jr.* and J.H. LaVail. Department of Anatomy, U.C.S.F., San Francisco, CA 94143.

Previous studies have indicated that the specific binding of wheat germ agglutinin (WGA) to neuronal surface membranes results in the retrograde axonal transport of this lectin. We have investigated the axonal transport of ^{125}I-WGA in the chick visual system and found that affinity purified ^{125}I-WGA is transported in an anterograde direction only. WGA was iodinated using a modification of the chloramine-T method of Hunter and Greenwood and applied to a Bio-Gel P-2 column to separate low molecular weight components. This sample was further purified by affinity chromatography on an N-acetylglucosamine (GlcNAc) column. The"active"fraction was eluted with 1 M GlcNAc and dialyzed extensively against 10 mM phosphate buffer (pH 7.5) to insure separation of the sugar from the lectin binding site. SDS gel electrophoresis and subsequent autoradiography of the gel served to identify the "active" fraction as a single band which co-migrates with cold WGA at 17,000 daltons.

Within 5 days of its preparation, 2-5 μCi of the "active" fraction (sp. act. 0.25 μCi/μg) were injected into the vitreal chamber of one eye in each of 5 chicks. After 21-23 hrs, the animals were fixed, and the eyes, superior cervical ganglia (SCG) and brains were prepared for autoradiography. The retinas and irises of all animals were heavily labeled. In addition, label was found over the Stratum opticum and layers a-f of the Stratum griseum et fibrosum superficiale, i.e., the retinoreceptive layers of the contralateral optic tectum. There was no labeling of the ipsilateral optic tectum. Moreover, neither the isthmo-optic nuclei nor SCGs contained labeled cells.

As a control, a fraction of the iodinated sample that failed to bind when passed over the affinity column was injected into one eye of each of 3 chicks. In all 3 animals, no labeling of the contralateral optic tectum was apparent, but a small population of labeled cells was identified in the ipsilateral SCG. The contralateral SCG contained no labeled cells. SDS gel electrophoresis and subsequent autoradiography of this fraction established multiple radioactive bands, one of which co-migrates with cold WGA.

In conclusion, in the chick visual system 1)"active"^{125}I-WGA is transported by retinal ganglion cells in an anterograde direction, 2)"active" ^{125}I-WGA does not appear to be transported in a retrograde direction by SCG or isthmo-optic neurons, and 3) there is a component of the iodinated sample, presumably "inactive" WGA, WGA fragments or a contaminant, that does not bind GlcNAc but is transported in a retrograde direction by SCG neurons. (Supported by USPHS grants RO1 NS13533 and T32 GM07618).

175.1 EXTRAHIPPOCAMPAL CORTICAL PROJECTIONS FROM THE ENTORHINAL CORTEX IN THE RAT AND MONKEY. K.C. Kosel and G.W. Van Hoesen, Depts. of Anat. & Neurol., Univ. of Iowa, Iowa City, IA 52242.

In recent years it has been documented in several species that the subicular cortices are the major output structures of the hippocampal formation. In fact, these cortices account for nearly all of the diversity of hippocampal output, since the Ammonic pyramids contribute largely to only intrinsic and commissural circuitry within the hippocampi, and one major extrinsic projection to the septum. The subicular cortices project widely to several cortical areas, but have an especially strong projection to the deeper layers of the nearby entorhinal cortex. This reciprocates an entorhinal projection which terminates in the molecular layer of the subiculum. We have noted consistently in HRP experiments that when cortical injections are made lateral to the rhinal sulcus, a distinct pattern of labeled neurons within the entorhinal cortex can be observed. This suggests that the direct subicular projections to the cortex may represent only one of many potential output pathways of the hippocampal formation, and that others may relay in the entorhinal cortex. In both rats and monkeys, small injections of 20% horseradish peroxidase (Sigma type VI) were made into areas 35 and 36. After a 24-48 hour survival period, the animals were perfused with aldehyde fixatives and reacted according to Mesulam's tetramethyl benzidine procedure. The sections were examined microscopically using bright and darkfield illumination. In the rat, labeled neurons were observed in layers II and III of the lateral entorhinal cortex. Consistent with the topography of this cortex, they were more numerous ventrally and diminished in number dorsally. In the monkey, labeled neurons were also observed in the lateral entorhinal and prorhinal cortices. However, unlike the rat, these neurons were largely confined to layer III, although occasional labeling was observed in deeper layers. In both species labeling in the medial part of the entorhinal cortex was not observed. Thus, these results suggest that the lateral entorhinal cortex projects not only to the hippocampus and dentate gyrus, but to adjoining parts of the neocortex as well. These projections provide an additional indirect output pathway for the hippocampal formation for the influence of cortical areas in the temporal region. (Supported by grant NS 14944.)

175.2 LIMBIC CORTICAL FORMATIONS OF TEMPORAL LOBE IN THE BOTTLENOSE DOLPHIN. Myron S. Jacobs, Peter J. Morgane and Willard L. McFarland. Dept. Pathobiol., NYU Coll. Dent., New York, NY 10010; Worcester Found. Exp. Biol., Shrewsbury, MA 01545; NIH, Bethesda, MD 20014.

In the temporal lobe (TL) of land mammals, the entorhinal area and presubiculum are transitional neocortical formations of the limbic lobe that relay information to the hippocampus from diverse sources. As part of an overall study of the limbic cortical formations in the purely aquatic cetacean Order of mammals, we were particularly interested in TL limbic formations because of the small size, both absolutely and relatively, of the elements of the temporal archicortex (fascia dentata, hippocampus and subiculum). Overall, the periarchicortical entorhinal area (Ent) and presubicular area (Psb) in the dolphin are far better developed than the archicortical formations. The Ent begins considerably more anterior to the Psb, and the latter terminates more posterior to the Ent than usually occurs in land mammals. Considerable cytoarchitectonic differentiation of the Ent is present. Anteriorly it exhibits a medial transition with the periamygdalar area (PAm) of the paleocortex and a lateral transition with typical 6-layered neocortex. Distinct medial and lateral subareas of Ent are present, with the cortical plate being about 40% wider and the clearing zone (lamina dissecans, LmDis) between superficial and deep cell layers more distinct medially than laterally. At mid-temporal levels the Ent exhibits, medially, a partial superlamination over the subicular plate, which has replaced the PAm. The superficial cell zone is broken and comprised of medium sized cells. The deep cell zone is more regular, wider and contains larger cells. Laterally, the Ent exhibits further architectonic division into two subareas which differ chiefly in the distinctness of the LmDis, in the differentiation as well as presence or absence of breaks in the superficial cell zone, and in the degree of compactness of the deep cell zone. Further posteriorly in the TL, the Psb appears between the subiculum and the medial Ent and gradually expands mediolaterally as the Ent attenuates. It differs from the medial Ent by having a uniformly narrower cortical plate, by the presence of more densely packed, smaller cells in its superficial cell zone and by a less prominent LmDis. Superficial papillarity, a characteristic of the Psb in many terrestrial mammals, is absent. Although numerous differences in cytoarchitectonic details are present, cetacean limbic cortical formations exhibit an overall striking similarity to these formations in land mammals.
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175.3 NEURONAL DEVELOPMENT OF THE VENTROMEDIAL HYPOTHALAMUS AND THE AMYGDALA IN THE GOLDEN HAMSTER: A QUANTATIVE GOLGI STUDY. Chia-Hung Hsü*, C. Sue Carter, William T. Greenough, Sharon Manne* and Lois Drake* Depts. of Psychology and Ecology, Ethology and Evolution and Program in Neural and Behavioral Biology, University of Illinois, Champaign-Urbana, 61820.

The purpose of this study was to analyze quantitatively neuronal morphology during postnatal development in the golden hamster. Brain tissue was stained using the Golgi-Cox procedure and 100 micron sections through the ventromedial hypothalamus (VMH) and cortico-medial amygdala (CMA)) were examined. Neurons were sampled from males and females at 5, 10, 20 and 30 days of age. There were 6 littermate pairs for each age. Approximately 20 well-stained neurons were sampled from each area of each brain. The neurons were traced on paper by camera lucida at 500X. All drawing and scoring was performed on coded slides which did not reveal sex.

In the VMH, soma size peaked on day 10. Total dendritic length increased until 20 days of age. The number of first order branches (branches from the soma) was highest on day 5 and decreased significantly after 10 days of age, and the number of higher order branches increased with age. The percentage of neurons with visible growth regions increased from day 5 to day 10 and returned to near zero on day 20 and day 30. Preliminary results indicated higher frequencies of growth regions for males than females. The percentage of neurons with multifurcation branching (more than 2 branches at the same point) slightly increased from day 5 to day 10 and then decreased to almost zero at day 30.

In the CMA, soma size also peaked on day 10, and then showed a greater decrease in females than in males such that males had a larger average soma size than females at day 30. Both total dendritic length and field size increased until day 20 and then leveled off. The number of first order branches did not change, while those of higher orders showed increases over the ages sampled. The percentage of neurons with growth regions and multifurcation branching was highest at day 5 and decreased greatly thereafter.

In general, the preoptic area (Hsü, Carter & Greenough, Abst. Soc. Neurosc. 4:115, 1978) and VMH share a similar developmental pattern. CMA differed from the other two areas in terms of soma size development patterns and the absence of an age-related decline in the number of first order branches. (Supported by NIH grant - NS 13421).

175.4 THE PROJECTION OF THE SUPRAMAMMILLARY REGION UPON THE DENTATE GYRUS IN NORMAL AND REELER MUTANT MICE. B.B. Stanfield, J.M. Wyss and W.M. Cowan. Dept. of Anatomy and Neurobiology, Wash. Univ. Sch. of Med., St. Louis, MO 63110.

The distribution of the hypothalamo-dentate projection in normal and reeler mice was investigated by making injections of a mixture of ^{3}H-proline and ^{3}H-leucine into the posterior hypothalamus of animals heterozygous (rl/+) or homozygous (rl/rl) for the reeler mutation, and subsequently processing the brains for autoradiography.

In the normal mouse (rl/+) the distribution of the hypothalamo-dentate projection is essentially the same as that which has been described previously in the rat and cat. Following injections which include the supramammillary region, silver grains indicative of anterograde transport in the hypothalamo-dentate fibers are restricted to a band which overlies the granule cell layer and the inner 10-15 μm of the molecular layer of the dentate gyrus of both sides. The grain density is consistently higher over the suprapyramidal than over the infrapyramidal blade, and is higher on the ipsilateral than the contralateral side.

The hypothalamo-dentate projection in the reeler mutant mouse (rl/rl) has some of these same features - it is heaviest over the dentate gyrus on the side ipsilateral to the injection site and is consistently heavier over the "suprapyramidal" part of the granule cell zone. However, in contrast to the narrow band of silver grains seen in the normal dentate gyrus, after an isotope injection into the supramammillary region of the reeler mouse, transported label is found throughout the zone containing granule cell somata, including not only the ill-defined granule cell layer, but also the "hilus" deep to it in which there are many ectopic granule cells. No label is seen over the molecular layer.

This dispersed distribution overlaps that which we have previously described for the commissural and associational inputs to the reeler dentate gyrus. However, whereas the latter are more concentrated at the interface between the granule cell and molecular layers and in the granule cell-free zones of neuropil in the hilar region, the hypothalamic afferents are more uniquely distributed throughout the zone containing granule cells but tend to be aggregated around the granule cell somata. Thus, despite the extensive overlap among these three afferent systems in the mutant, they appear to exhibit the same target site specificity which they display in the normal.

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175.5 EFFECT OF ANISOMYCIN ON POST-TETANIC CHANGES IN THE DENTATE FASCIA. Eva Fifková, Carol L. Anderson* S. J. Young*and A. Van Harreveld. Dept. of Psych., Univ. of Colorado, Boulder, CO 80309, and Div. of Biol., Caltech, Pasadena, CA 91125.

Tetanic stimulation of the entorhinal area has been shown to induce in the dentate molecular layer long-lasting enlargement of dendritic spines. Increased protein synthesis was postulated to be the mechanism underlying this change. Therefore, an attempt has been made to suppress the spine enlargement by a protein synthesis blocking drug - anisomycin (Ani). Two groups of 14 and 13 mice survived the tetanic stimulus to the medial and lateral entorhinal area for 4 and 90 min, respectively; a control group (11 mice) had a sham procedure. Fifty-three mice received Ani subcutaneously (0.4 mg/kg) 15 min prior to the treatment. The Ani animals were divided into four groups: two of them were stimulated and were sacrificed 4 min and 90 min later. The other two groups were sham controls with survival periods similar to those of the stimulated groups. Spines in the dentate molecular layer of stimulated mice without Ani were enlarged in the 4 min poststimulation interval in the middle and distal third of the dentate molecular layer by 20% and 34% and in the 90 min interval by 28% and 32%, respectively. Ani suppressed the spine enlargement significantly in the 4 min but not in the 90 min poststimulation interval. Protein synthesis is maximally blocked between 10-90 min after application of a single dose of Ani and then it quickly recovers. Long-surviving stimulated mice were under Ani for 105 min, which is outside the maximal effective range of the drug. This could explain why the spine reaction was no longer suppressed in the 90 min interval. Since suppression of the spine reaction to stimulation and its recovery coincides in time with the blocking effect of Ani on protein synthesis, it can be concluded that protein synthesis might be the mechanism underlying the dendritic spine enlargement induced by tetanic stimulation. (Supported by NIMH Grant MH 27240-06.)

175.6 LIMBIC KINDLING: INHIBITORY INTERACTIONS IN THE TRANSFER EFFECT. Michael S. Duchowny, Frank H. Duffy, James L. Burchfiel. Seizure Unit & Dept. of Neurology, Children's Hospital Medical Center & Harvard Medical School, Boston, MA 02115.

To investigate limbic mechanisms involved in the transfer of kindled activity, we analyzed the growth of hippocampal seizures induced by epileptiform afterdischarges (ADs) arriving from two anatomically distinct hippocampal input pathways. Electrodes were implanted into the septal region and entorhinal cortex of adult rats to activate the fornix and perforant path hippocampal afferents respectively. Generalized motor convulsions were kindled independently from each site and complete mutual transfer was demonstrated between both hippocampal inputs. That is, prior kindling of one pathway significantly reduced the number of stimuli required to elicit a generalized convulsion from the other. In comparing these results to a paradigm in which stimulation of the two pathways was alternated on a trial-by-trial basis, we found markedly different outcomes. In contrast to the mutual transfer seen with massed stimulation, alternating stimulation resulted in generalized seizures being kindled from only one of the two sites. Stimulation of the other site elicited local AD activity but no progression to motor seizures, even after an excessive number of trials. Which pathway became dominant appeared to be random and not predictable by AD characteristics. There was a difference however in the characteristics of kindling depending upon which pathway became dominant. When the fornixpathway was dominant, there was considerable facilitation between the two inputs and the rate of kindling was faster than with stimulation of either pathway alone. When the perforant pathway input was dominant, the number of trials required for generalized convulsions was almost twice that for massed stimulation, as if fornical input had no effect at all.

Our results indicate that the propagation of AD activity believed to underly the kindling phenomenon is more complex than the simple summation of excitatory influences. Inhibition appears to play an important role in determining the route of propagation. When competing pathways are stimulated in alternation, one becomes dominant for kindling and the other is suppressed. Moreover, when competing pathways are active, the kindling process varies as a function of which pathway is dominant.

175.7 RESIDUAL ACHE STAINING IN THE HIPPOCAMPAL FORMATION FOLLOWING SEPTAL LESIONS: THE TOPOGRAPHY OF ACHE POSITIVE NEURONS AND ZONE 31. J.R. West, G.W. Van Hoesen, L.L. Chen,* and C.L. Barnes.* Depts. of Anat. & Neurol., Univ. of Iowa, Iowa City, IA 52242.

Following transection of the fimbria fornix and/or a lesion of the septum, both acetylcholinesterase (AChE) and choline acetyltransferase (ChAc) activity in the hippocampal formation drop precipitously. The results of several investigations place the depletion at 80-85%. The precise morphological elements which contain the residual 15-20% of the enzyme are unknown, but likely candidates exist. For example, some have reported the persistance of AChE positive neurons in the hippocampal formation following a lesion of the septum. And moreover, all agree that terminal staining persists in a portion of the molecular layer of the subiculum and CA 1 field. This pattern of staining conforms in part to Storm-Mathison and Blackstad's zone 31. We have examined the topography of residual AChE staining in the hippocampal formation of the rat following septal lesions. For all cases, the animals survived for 2-3 weeks and were perfused with saline and 4% formalin. The brains were sliced on a freezing microtome and serial sections were stained for AChE according to Genneser-Jensen and Blackstad's method. Ethopropazine was added to the incubation medium to suppress unspecific cholinesterase. In nearly all cases, AChE positive neurons in varying quantities were detected in many parts of the hippocampal formation. Their most frequent location was in the hilus of the dentate gyrus and in the subiculum, although stained neurons were also observed in the stratum oriens and radiatum of the CA 3 region and in the entorhinal cortex. In general, neurons containing AChE were more numerous in the septal end of the hippocampal formation, although they had a wide septo-temporal extent. In all cases with septal lesions, residual AChE staining was observed to persist in the molecular layer (stratum moleculare) of the inner one half of the subiculum and the outer one half of the CA 1 zone. This staining formed a conspicuous wedge-shaped or triangular pattern with the base located in the subiculum and the apex in the hippocampal CA 1 zone. On each end, the stained area was contiguous with the hippocampal fissure and did not extend across it into the molecular layer of the dentate gyrus. This pattern, present throughout the extent of the subiculum and CA 1 zones, seems to represent a part of Storm-Mathison and Blackstad's AChE zone 31 in the normal rat. The source of this staining pattern is unknown. However, it may represent the stained axon terminals of AChE neurons intrinsic to the entorhinal or subicular cortices. (supported by grant NS 14944).

175.8 RESIDUAL ACHE STAINING IN THE HIPPOCAMPAL FORMATION FOLLOWING COMBINED SEPTAL AND ENTORHINAL LESIONS: REINNERVATION OF THE PERFORANT PATHWAY TERMINAL ZONE. G.W. Van Hoesen, J.R. West, C.L. Barnes,* and L.L. Chen.* Depts. of Anat. & Neurol., Univ. of Iowa, Iowa City, IA 52242.

Reinnervation of the various terminal zones in the hippocampus and dentate gyrus following deafferentation is now a well-known phenomenon. The most extensively studied model concerns the proliferation of AChE containing axons in the outer part of the molecular layer of the dentate gyrus after removal of the entorhinal cortex. The source of axons that reinnervate this zone has yet to be shown, but it is likely that they reside in the septum, or in nearby areas that send axons through the septum enroute to the dentate gyrus. This is predicated on the fact that septal lesions and fornix transections diminish the AChE content of the dentate gyrus in both normal animals and experimental animals having a prior entorhinal lesion. While this cannot be disputed, discernible AChE staining persists in the hippocampal formation in spite of a septal lesion. We report evidence that these residual AChE positive elements are capable in and of themselves of reinnervating the perforant pathway zone in both the hippocampus and dentate gyrus following combined septal and entorhinal lesions. In 18 adult rats bilateral lesions were made in the septum. Entorhinal ablations were made by aspiration immediately thereafter. After 21 days, the rats were perfused with saline and 4% formalin and the brains were sliced on a freezing microtome. Serial sections were stained for AChE according to Genneser-Jensen and Blackstad's method. Ethopropazine was added to the incubation medium to suppress unspecific cholinesterase activity. Bilaterally AChE staining was reduced greatly. On the side with the intact entorhinal cortex, the normal residual compliment of AChE was observed in zone 31, and in some instances, AChE positive neurons were observed in the hilus of the dentate gyrus and subiculum. In contrast, on the side of entorhinal lesion, AChE staining spread from zone 31 along the stratum moleculare of the hippocampus and was detectable in the outer part of the molecular layer of the dentate gyrus. This pattern was observed at all levels of the hippocampal formation, but was especially impressive in the septal end of the structure. In all respects, the zone of reinnervation resembled the known distribution of the perforant pathway. These results suggest that non-septal AChE containing axons are capable of reinnervating sizable terminal fields in both the hippocampus and dentate gyrus and traverse long distances to do so. The source of the AChE appears to be derived from the subiculum, although a contribution from hilus neurons cannot be ruled out. (supported by grant NS 14944.)

175.9 MOST ENTORHINAL CORTEX (ERC) NEURONS PROJECTING TO THE RAT DENTATE GYRUS (DG) CONTAIN ACETYLCHOLINESTERASE (AChE). Konrad Talbot[1], Barry Fass[1], and Larry L. Butcher[1,2]. Department of Psychology[1] and Brain Research Institute[2], UCLA, Los Angeles, CA 90024.

Using the pharmaco-histochemic protocol for cholinesterases (Butcher et al., J. neural Trans. 37:127, 1975), we have discovered AChE-reactive stellate neurons throughout layer II of the rat ERC, especially at dorsal levels of its medial sector. Their morphology, density, and location match that reported by Steward and Scoville (J. comp. Neurol. 169:347, 1976) for ERC neurons innervating the rat DG. We summarize here an experiment confirming that entorhino-dentate nerve cells possess AChE.

In order to retrogradely label ERC neurons projecting to the DG, 0.20-0.25µl of 30% Evans Blue (EB) fluorescent dye was infused unilaterally into the dorsal half of the DG in 3 female, albino rats. To optimize results with the pharmaco-histochemic method for AChE, animals received 2.0mg/kg of diisopropylfluorophosphate (DFP) 12 hrs. prior to the end of the 2 day post-operative survival times allowed. Using 490nm primary and 530nm secondary filters, dark-field photomicrographs were taken of red fluorescing, EB-positive ERC cells. These were then counterstained on-the-slide in an incubation medium for AChE (see Butcher et al., ibid.) and photographed again under bright-field illumination.

The EB injection fields covered primarily the DG, including its outer molecular layer, resulting in retrograde labelling of numerous layer II stellate neurons throughout the ERC. A relatively small number of EB-positive nerve cells also occurred in ERC layers III and VI of Lorente de No (J. f. Psych. & Neurol. 45:381, 1933). Comparison of photomicrographs of labelled layer II neurons taken before and after enzyme counterstaining revealed that many, but not all, entorhino-dentate nerve cells contained AChE. Such cells are probably not cholinergic (e.g., see Storm-Mathisen, Br. Res. 80:181, 1974). Their AChE content may, however, reflect a cholinoceptive nature, especially since layer II of the rat ERC appears to be innervated by AChE-rich septocortical fibers (see Srebro & Mellgren, Br. Res. 52:19, 1973), the hippocampal terminations of which are known to be cholinergic (see Kuhar & Yamamura, Br. Res. 110:229, 1976). [Support: NS 10928 to L.L.B.]

175.10 NEURAL MODULATION OF THE IMMUNE RESPONSE. R. J. Cross*, W. R. Markesbery,*W. H. Brooks*and T. L. Roszman*(SPON:B.Peretz) Dept. of Pathology, Univ. of Kentucky Med. Ctr., Lexington, Ky. 40536.

Our recent studies have shown that the hypothalamus has a modulating effect on the immune response. This is manifest by a decrease in spleen and thymus cell number and reduced in vitro cell-mediated responsiveness in anterior hypothalamic (AHT) lesioned rats. The latter is due to the induction of suppressor macrophages which qualitatively differ from macrophages from control rats. It was demonstrated that these effects were not due to stress-induced corticosteroid release. In the present studies, bilateral electrolytic lesions were placed in the medial hypothalamus, mammillary body, hippocampus or amygdala of Fischer 344 rats. Four days after lesioning the spleens were removed, single cell suspensions prepared and their blastogenic reactivity to concanavalin A (Con A) determined. Spleen cells from animals with lesions in the mammillary bodies, hippocampus or amygdala exhibited significantly enhanced responsiveness to Con A as compared to the responses obtained with spleen cells from animals with frontal cortex lesions. Thus, our data indicates that lesions in the limbic system enhance immune responsiveness whereas AHT lesions suppress this responsiveness. These studies support the concept that the central nervous system plays a major role in regulating the immune response. This work was supported in part by NIH grants CA-18234, CA-17786, and NS-14221.

176.1 Control of Muscle Mass I: Changes in Glucocorticoid Receptor in Experimental Atrophy. Richard R. Almon and Debra C. DuBois*. Division of Cellular and Molecular Biology, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York, 14214.

Glucocorticoids have been shown to have a profound catabolic effect on skeletal muscle. In the present report we examine the hypothesis that selective muscle atrophy, regardless of cause, is due to an increase in the sensitivity of a particular muscle to normal circulating levels of glucocorticoids.

The glucocorticoid receptor population of muscle cytosol in three experimental atrophy conditions was examined using [^{3}H]-Dexamethasone and a column chromatography assay.

The results show significant increases in receptor number with no change in affinity in the following three atrophy conditions: 1) the levator ani muscle after castration; 2) the gastrocnemius muscle after denervation and; 3)the gastrocnemius muscle after immobilization.

176.2 Control of Muscle Mass II: Changes in Glucocorticoid Receptor in Dystrophic Animal Models. Debra C. DuBois* and Richard R. Almon. Division of Cellular and Molecular Biology, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York, 14214.

Glucocorticoids have been shown to have a profound catabolic effect on skeletal muscle. In the present report we examine the hypothesis that general muscle atrophy in pathological states is due to hypersensitivity of the muscle to glucocorticoids.

The glucocorticoid receptor population of muscle cytosol from two dystrophic animal models was examined using [^{3}H]-Dexamethasone and a column chromatography assay.

The results show that in both chicken and mouse models, muscles from the dystrophic animals contain significantly higher levels of receptor than do normal controls.

176.3 INCREASED GLUTAMATE FORMATION AFTER PORTOCAVAL ANASTOMOSIS. F. Moroni, C. Paparozzi, D. Pellegrini and C. Cortesini. Depts of Pharmacology and Experimental Surgery, University of Florence, Florence, Italy.

Rats with a surgically-constructed (end-to-side) portocaval anastomosis are considered a good experimental model for the study of behavioural and biochemical changes which occur during chronic liver disease. In this animal model, blood and brain GABA, glutamate (GL) and glutamine (GLN) content were measured by a mass fragmentographic method. Brain glutamate synthesis and brain GABA turnover were also measured by infusing the animal with ^{13}C-glucose 50 µmol/Kg/min (uniformly labelled Kor Isotopes, Cambridge Mass) and by measuring the incorporation of ^{13}C in the aminoacid molecules (Moroni, F. et al., J. Pharmacol. Exp. Ther., 207: 870, 1978). Four weeks after PCA blood GABA, GL and GLN concentrations were increased by 350, 30 and 290% respectively. Regional brain GABA content and the turnover rate of GABA showed no changes, while the GL content was increased in the parietal cortex, in n.caudatus, in the hypothalamus and in the hyppocampus by 20 - 35%. The incorporation of $^{13}C_2$ derived from ^{13}C glucose in the glutamate molecule was approximately doubled. Glutamine concentration was increased in several areas by 250 - 300%. Since administration of high doses of GL may cause convulsions, selective neuronal degeneration and gliosis, it is possible that the increased glutamate synthesis we observed plays a role in the pathogenesis of chronic hepatic encephalopathy.

176.4 EFFECT OF MORPHINE ON PRL RELEASE IN MIGRAINE PATIENTS. G.Bussone, A.Boiardi, A.M.Di Giulio, E.Tansini, B.Merati and A.E.Panerai. Istituto Neurologico "Besta" & Dept. of Pharmacology University of Milan, Italy.

It is well known that morphine has not an analgesic effect in migraine patients. It has been suggested that this lack of effect is due to a deficiency of the serotoninergic system in these patients. Morphine releases prolactin through a serotoninergic mechanism. We have treated ten female migraine patients with 10 mg s.c. of morphine hydrochloride. In these patients, morphine elicited a clear cut release of prolactin (basal values 6.3 ± 0.5 ng/ml; peak values 72.5 ± 15.6 ng/ml) similar to the one observed in normal subjects. Pretreatment with metergoline, a specific antiserotoninergic drug, (2.5 mg x 3/die for 3 days) completely inhibited the prolactin releasing effect of morphine. This observation indicates that the serotoninergic system is well tuned in migraine patients. Consistently with this hypothesis, the administration of L-tryptophan (70 mg/kg p.o.) induces a release of prolactin and growth hormone similar to the one observed in normal subjects. This observation indicates the lack of a deprivation supersensitivity to serotonin as suggested by some authors.

The deficiency of the serotoninergic system therefore cannot be advocated to explain the lack of the analgesic effect of morphine in migraine patients. It has been suggested that the lack of the analgesic effect of morphine in migraine patients is due to a functional deficiency of the opiate receptor. In our subjects, the specific opiate antagonist naloxone (5 mg i.v. over a 30 min period) completely abolished the prolactin releasing effect of morphine.

This observation indicates that in these patients the opiate receptor is present and can be activated or inhibited in order to modulate prolactin release.

Explanations other than a deficiency of the opiate receptor or the serotoninergic system have to be searched in order to interpret the lack of analgesic effect of morphine in migraine patients. The role of GABA and acetylcholine are being investigated.

Differently from what observed in migraine patients, the prolactin releasing effect of morphine is blunted in "cluster" headache patients. This observation indicates a specific functional difference between the two syndromes.

176.5 GLUTAMATE UPTAKE INTO HUMAN PLATELETS: CHARACTERIZATION AND OBSERVATIONS IN HUNTINGTON'S DISEASE. Richard M. Mangano* and Robert Schwarcz (SPON: N. Khazan). Maryland Psychiatric Research Center, Baltimore, Md. 21228

Blood platelets have been repeatedly used as a model of putative neurotransmitter uptake systems in brain tissue. Extending an early report on the energy-dependent uptake of amino acids into platelets (Zieve and Solomon, Am.J.Physiol., 214,58,1968) we have now studied the uptake of L-glutamate, a neurotransmitter candidate thought to be etiologically implicated in the neurodegenerative disorder Huntington's disease (HD; Coyle et al., Prog. Neuro-Psychopharmacol., 1,13,1977), in human platelets.

L-glutamate uptake into human platelets revealed two components: a high-affinity system (K_{mH} = 3.1 µM), which was sodium-dependent and a low-affinity site(K_{mL} = 88 µM) displaying temperature- rather than sodium-dependency. These kinetic properties, along with pH-dependency and pharmacological characteristics of the platelet system (see below), were similar to those found in synaptosomal preparations and brain slices. However, V_{max}-values were far higher in brain (V_{maxH} = 325 ± 96, V_{maxL} = 3759 ± 1116 pmoles/mg wet weight x min) than in platelets (V_{maxH} = 14 ± 6, V_{maxL} = 313 ± 63 pmoles/mg platelet protein x 10min), indicating a denser population in brain than in platelets of qualitatively similar sites.

Pharmacological analysis substantiated the resemblance of nerve endings and platelets: the specific uptake inhibitors threo-3-hydroxy-DL-aspartate and DL-aspartate-β-hydroxamate as well as D- and L-glutamate and L-aspartate showed similar- though not identical - IC_{50} values in both preparations; and a spectrum of compounds devoid of inhibitory effects in synaptosomes also did not interfere with glutamate uptake in platelets.

Uptake parameters were studied in a population of human volunteers to determine the variability of platelet glutamate uptake. Whole blood could be stored up to six hours after venipuncture without any appreciable change in experimental values. Coefficients of variation between 0.09 and 0.28 for three repetitive (weekly) assays in single subjects indicated that uptake measurements were sufficiently suited for clinical studies.

Finally, platelets from HD-patients were analyzed for changes in glutamate uptake in order to test a possible etiological role of (a reduction in) this parameter in HD. No significant changes occured in HD-platelets compared to control values when uptake was performed at 10^{-7}M glutamate (high-affinity uptake conditions; N = 15 per group). However, preliminary kinetic data indicated a trend towards higher affinity (lower K_m) in the patient population.

This work was supported by a grant from the Wills Foundation.

176.6 INCREASED EXCRETION OF HOMOVANILLIC ACID (HVA) IN URINE BY YOUNG CHILDREN WITH INCREASED LEAD ABSORPTION. J.J. Chisolm, Jr.* and E.K. Silbergeld, (SPON: R. Irwin) Dept. of Pediatrics, Johns Hopkins Medical School, Baltimore, MD, and NINCDS, NIH, Bethesda, MD 20205.

Studies in neonatal rodents have revealed a disturbance in central dopamine metabolism, due to lead exposure, which is associated with increased levels of HVA in brain and urine (Silbergeld and Chisolm (1976) Science 192:153). This study was undertaken to test the relevance of the animal work to increased lead absorption in children. A statistically significant positive linear relationship between blood lead (PbB) concentration and the 24-hr excretion of HVA in urine was found in 25 children with PbB in the range of 38-72 µg/100 ml whole blood ($r=.729$, $p<.001$). Eleven children were restudied 13-60 months after chelation therapy when their PbB levels were in the range of 20-42 µg/100 ml whole blood. There was a corresponding decrease in levels of urinary HVA, suggesting reversibility of the effects of lead on dopamine metabolism. These data provide the first evidence in humans for a dose-effect relationship between blood lead concentration and a biochemical indicator of an effect of lead on the nervous system. Because indicators of altered heme synthesis are widely used in the diagnosis of lead intoxication, the relationships between HVA, erythrocyte protoporphyrin and urinary δ-aminolevulinic acid (ALAU) were evaluated. There were no significant correlations between HVA, ALAU, or erythrocyte protoporphyrin, or the excretion of lead in urine after chelation. The data thus suggest that evidence of disturbance in heme synthesis due to lead may not reliably predict the effects of lead on the nervous system in children. The PbB "threshold" appears to be between 30-40 µg/100 ml for significant increases in both HVA and ALAU. This is consistent with other clinical studies suggesting that increased lead absorption associated with PbB>30 µg/100 ml is potentially neurotoxic for young children.

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176.7 TRIETHYLTIN INTOXICATION ALTERS ACETYLCHOLINE RELEASE FROM RAT PHRENIC NERVE-DIAPHRAGM. G.G. Bierkamper*, A.M. Goldberg, J.J. Valdes, Division of Toxicology, The Johns Hopkins University, Baltimore, MD 21205

Organotin compounds are manufactured for use in chemical synthesis, marine anti-fouling paints, wood preservation, pesticides, plastics, fungicides and other industrial processes. Mammalian exposure to triethyltin (TET), the most toxic trialkyltin compound, may produce cerebral edema, affective disturbances, muscular weakness and damage to the myelin sheath. The purpose of this preliminary study was to determine the effect of acute and chronic TET exposure on unstimulated and stimulated release of endogenous acetylcholine (ACh) from a neuromuscular model system--the vascular perfused rat phrenic nerve-hemidiaphragm preparation (Bierkamper & Goldberg, JEPT 6:40-46, 1978).

Adult male Long-Evans hooded rats (250-350g) were exposed acutely to one injection of TET (10 mg/kg, i.p.) or chronically to TET (30 mg/L) in their drinking water. Hemidiaphragms were obtained from treated and age-matched control rats 24 hrs after acute exposure or 1, 2 and 3 weeks after initiation of the chronic exposure regimen. Twenty-four hours after the acute dose of TET, rats were completely incapacitated and usually died within 48 hrs; however, unstimulated and stimulated release of ACh were unaltered in the edematous hemidiaphragms from these animals. Chronically exposed animals progress through an increasingly compromised state beginning with mild ataxia and hindlimb weakness after one week of exposure to hindlimb paralysis, piloerection, penile erection, exopthalmos, and aggressive responsiveness by 3 weeks. Spontaneous (unstimulated) ACh release was 1.21±0.20 pm/min/hemidiaphragm (mean ± SEM; n=5) in control preparations and did not vary in TET-preparations. Stimulated release of ACh (via phrenic n.) was 6.83±0.57 and 9.89±1.02 pm/min after 45 min of 7 Hz and 20 Hz stimulation, respectively, in control preparations. Stimulated release from TET-preparations was 5.57±0.91, 4.32±0.86, 4.40±0.94 pm/min after 45 min at 7 Hz and 9.95±1.73, 6.78±1.31, 3.02±0.88 pm/min after 45 min at 20 Hz for 1, 2 and 3 weeks of exposure, respectively. These results clearly demonstrate that chronic, but not acute, TET intoxication results in a time-dependent decrease in stimulated ACh release. Interestingly, TET-preparations also had a reduced capacity for maintaining stimulated ACh release over longer periods even in the presence of 10 µM choline.

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176.8 SUBNORMAL CONCENTRATIONS OF BRAIN CIRCUMVENTRICULAR ANGIOTENSIN II RECEPTORS IN HEREDITARY DIABETES INSIPIDUS. M. van Houten*, E.L. Schiffrin* and B.I. Posner*. (SPON: R. Hirsh). Dept. of Medicine, McGill University and the Clinical Research Institute of Montréal, Quebec H3A 2B2.

In a previous study (Brain Research 186:480, 1980) we localized specific binding sites for blood-borne 125I-angiotensin II (AII) to the circumventricular organs (CVO) of the rat brain, using quantitative in vivo light microscope radioautography. We observed that the greatest concentration of AII receptors occurred in the subfornical organ, a key region implicated in AII-mediated thirst. In the median eminence AII receptors were concentrated in the medial palisade zone, rich in vasopressin. In the present study we evaluated the binding capacity of the CVOs for blood-borne 125I-AII in an animal model of hereditary diabetes insipidus, exhibiting chronically elevated circulating AII, insatiable thirst, and a genetically-determined inability to synthesize vasopressin. 125I-AII was injected systemically into male Long-Evans and homozygous Brattleboro rats, some of which were infused with saralasin (10nmol/Kg/min.) during and for 30 minutes prior to 125I-AII injection to determine levels of non-specific 125I-AII binding. Three minutes after injection brains were fixed by vascular perfusion for light microscopic quantitative determination of 125I-AII-specific binding, as described in the article cited above. Briefly, 125I-AII-specific binding in all of the CVOs of the homozygous Brattleboro rat was 55-92% below control levels, although levels of non-specific binding were normal. Deficiencies in AII-specific binding capacity were most severe(80-92% subnormal) in regions of the CVOs normally containing the greatest concentration of AII receptors. AII receptors in the pars nervosa, concentrated peripherally along the interface with the pars intermedia in normal rats, were undetectable in Brattleboro rats. These observations reveal a remarkable paradox: elevated circulating AII, insatiable thirst, and profoundly subnormal concentrations of brain circumventricular AII receptors. Studies are in progress to determine whether these deficiencies in brain AII receptors represent a reversible consequence of diabetes insipidus and elevated AII levels, or an irreversible brain AII receptor lesion.

176.9 CATECHOLAMINE METABOLITES IN DEPRESSED AND NON-DEPRESSED HYPERACTIVE BOYS. W.O. Shekim, J. Javaid, H. Dekirmenjian, J. Kashani*, K. K. Hodges*, L. Cytryn*, D. McKnew*, J.M. Davis. Clinical Research Center, University of Missouri Medical Center, Columbia, MO 65201.

The diagnostic category of childhood hyperactivity is generally thought to consist of heterogeneous groups. It is considered by many to represent masked depression or a depressive equivalent. Some hyperactive children are known to present with low self-esteem, feelings of worthlessness, self-deprecatory ideations, helplessness, feelings of loss of control, and isolations from peers.

We compared the urinary catecholamine metabolites, 3-methoxy-4-hydroxyphenylglycol(MHPG) and Homovanillic acid(HVA), in two groups of hyperactive boys: one group diagnosed as depressed on the basis of the Research Diagnostic Criteria(RDC) and the other group diagnosed as non-depressed. Twenty-five hyperactive boys ages 7-12 with full scale IQ of over 80 on the WISC and who were free from any neurological abnormality were involved in the study. The children met the diagnostic criteria for hyperkinetic Reaction of Childhood in the Children Diagnostic Scale(CDS) and the Children Diagnostic Classification(CDC). The diagnosis of depression on the RDC was made from the parents' responses on the Conners Parents Questionnaire and the Deveraux Parents Symptoms Checklist and from the psychiatric evaluation, including a mental status and nurses' observation notes during the children hospitalization in the Clinical Research Center. Three 24-hour urine samples were collected from each child on ice and aliquots were frozen and then were analyzed for MHPG according to the method of Dekirmenjian and MAAS(1970) and for HVA according to the method of Dziedzic et al. (1972).

Neither hyperactive children, depressed hyperactives nor non-depressed hyperactives differed from healthy control children or from each other in age, 24-hour urinary creatinine or body surface (m^2). Hyperactive children excreted lower levels of MHPG than controls (693 $\pm$ 237 vs 922 $\pm$ 168 ug/24 hrs/m^2, $p<0.01$) while their HVA excretion did not differ (2814 $\pm$ 723 vs 2959 $\pm$ 355 ug/24 hrs/m^2, p=ns). Depressed and non-depressed hyperactives did not differ in MHPG excretion (653$\pm$206 vs 709$\pm$246 ug/24 hrs/m^2, p=ns). The non-depressed hyperactives excreted lower levels of HVA than depressed (2639$\pm$706 vs 3265$\pm$575 ug/24 hrs/m^2, $p<0.05$.

The non-depressed hyperactive children excreted lower levels of MHPG & HVA than controls while depressed hyperactive children excreted lower levels of MHPG only. The findings are discussed in terms of the heterogeneity of hyperactive children and in terms of dopamine and norepinephrine theory of hyperactivity and norepinephrine theory of depression of childhood.

176.10 ENKEPHALIN ACTIVITY IN AN ANIMAL MODEL FOR HUNTINGTON'S CHOREA. B.I. Diamond, G.S. Sudakoff*, H.S. Havdala* and R.L. Borison. Mount Sinai Hospital, Rush Medical College, and Illinois State Psychiatric Institute, Chicago, IL 60608 & 60612.

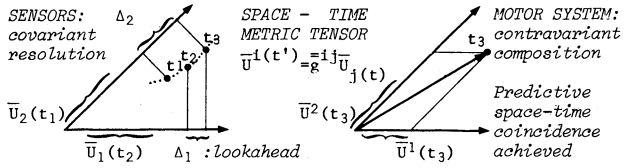
We have previously demonstrated evidence for a presynaptic facilatatory and postsynaptic inhibitory enkephalinergic input into the nigrostriatal system. Since this report, clinical studies have shown the efficacy of the opiate antagonist naloxone in Parkinson's disease and synthetic enkephalins in tardive dyskinesia. We now present experimental evidence for the role of the opiates in Huntington's Chorea (HC). Experiments were conducted using white male Sprague-Dawley rats which received intracaudate injections of kainic acid (KA). It has been shown that KA is neurotoxic to cell bodies, while sparing nerve terminals and axons in passage. Both the biochemistry and pharmacology of KA treated animals closely mimics that of HC. One week after KA injection, animals received injections of d-amphetamine (2 mg/kg) and stereotyped behavior was quantitated in the presence of drugs affecting the brain opiate system. We found that morphine (5 mg/kg, ip) produced a significant increase in stereotypy as compared to saline control. In contrast, D-phenylalanine (250 mg/kg), which is believed to block enkephalin degradation, failed to affect behavior. We also observed that methionine-enkephalin (2 mg/kg,ip) produced a significant increase in stereotypy. The administration of naloxone (20 or 40 mg/kg) produced a significant decrease in stereotypy in KA treated animals. These results differ from those found for the animal model of tardive dyskinesia, and may be reconciled by the fact that in KA treated animals there is a loss of the postsynaptic inhibitory enkephalinergic input. Our results would further suggest that opiate antagonists may have potential efficacy in the treatment of HC.

(Supported by Mount Sinai Hospital Medical Center Research Foundation, Chicago)

177.1 TENSORIAL REPRESENTATION OF SPACE-TIME IN CNS: SENSORY-MOTOR COORDINATION VIA DISTRIBUTED CEREBELLAR SPACE - TIME METRIC. A. Pellionisz and R. Llinás. Dept. Physiology & Biophysics, New York University Med. Ctr., 550 First Ave., New York 10016

Locating and intercepting moving objects is a coordinated sensorimotor act, aimed at achieving a coincidence of the interceptor and the target. While both sensory and motor functions relate to space-time, the conceptual basis of how such union is established by the brain is ill-understood; e.g. usually *separate time and space representations are assumed* and timing is linked to the concept of *simultaneity* (established by a centralized clock). However, the brain cannot use this timing principle, since there is no instantaneous signal capable of establishing such simultaneity.

The proposed space-time concept is based on the tensor network theory of CNS (*Neuroscience* 4:323,1979), which states that brain function is tensorial; i.e. brain activity vectors, assigned to objects of the external world, have reference-frame invariant properties. We further indicated (*Neuroscience* in press,1980) that in the oblique CNS reference-frame sensory information is resolved into *covariant* vectorial components, while motor execution is composed of *contravariant* components. Thus, coordination was defined as the geometrical transformation of the motor vector from covariant to contravariant expression; the first featuring intention, the latter allowing execution.



This scheme provides a geometrical interpretation of the unified space-time operation in CNS. Because of the different delays in the pathways sensory information relates not only to the space coordinates of the target, but also to the time of occurrence of the event, *in the past*. This information is coded in the signal itself. Given the different delays inherent in the sensory covariants a set of transformations must take place to ensure appropriate motor response: (1) Motor execution must be contravariant and (2) must refer to a *future* space-time coincidence. *The above scheme is a unification of the notion on temporal lookahead by Taylor expansion and the notion of cerebellar function as a metric tensor:* the covariant, distributed space-time components are (1)extrapolated by a "lookahead" (2)transformed by the space-time metric tensor. (Supported by USPHS grant NS13742 from NINCDS)

177.2 RESPONSES OF MOSSY FIBER UNITS IN THE MONKEY FLOCCULUS TO BACKGROUND MOVEMENTS DURING STEADY FIXATION. Hiroharu Noda. Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA 90024

The existence of visual inputs through mossy fibers to the monkey flocculus has been known. However, partially because of the fibers carrying visual information comprise only a small proportion of the afferent fibers, the properties of visual input signals to the monkey flocculus are mostly unknown. Single unit activity was recorded from the white matter of the flocculus of monkeys trained to fixate a visual target and to track the target when moved slowly. Mossy fibers were identified by eliminating fiber units of known Purkinje cell discharge patterns and climbing fiber units which discharged only once or twice per second.

A common feature of the responses of visual mossy fiber units was directional selectivity. Each unit had its own preference in the direction of stimulus movement and showed a vigorous response only when the stimulus was moved in the preferred direction. Stimulus movements in the other directions were usually not associated with discharges. The second and perhaps more important feature of the responses seen in a portion of mossy fiber units was their sensitivity to the velocity of stimulus movements. Within a range, the responses were positively related to the velocity of stimulus movements. Interestingly, their preferred velocity was within the range of that of smooth pursuit eye movements of the monkey. Responses of these fibers reflected the changes in the velocity when tested with sinusoidal background movements, showing the peak responses at approximately or slightly after the peak velocity of the movements. Most units studied in the present experiment responded optimally to sinusoidal stimulus movements whose peak velocity ranged from approximately 10 to 50 deg/sec. In response to the changes in the direction of ramp stimulus movements, mossy fiber units discharged with a mean latency of about 108 msec. In response to momentary illumination of the tangent screen with flashes of light, mossy fibers discharged with a mean latency of about 70 msec. Climbing fibers, including complex spikes which are thought to be Purkinje cell responses to climbing fiber inputs, did not show responses to visual stimulus movements in the range of slow eye movements of normal monkeys. (Supported by NIH Grant RO1 EY01051).

177.3 CEREBELLAR TARGETS OF VISUAL PONTOCEREBELLAR PROJECTIONS IN THE CAT. F. Robinson*, J. L. Cohen*, J. G. May*, and M. Glickstein (SPON: J. McIlwain). Walter S. Hunter Lab. of Psychology, Brown University, Providence, RI 02912.

The superior colliculus of the cat projects to the dorsolateral nucleus of the pons. Visual areas of the cat cortex project to a non-overlapping region of the rostral pons which is adjacent to the pyramidal tract on its medial and ventral borders.

Cells in these two pontine areas can be driven vigorously by appropriate visual targets. Previous studies (Mower, et al., J. Neurophysiol. 43:355, 1980) of antidromic invasion following electrical stimulation of the cerebellar cortex and retrograde transport of horseradish peroxidase have revealed that collicular-driven cells in the dorsolateral pons project to the vermis and the ipsilateral cerebellar hemispheres. The cortically-activated pontine visual cells project to the contralateral cerebellar hemispheres.

To study the projections of cells in these two pontine visual areas in more detail, we injected tritiated leucine for autoradiography. In one case, we injected the dorsolateral, collicular-driven area. In three cases, we injected H^3leucine amongst cells in the medial, cortically-driven area. The two visual areas in the pons were first identified by recording visually activated cells and noting their position. We then removed the microelectrode and replaced it with the needle of a microsyringe, locating the needle tip accurately in the visual pontine areas by using a microscopic zeroing stand. The animals were allowed to survive four days and their brains were processed for autoradiography.

Confirming our previous observations, the principal target of the dorsolateral visual area of the pons is the caudal vermis and ipsilateral hemisphere. The principle target of the medial, cortically-activated visual area of the pons is the contralateral cerebellar hemispheres. Within the contralateral hemisphere, the medial pontine visual area sends an especially dense projection to the dorsal and ventral paraflocculi. We also found that the medial visual area projects to the contralateral half of lobule IX of the vermis, the uvula. Though both the paraflocculus and uvula receive visual information from the medial pons, their non-visual inputs differ and they do not project to the same cerebellar nuclei. These two areas of the cerebellum may mediate visual control of two different classes of movement.

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177.4 PONTOCEREBELLAR PROJECTIONS IN THE CAT. AN AUTORADIOGRAPHIC STUDY. K. Kawamura and T. Hashikawa*. Dept. of Anatomy, Iwate Medical Univ., Morioka 020, Japan.

After unilateral injections of a small amount (0.5µl, 10µCi) of tritiated amino acids into various parts of the pontine nuclei proper and the tegmental reticular nucleus (N.r.t.) in 34 adult cats, labeled terminals of pontocerebellar fibers were found in the granular layer of the cerebellar cortex. No evidence was obtained of labeled fibers in the molecular layer.

In general, the pontocerebellar projection is bilateral with a contralateral preponderance. Part of the dorsolateral pontine nucleus and that of the N.r.t., however, demonstrate a stronger ipsilateral projection. The projection shows complex patterns of organization, characterized by both convergence and divergence. Thus, while a small region in the cerebellum receives fibers from several pontine areas, a small pontine area sends fibers to extensive cerebellar parts with a certain degree of preferential terminations in particular lobules or folia. For example, in addition to a strong projection from the dorsolateral nucleus to lobule VII, weak projections exist as well to lobules VI, VIII and IX, crus I and II, the paraflocculus and the paramedian lobule.

Small adjoining areas within the pons have different patterns of projections. For example, fibers from the lateral, central, and medial parts of the dorsolateral nucleus have preferential terminations in lobule VII, crus IIp plus dorsal paraflocculus, and the paramedian lobule, respectively. Parasagittal terminal zones of mossy fibers appear to exist in part of the anterior lobe and the paramedian lobule.

Although principles of the projection are similar, cerebellar projections from the N.r.t. appear to be more extensive in the fields of termination than those from the pontine nuclei proper.

177.5 CONGRUENCE OF SPATIAL PATTERNS OF RECEPTIVE FIELD PROJECTIONS TO PURKINJE CELL AND GRANULE CELL LAYERS IN THE CEREBELLAR HEMISPHERES OF THE RAT. James M. Bower, Donald C. Woolston,* and John M. Gibson, Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Tactile projections to the cerebellar granule cell (GC) layer of rats exhibit a precise, patchy, mosaic pattern of organization on several folia of the hemispheres (Shambes et al., BBE 15 : 94, 1978). The purpose of this study was to compare this novel spatial projectional pattern with that of the overlying (GC activated) Purkinje cells (PC's). Micromapping methods were used to sample both single-units in the PC layer and multiple-units in the underlying GC layer in the tactile hemispheres of rats anesthetized with ketamine hydrocloride. A mechanical stimulator delivered discrete tactile stimuli to a vibrissa or skin surface while spike activity of single PC's, identified by the presence of climbing fiber responses, was recorded. Dot rasters and PST histograms were used to judge the presence of initial short-latency excitatory PC responses. Two different procedures were used to determine the receptive field (RF) projectional pattern to the PC layer: 1) A single PC was isolated and the peripheral area localized which activated it; then the tactile stimulating probe was systematically placed at different points in and around that area until the exact RF of the PC was determined. 2) The probe remained in a fixed location on the skin while many single PC's in the PC layer were systematically sampled, thus determining the spatial distribution of PC's responding to stimulation of that peripheral locus. In both procedures the RF of GC's immediately underlying each PC was defined and later compared to the RF of that PC. Major results include: Tactile stimulation of the appropriate RF activates GC's at a latency of 4-6 msec and overlying PC's at a latency of 7-10 msec. With regard to excitatory peripheral RF projections, the PC layer is similar to the GC layer in the following respects: 1) The excitatory RF of each PC is very similar or identical to the RF of the subjacent GC locus; 2) the boundaries of the RF projections to the PC layer coincide with those of the RF projections to the subjacent GC patch; 3) the PC's overlying adjacent GC patches are activated by stimulation of the different RF's which project to those patches; 4) the ensemble of PC's responsive to a punctate skin stimulus is as small as the tiny GC patches lying beneath them; 5) PC's were found to be organized in a patchy, mosaic pattern which corresponds exactly with the pattern of RF's in the underlying GC layers. (Supported by NSF Grant BNS-16230 and NIH Grant NS-14748).

177.6 EFFECT OF NATURAL PERIPHERAL STIMULI ON THE PATTERNING OF SIMPLE SPIKE ACTIVITY IN PURKINJE CELLS. T. J. Ebner and J. R. Bloedel. Dept. Neurosurg. and Physiology, Univ. of MN, Minneapolis, MN 55455.

Many investigators have documented that Purkinje cells can increase or decrease their simple spike firing rate in response to natural proprioceptive and exteroceptive stimuli. However, the data processing techniques commonly employed for these analyses provide no information regarding the effects of these stimuli on the temporal dependency of the unitary activity. This study was undertaken to demonstrate that natural peripheral stimuli can evoke marked changes in the "temporal patterning" present in the simple spike activity of Purkinje cells located in the cerebellum of decerebrate, unanesthetized cats. Exteroceptive stimuli consisted of sinusoidal indentation of the skin in the animal's paw or forearm. Proprioceptive input was provided by step-like stretches of the gastrocnemius-soleus. Extracellular unitary activity of Purkinje cells was recorded in the ipsilateral vermal and paravermal region of the anterior lobe. Identification of a Purkinje cell was based on the presence of spontaneous responses to climbing fiber inputs. The autocorrelation of the simple spike activity was constructed during continuous sinusoidal cutaneous stimulation of the forearm at several frequencies as well as during spontaneous activity. Type 1 autocorrelations, characterized by a positive correlation at short lag time which decayed slowly to baseline, could be modified by exteroceptive stimuli. Both the magnitude and decay time of the positive correlation could be altered. For Purkinje cells with Type 2 autocorrelations (positive correlation at short lag time which rapidly returned to baseline), exteroceptive stimuli could alter the patterning in the spike train to produce an autocorrelation with Type 1 characteristics. These alterations in the autocorrelograms were independent of any modulation in the firing rate of the cell produced by the sinusoidal exteroceptive stimuli. Use of an additional autocorrelation technique developed for analyzing non-stationary stochastic processes allowed an examination of the effects of short duration proprioceptive or exteroceptive stimuli applied at low frequencies (0.5-1.0 Hz.) on the correlation as well as the firing rate in the simple spike activity of these cells. Some Purkinje cells were found to undergo dramatic increases in the positive correlations within their simple spike activity during the natural peripheral input, often with only minimal modulation of their firing rate. These findings demonstrate that cutaneous and proprioceptive inputs can modify the temporal patterning in the simple spike activity of Purkinje cells independent of changes evoked by the same stimuli in the firing rate of neurons. (Supported by NIH Grant No. 2RO1-NS-09447-10.

177.7 CEREBELLAR CORTEX OF THE RAT: PRESUMED INHIBITORY INTERNEURONS ARE ACTIVATED EARLIER THAN PURKINJE CELLS BY A PARALLEL FIBER VOLLEY. H. Axelrad and H. Korn, INSERM U3, CHU Pitié-Salpêtrière, Paris 13° France.

Extracellular recordings were obtained with glass microelectrodes from the cerebellar cortex of lightly nembutalized albino rats. 17 cells exhibiting diphasic spikes were discriminated as Basket cells (Bcs) since 1) they were located in the lower half of the molecular layer, 2) they responded to a parallel fiber volley (pfv) produced by a local (Loc) stimulation with 1-5 spikes, the latency of which decreased at increased stimulus intensities, 3) following a juxta fastigial (JF) stimulation spikes were only induced at long latencies and during the thereby evoked mossy fiber field potential, 4) in addition to these classical criteria (Eccles, Llinas, Sasaki, Exptl. Brain Res., 1966, 1:1-16) no collision was observed between the long latency action potentials of these neurons and those of Purkinje cells (Pcs) even when the JF stimulus was adjusted to produce a maximum amplitude antidromic field potential. Purkinje cells encountered along the same tracks were identified on the basis of their large and short latency antidromic spikes produced by JF stimulation and/or their climbing fiber responses. Two unexpected observations were made: 1) the threshold i.e. the lowest Loc stimulus strength adequate to activate Basket cells (iBc) was consistently lower than that for Purkinje cells (iPc) activation ($\bar{m}$ of iBc/iPc = 0.7; range 0.67-0.74), 2) for a given Loc stimulus, Bcs fired earlier than Pcs, with a difference which amounted to $\bar{m}$ = 1.14 msec (SD = 0.28; n= 15). However, this value does not reflect exactly the difference in latencies of activation of the two groups of cells by pf fibers which for each of them must be computed with respect to the time of arrival of the presynaptic volley. Superficially this parameter is indicated by the positive peak of the triphasic Loc evoked field described by Eccles, Llinas and Sasaki (Exptl. Brain Res., 1966, 1:17-39); in our experiments, this positive dip appeared earlier at deeper levels, with measured conduction velocity for superficial and deeper parallel fibers thus calculated being of respectively 0.33 and 0.38 m/sec. Even if morphological data showing 1) a constant gradient increase of pf diameter from the surface to deeper levels of the molecular layer 2) a different space distribution of Bc and Pc soma and dendrites (Palay and Chan Palay, The Cerebellar Cortex, Springer Verlag, 1974) were taken in consideration the deep volley→Bc firing latency was still shorter than that from the superficial volley→Pc firing with a difference reduced to only 0.49 msec (SD=0.33; n=15). These data strongly suggest that Bcs are activated by a set of pfs where deeper and faster conducting ones are statistically more numerous than those exciting the correspondingly located Pcs.

177.8 OLIVOCEREBELLAR PROJECTIONS TO THE NODULUS IN THE RAT. K.D. Phelan*, J.A. Rubertone* and W.R. Mehler (SPON: R. Snyder) NASA Ames Res. Ctr., Moffett Field, CA., 94035 and Univ. Calif. San Francisco, CA., 94143.

The topographical projection of the inferior olivary nucleus onto the nodulus in the rat was investigated using the retrograde axonal transport of horseradish peroxidase (HRP). Single iontophoretic injections (1.0-3.0 ua, 20-60 um pipette tips) of HRP (Sigma Type VI) were stereotaxically placed in the nodulus of adult rats. Following 24-48 hr survival times, each animal was perfused (1.0% Paraform-:1.5% Glutar-aldehyde), 50 um transverse serial frozen sections cut and treated with benzidine dihydrochloride (BDHC, Mesulam, '76) or tetramethylbenzidine (TMB, Mesulam, '78).

These experiments indicate that the nodulus in the rat receives afferent projections mainly from cells in the dorsal cap of Kooy (dc) and the ventrolateral outgrowth (vlo). Some cells in nucleus beta (B) and the dorsomedial cell column (dmcc) may also contribute to this projection. These findings are similar to HRP labeled cell patterns reported from this lab in an earlier investigation in the cat (Phelan and Mehler, Soc. Neurosci. Abstr., Vol. 5,p.106,'79) and other studies in the rabbit (Alley et al., Brain Res. 98: 582-589,'75; Hoddevik and Brodal, J. Comp. Neur. 176: 269-80,'77). Some minor species differences might exist in the olivocerebellar projections to the nodulus in the rat, cat and rabbit and will be discussed.

Lateral injections, with spread primarily confined to the lateral third of the nodulus only, result in HRP labeled cells limited to the contralateral vlo, while in more medially located injections labeled cells were found chiefly in the dc and vlo,and possibly B and dmcc. These preliminary results seem to indicate that a similar longitudinal distribution of olivocerebellar projections to the nodulus may also exist in the rat, as has been reported in autoradiographic studies in the cat (Groenewegen, Voogd and Freedman, J. Comp. Neur.183: 551-602,'79)

Injections involving the uvula result in labeled cells in B, dmcc, the medial accessory olive and the principal olive, a cell pattern similar to that found in the cat (Brodal, J. Comp. Neur. 166:417-26,'76;Phelan and Mehler,'79).

The distribution and size of labeled cells within the various subdivisions of the inferior olive will be discussed.
Supported by NASA-Task 199-05-02-07.

177.9 PATTERNS OF TERMINATION OF THE CEREBELLOTHALAMIC PATHWAY IN THE MONKEY. C. Asanuma, W.T. Thach and E.G. Jones. Departments of Anatomy and Neurobiology and Neurology and Neurosurgery, Washington University School of Medicine, St. Louis, MO 63110.

The cerebellar relay area in the monkey thalamus is more restricted than customarily thought. It is confined to the relay to area 4: the common VPLo-VLc nucleus, with lesser inputs to the central lateral (CL) nucleus. Single or multiple [^{3}H] amino acid injections were made into parts of dentate n., n. interpositus or n. fastigius and, for comparison, into the dorsal column nuclei. Patterns of cerebello-thalamic label are continuous across the "border" of VPLo and VLc. Label extends into anterodorsal extensions of VLc, but no terminal labelling overlies the dense cellular clusters of the VLo nucleus. Posteriorly there is no overlap into the terminal territory of the medial lemniscus in VPLc and VPM. The frequency of isolated patches of label in CL appears highest following dentate injections.

A lamellar organization is apparent within VPLo-VLc following injections of all deep cerebellar nuclei but is particularly obvious following n. interpositus injections. Parasagittal sections reveal rows of evenly spaced labelled clusters extending dorsally within VPLo and curving posteriorly within VLc. These clusters of grains are also aligned in rows in the mediolateral dimension, thus defining curving planes within which grain clusters are distributed in a checkerboard arrangement. Such arrays can be readily seen in tangential sections through these lamellar planes. Anteroposteriorly oriented rods and isolated clusters of terminal labelling result following injections of the other deep cerebellar nuclei. These rods have a tendency to intersect the lamellae. The lamellar organization corresponds to the distribution of thalamocortical relay cells: following punctate horseradish peroxidase injections into area 4, retrogradely labelled clusters of cells are aligned mediolaterally within similar lamellae extending through VPLo-VLc.

The results indicate that VPLo and VLc form a common nucleus which is distinct from the lemniscal relay nucleus, VPLc, and from the pallidal relay, VLo, and projects only to area 4. Within VPLo-VLc, there is a finer organization of topographic lamellae and subsidiary clustering of inputs and of relay cells.

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177.10 CEREBELLAR CORTICAL EFFERENT FIBERS OF THE DORSAL CULMINATE LOBULE (ANTERIOR LOBE - LOBULE V) OF THE SQUIRREL MONKEY, SAIMIRI SCIUREUS. D.E. Haines and G.W. Patrick. Dept. of Anatomy, West Virginia Univ. Sch. of Med., Morgantown, WV 26506.

The efficacy of chronic cerebellar stimulation (CCS) as a viable treatment for movement disorders is debated in the literature. Although primates have been utilized as animal models in a number of investigations designed to ascertain the value of CCS little data is available concerning the spatial organization of corticonuclear fibers in primates. This study details the arrangement of cortical efferent fibers from the dorsal culminate lobule (anterior lobe) in the squirrel monkey. Lobule V was selected for this study since, in human, stimulating electrodes are frequently placed on this region of cortex.

A total of 14 lesions were made in lobule V. Additional lesions were placed in the immediately adjacent cortex of lobule VI (1 case) and lobule IV (3 cases). Following survival times of 4-12 days all animals were killed via perfusion with .9% heparinized saline followed by 10% formalin. Brainstems with cerebelli intact were cut at 40 µm is coronal, horizontal and parasagittal planes and impregnated with the Fink-Heimer method.

From lesions located in approximately the lateral one-third of the lobule, degenerated fibers enter rostrolateral and rostral portions of the lateral cerebellar nucleus (NL). This terminal field within NL may be divisible into two portions. Corticonuclear fibers arising from more medial regions of the lobule (so-called intermediate cortex) terminate primarily in portions of the anterior (NIA) and posterior (NIP) interposed nuclei. Lateral areas of intermediate cortex (IC) are related mainly to lateral and rostrolateral NIA while medial regions of IC project into medial NIA with a lesser contribution entering the caudally adjacent NIP. The pattern of degeneration in NL, NIA, NIP following lesions of lateral and intermediate cortex indicate that cortical zones D (related to NL) and C_{1-3} (related to NIA and NIP) are present in squirrel monkey. The vermis, as reflected by the trajectory of its efferent fibers, extends about 2.5 mm lateral from the midline. Lateral portions of vermal cortex project into the vestibular complex while its medial areas are related to the medial cerebellar nucleus. These represent cortical zones B and A respectively. Although specific regions of cortex project primarily into identifiable areas of their respective nuclei there is some overlap at the interface of adjacent zones. These results show that corticonuclear fibers in the primate project into the deep nuclei in a complex and highly organized manner. The sometimes capricious results of CCS may be partially related to electrode position. More precise placement of electrodes in relation to cortical zones may precipitate more consistently beneficial results. (Supported by USPHS Grant NS 11327).

177.11 VESTIBULOCEREBELLAR PROJECTIONS TO THE PARAMEDIAN LOBULE OF THE TREE SHREW (TUPAIA GLIS). G.W. Patrick and D.E. Haines, Dept. of Anatomy, West Virginia Univ. Sch. Med., Morgantown, WV 26506.

Secondary vestibular connections with the cerebellum have been described in many animal species. The vast majority of vestibulocerebellar fibers end in archicerebellar regions, that is the vermis and flocculonodular lobe. In the course of another study concerned with brainstem afferents to the paramedian lobule (PML), a moderate number of retorgradely labelled somata were found in the vestibular nuclei. This report describes the distribution of these cells. A total of ten tree shrews (Tupaia glis) were utilized in this study. Under ether anesthesia, the skull overlying the paramedian lobule was removed and a small sliver of crystalline horseradish peroxidase (HRP) was implanted. Following survival times of 22-38 hours, the animals were killed by transcardiac perfusion. The brainstems and cerebelli were cut in frozen section at 50µ in transverse plane. The tissues were subsequently processed according to both TMB or DAB techniques. HRP positive cells are present in both the medial (MVN) and spinal (SPVN) vestibular nuclei. Lateral and superior vestibular nuclei are completely devoid of HRP labelled cells. HRP-reactive somata are located primarily in the caudal two-thirds of the SPVN and MVN. The total length of these nuclei which contain labeled somata is approximately 900-1400µm. Both ipsilateral and contralateral MVN and SPVN project to paramedian lobule. HRP implants in more lateral regions of PML resulted in fewer labelled cells in the vestibular nuclei on both sides. This would suggest a diffuse medial to lateral topographic relationship fewer between the SPVN and MVN bilaterally and the PML. Since SPVN and MVN receive direct input from portions of flocculonodular lobe to which they project, it was considered prudent to check for reciprocity in the above described vestibulocerebellar pathway. In 10 animals lesions were placed in the PML and tissue was processed by the Fink-Heimer technique. The lack of terminal debris in SPVN and MVN indicates no direct reciprocity from PML to these nuclei. These results suggest that although the PML receives afferents from the MVN and SPVN, this portion of cerebellar cortex may play no direct role in motor coordination via circuits through the vestibular complex. (Supported by USPHS Grant NS 11327-5,6 from NINCDS.)

177.12 IMPROVEMENT OF ABNORMAL PROPRIOCEPTIVE REFLEXES IN SPASTIC MONKEYS BY CEREBELLAR STIMULATION. J. L. Vitek*, T. J. Ebner, A. Schwartz* and J. R. Bloedel. Dept. Neurosurg., Univ. of Minnesota, Minneapolis, MN 55455.

The effects of stimulating the cerebellar surface on abnormal segmental reflexes was examined in monkeys (Macaca mulatta and Cercopithecus aethiops) rendered spastic by a two stage bilateral decortication of areas 1, 2, 3, 4 and 6. The rectified, integrated EMG from the biceps and triceps as well as the torque were recorded and averaged during several successive flexion-extension movements of the arm produced by a displacement controlled torque motor. Two passive movement paradigms were employed. The first, a ramp-and-hold paradigm, consisted of a rapid 30-45 degree flexion of the forearm, a 2 sec. period during which the limb position was held constant, followed by a rapid extension returning the limb to the initial position. In the second paradigm, the forearm position was modulated sinusoidally at several different frequencies. A cerebellar stimulating electrode was placed over the pars intermedia and medial cerebellar hemispheres of the anterior lobe, although the anterior part of the posterior lobe was also contacted. Each electrode consisted of three platinum discs (each 7.6 mm.^{2}). Different frequencies (10-300 Hz.) and charge densities (1.5-10 u coul/cm.^{2}) were used to maximize the physiological effects described below. In each experimental trial short periods of cerebellar stimulation (10-30 min.) were interspersed between control periods. The findings from each experimental animal were extremely similar. As in some types of clinical spasticity, flexion or extension of the extremity was capable of evoking co-activation of flexors and extensors. Cerebellar stimulation reduced the amplitude of the phasic and tonic stretch reflexes recorded from the triceps during flexion and decreased the abnormal triceps response during its shortening. The biceps response to stretch was increased by cerebellar stimulation. However, the abnormal response during flexion was decreased. Cerebellar stimulation was also capable of improving the organization of segmental reflexes, producing a more normal reciprocal relationship in the EMG activity evoked in biceps and triceps. In the absence of cerebellar stimulation the activation of agonist and antagonist occurred at approximately the same phase during sinusoidal limb displacement. During cerebellar stimulation, the peak activity in the flexor and extensor became approximately 180° out of phase. Therefore, stimulation of the cerebellar surface can produce dramatic physiological effects in spastic monkeys, including improvement in the organization of activity evoked during the stretch reflex. This research was supported by NIH Contract No. NO1-NS-4-2332, and NIH Grant No. 2RO1-NS-09447-10.

177.13 IN VITRO STUDIES OF THE BRAIN STEM-CEREBELLAR SYSTEM PERFUSED VIA THE BASILAR ARTERIAL SYSTEM. R. Llinás, Y. Yarom* and M. Sugimori*. Dept. Physiology & Biophysics, New York Univ. Med. Ctr., 550 First Ave., New York 10016.

Last year a set of experiments on isolated brain stem in guinea pigs was reported (Yarom and Llinás, Soc. Neurosci. Abst. 5: 109, 1979). Recently we have developed the technique to include the brain stem and the cerebellum en block. This tissue may be kept alive for as long as 12 hours after Ringer solution perfusion via the arterial system while keeping the tissue under a rapidly moving Ringer solution in the external bath. The present studies indicate that both brain stem and cerebellum can survive such isolation if the perfusion fluid is kept under strict osmotic and pressure conditions (Yarom and Llinás, 1979) and oxygenated with 0.001% H_2O_2 in Ringer (Llinás and Sugimori, J. Physiol. 305: 1980 in press). Studies in the detailed electrophysiological analysis of the cerebellar cortex indicate that antidromic, climbing fiber, and mossy fiber/granule cell activation of Purkinje cells may be observed and are in every way similar to the records obtained in vivo. In addition, Golgi and stellate-basket cell inhibition have all the spatial and temporal properties found in normal circumstances. The study has allowed a definition of field potentials and intra- and extracellular unitary recordings, all of which indicate that the known properties of the normal circuitry are intact in the present condition.

The preparation is determined extremely useful for the study of pharmacology and of nerve nets under conditions of high stability. Concomitant with the electrophysiological studies, measurement of oxygen levels was obtained using platinum microelectrodes. The measurements indicate that while the oxygen levels in the extracellular medium are close to normal in the presence of H_2O_2, the firing of the neuronal components either by direct or reflex stimulation does not fall as low as in the brain perfused without such oxidating agent. In addition, in the presence of H_2O_2 the extracellular level of oxygen recuperates quickly to the normal baseline. This change in oxygen levels correlates well with the ability of the nervous system to follow stimulation at high frequencies and to show depletion following protracted stimulation. (Supported by USPHS grant NS-13742 from NINCDS)

178.1 SYNAPTOSOMAL CONVERSION OF RADIOACTIVE PYRUVATE INTO RADIOACTIVE ACETYLCHOLINE. A.M. Benjamin* and J.H. Quastel. Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

Whereas the presence of CoA alone (0.1 mM) in the incubation medium has little effect on the conversion of [2-^{14}C]-sodium pyruvate (5 mM) into ^{14}C-acetylcholine in the presence of choline (5 mM) by a P_2 fraction (4-6 mg protein) of rat brain, the addition of $CaCl_2$ (1 mM) causes a marked acceleration of the reaction, producing 200 nmols ACh per 100 mg P_2 protein per hour. The addition of $CaCl_2$ (1 mM) alone has no effect. With Triton X-100 (0.1%) present at a concentration that ruptures mitochondrial membranes, the addition of CoA (0.1 mM) alone to the P_2 fraction produces over a five-fold increased rate of ^{14}C-ACh conversion, the further addition of $CaCl_2$ (1 mM) having a small (20%) retarding affect. It would appear that the presence of Ca^{++} facilitates the entrance of CoA into the mitochondria where the conversion of pyruvate into acetyl-CoA occurs and/or the efflux of acetyl-CoA from the mitochondria into the synaptoplasm containing choline acetyltransferase. Whereas the addition of NAD (1 mM) to the incubation medium has little or no effect on the conversion of pyruvate to ACh in the P_2 fraction, it has a marked (30-fold) effect when Triton (0.1%) is also present. This is possibly due to restoration of the NAD concentration present originally in the mitochondria. Triton (0.1%) blocks the respiration of the P_2 fraction, but the corresponding amount of acetyl-CoA spared from respiration is only partially (about 20%) converted into ACh. Phospholipase A_2 (venom) causes release of CoA + acetyl-CoA from the P_2 fraction, presumably by affecting the permeability of the mitochondrial membrane. It would seem that the permeability effect of Triton is proportional to its concentration (0.02%-0.06%). The maximum amount of ACh derived from pyruvate in a P_2 fraction (in absence of Triton) occurs at about 1 mM pyruvate. Sodium oxaloacetate is a potent inhibitor of ACh formation from pyruvate or from acetyl-CoA, almost complete inhibition occurring at 2 mM. Sodium pyruvate (5 mM) exercises a smaller inhibitory effect from acetyl-CoA.

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178.2 ACETYLCHOLINE HAS A POWERFUL DISINHIBITORY ACTION IN THE HIPPOCAMPUS. R.J. Reiffenstein*, K. Krnjević and N. Ropert* (SPON: D. Bindra). Anaesthesia Research and Physiology Depts., McGill University, Montreal, Canada H3G 1Y6.

It is known that ACh has a slow, prolonged muscarinic excitatory action on some hippocampal neurons (Biscoe & Straughan, J. Physiol. 183,341,1966). We describe here a different excitatory action of ACh seen in the superficial pyramidal layer during fimbrial stimulation. Our experiments were done on urethane-anaesthetised rats, using multibarrelled micropipettes to record field responses evoked in CA1 and CA3 areas of dorsal hippocampus by fimbrial shocks. At low frequencies of stimulation (< 2/s) the main response is a relatively large, slow positive wave (peak latency about 10 ms) in the pyramidal cell layer. This reflects the powerful IPSPs generated by recurrent collaterals of pyramidal axons (Andersen et al., J.Neurophysiol. 27,592,1964). Stimulation at higher frequencies rapidly leads to the appearance of single or multiple negative "population" spikes. Recent evidence (Ben-Ari et al., Can.J.Physiol.Pharmacol. 57, 1462,1979) suggests this "release" of firing is due to a reduced efficacy of the recurrent inhibitory pathway. We now report that a similar release of firing can be produced by iontophoretic application of ACh (≥ 10 nA) during low-frequency fimbrial stimulation. At the same time the amplitude of the positive field diminishes. That this effect is due to disinhibition, rather than direct stimulation, is strongly supported by the following: 1) ACh is effective only where the stimulus generates the largest positive field - that is, near the pyramidal cell bodies where inhibitory synapses are concentrated; 2) ACh only generates spikes at fimbrial stimulus strengths which also evoke spikes at higher stimulus frequencies; 3) the onset and offset of the ACh action is much more rapid than the time course previously described for single unit excitation; 4) L-glutamate, a powerful excitant of pyramidal cells, has no comparable effect; 5) but agents known to antagonise GABA show a similar effect (Ropert et al., 1980, this meeting). The disinhibition is muscarinic in nature since methacholine and bethanechol are also effective, while tetramethylammonium and butyrylcholine are not. However, both nicotinic and muscarinic antagonists can generate a similar excitation, possibly by blocking GABA receptors. Since eserine enhances the population spikes, as well as potentiating the effects of ACh, it appears that cholinergic inputs may play an important role in the control of hippocampal pyramidal cell firing, by reducing inhibition normally operating.

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178.3 ACETYLCHOLINE: A NEUROMODULATOR OF HIPPOCAMPAL PYRAMIDAL NEURONS. L.S. Benardo and D.A. Prince. Dept. of Neurology, Stanford University Medical School, Stanford, CA 94305.

The mode of action of acetylcholine (ACh) in the mammalian central nervous system has not been studied extensively. The hippocampus is known to receive a cholinergic input from the septal nuclei, but the mechanism of excitation by ACh and the related changes in cellular behaviors have not been completely described. Intracellular recordings were obtained from a total of 105 hippocampal CA1 pyramidal cells (HPCS) utilizing the guinea pig hippocampal slice preparation. Normative data were collected from 74 HPCS. In 46 of these neurons the action of ACh was investigated, using either focal pressure application (1-200 mM) or iontophoresis (.1-1 M) onto dendrites in stratum oriens or stratum radiatum. In all HPCS, ACh induced depolarizations of membrane potential (V_m) of up to 28 mV coincident with increases in input resistance (R_N) of up to 300%. When R_N was compared at various levels of V_m before and after ACh application, increases in R_N were noted to have both voltage dependent and independent components, perhaps corresponding to actions on a delayed rectification and a resting K^+ channel, respectively. In 18 cells an initial hyperpolarization of up to 10 mV lasting 2-30 seconds, and associated with decreases in R_N, was noted to precede these events.

The increases in R_N were quite prolonged, as judged by comparisons of membrane properties in populations of neurons before and following ACh exposure, and outlasted changes in V_m. Elevations in R_N seemed to underlie the striking long-term alterations in cellular behaviors subsequently noted. Following ACh application HPCS shifted from a single spike firing mode to one of burst generation. Bursts occurred spontaneously and could be evoked by afferent or intracellular stimulation. These changes persisted for the duration of an experiment (hours).

Following perfusion of slices with solutions containing atropine (10^{-7}-1.5 x 10^{-6} M) (n = 24) or scopalamine (10^{-6} M) (n = 7) all responses noted above were blocked, indicating that these effects of ACh were mediated through muscarinic receptors. In addition, exposure to muscarinic antagonists uncovered a tonic action of endogenous ACh on membrane properties of HPCS. Mean R_N in atropine was 21.9 ± 7.7 (S.D.) MΩ, while R_N in the control group of neurons was 37.6 ± 8.7 (S.D.) MΩ, though relatively little change was noted in mean resting V_m. Thus spontaneous ACh release appears to have an important influence on resting membrane properties of HPCS. This remarkable finding considered along with the actions on voltage dependent conductance and the profound potentiation of cellular responses supports classification of ACh as a neuromodulator of HPC activities.

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178.4 TWO [^{3}H] QNB BINDING SITES IN RAT HIPPOCAMPAL MEMBRANES: CORRELATION OF ONE SITE WITH CHOLINERGIC STIMULATION OF PHOSPHATIDYLINOSITOL METABOLISM IN HIPPOCAMPAL SLICES. James N. Davis, William R. Tyor*, Keith A. Crutcher and Elizabeth Hoyler*, Dept. of Medicine (Neurology) and Pharmacology, Duke University Medical Center and Veterans Administration Medical Center, Durham, N.C. 27705.

[^{3}H] Quinuclidinyl benzilate (QNB) binds to sites on brain membranes with the characteristics expected for muscarinic cholinergic membrane receptors. Recent studies have demonstrated that [^{3}H] QNB binding is more complex than originally thought, since some cholinergic agonists compete with [^{3}H] QNB as if more than one population of membrane binding sites was present. We have correlated the binding of [^{3}H] QNB to membranes from the rat hippocampal formation with the ability of cholinergic agents to elicit or block [^{3}H] myo-inositol incorporation into hippocampal slice phospholipids. Using a computer modeling approach, two [^{3}H] QNB bindings sites (Site No. 1, 0.63 pmol bound/mg protein, and Site No. 2, 0.17 pmol/mg) could be distinguished in hippocampal membranes by differences in the affinity of agonists for [^{3}H] QNB binding. At site 1, the agonists displayed a typical muscarinic potency series with oxotremorine > acetylcholine > carbachol. The potency of these agonists in competing for [^{3}H] QNB binding to site 1 was the same as their potency in eliciting [^{3}H] inositol incorporation into hippocampal slice phospholipids. By contrast the affinity of the agonists for site 2 was carbachol ≥ acetylcholine ≥ oxotremorine. [^{3}H] QNB and atropine, both active as antagonists in the incorporation of [^{3}H] I into hippocampal slice phospholipids, had the same affinity for both sites. These data demonstrate the presence of two distinct [^{3}H] QNB binding sites in hippocampal membranes, one of which (site No. 1) appears to mediate cholinergic stimulation of phosphatidylinositol metabolism in hippocampal slices. The physiological relevance of the other site remains to be determined.

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178.5 TEMPERATURE DEPENDENCE OF AGONIST COMPETITION AT [^{3}H] QNB BINDING SITES IN RAT HIPPOCAMPAL MEMBRANES.
William R. Tyor, Andre De Leon, and James N. Davis
(SPON: R. Nashold), Department of Medicine (Neurology) and Pharmacology, Duke University Medical Center and Veterans Administration Medical Center, Durham, N. C. 27705.

Recent radioligand binding studies have suggested that the affinity of membrane receptors for agonists, but not antagonists is temperature dependent. We studied the temperature dependence of agonists and antagonists to compete with [^{3}H] QNB for binding in rat hippocampal membranes using a computer modeling approach. Membranes were incubated with [^{3}H] QNB at either 25°C (2 hours) or 4°C (17 hours) with or without a small series of cholinergic agonists and antagonists. [^{3}H] QNB bound to 0.80 ± 0.14 pmols of sites/mg protein in these membranes. The K_D at 25° (28 ± 1 pMolar) was significantly different ($p < 0.01$) from the K_D at 4° (108 ± 10 pMolar). By contrast the other antagonist tested, atropine, showed no difference in affinity for these sites when studied at 25° and 4° (K_D, 0.27 nM). The two agonists tested at 25° and 4° also demonstrated differences in their affinity for the [^{3}H] QNB binding sites. Acetylcholine and oxotremorine competed with [^{3}H] QNB binding as if two populations of sites were present. Oxotremorine demonstrated the same affinity for both sites at 25° (0.16 ± 0.03 μM) but a significantly ($p < 0.01$) different affinity for the two sites at 4°C (0.027 ± 0.001 μM). Similarly the affinities of acetylcholine for the two populations of sites at 25°C (0.03 ± 0.03 μM, 10 ± 1 μM) was significantly different ($p < 0.01$) than the affinities of acetylcholine at 4° (0.01 ± 0.02 μM, 4.7 ± 0.6 μM). From these studies we conclude that like some other radioligand binding sites, hippocampal [^{3}H] QNB sites demonstrate a greater affinity for agonists when binding is carried out at lower temperatures.

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178.6 INCREASED MUSCARINIC CHOLINERGIC RECEPTORS IN THE DEAFFERENTED HABENULA. Zehava Gottesfeld and Adriana Maggi*, Dept. of Neurobiol. & Anat., Univ. of Tex., Med. Sch., Houston; and Dept. of Cell Biol. Baylor Col. Med., Houston, Texas 77025.

It has been reported recently that the habenular nuclei (Hb) are innervated by cholinergic projections from the septal-diagonal band area via the stria medullaris (SM)(Gottesfeld & Jacobowitz, 1979). Ample evidence exists to show that drug-induced acetylcholine depletion causes supersensitivity that is associated with elevation of cholinergic receptor binding in the deprived regions. This work was undertaken to determine whether or not deafferentation of the Hb will result in an increased density of muscarinic receptors in these nuclei. Radiofrequency lesions were produced in the SM of Sprague-Dawley male rats (180-200g). Seven weeks post-lesion the animals were killed by decapitation and the Hb and the hippocampus were removed from frozen brain sections (300 μM thick). Sham-treated control rats underwent similar treatment, but lesions were not produced. The accuracy of the lesion placement was verified microscopically by observing 60 μM thionine-stained sections. Cholinergic muscarinic receptor binding was assayed using ^{3}H-Quinuclidinyl benzilate (^{3}H-QNB) as a ligand (Yamamura et al., 1974). Scatchard analysis plots carried out on the Hb of lesioned and sham-treated control rats, showed an increase of ^{3}H-QNB binding (Bmax) in the deafferented Hb by 36%, with no change in dissociation constant (Kd). In the hippocampus of control and lesioned animals, no difference was indicated in either the Bmax or Kd. Based on the present results, it appears that SM lesions induce an increase of muscarinic cholinergic receptors binding only in the Hb. Whether or not the observed elevated QNB binding is associated with acetylcholine supersensitivity in the Hb has yet to be determined.

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178.7 AUTORADIOGRAPHIC LOCALIZATION OF MUSCARINIC CHOLINERGIC RECEPTORS IN RAT BRAIN STEM. M. Lewis, J.K. Wamsley, W.S. Young, III and M.J. Kuhar
(SPON: R. Dismukes). Johns Hopkins School of Medicine, Departments of Pharmacology and Psychiatry, Baltimore, Maryland 21205.

Quinuclidinyl benzilate (QNB) is a potent muscarinic cholinergic antagonist and was used previously in our laboratory to provide the first anatomical localization of muscarinic receptors in the rat forebrain by *in vivo* autoradiography. We have now extended these studies to include the localization of muscarinic cholinergic receptors in the rat brain stem and have employed a new *in vitro* autoradiographic method (Young and Kuhar, Brain Res., 179:255-270). Initially we characterized the binding of ^{3}H-QNB to our tissue sections and examined the kinetics and specificity of this binding in order to determine that we were labeling the receptors as they were originally described in pharmacological studies. Using a 2 hr incubation in 1 nM ^{3}H-QNB followed by a 10 min rinse (blanks were generated on adjacent slides by incubating under the same conditions, but in the presence of 1 micromolar atropine) we were able to generate specific to nonspecific (signal to noise) ratios as high as 70 to 1. Thus, the *in vitro* method provides an easy and economical method with which we can label muscarinic cholinergic receptors with a high specificity not possible with conventional methods of autoradiography. Our results have demonstrated high concentrations of muscarinic cholinergic receptors in the superficial layers of the superior colliculus , in the pontine nuclei, parabrachial nuclei and the nucleus of the trigeminal nerve. The locus ceruleus showed a more moderate level of grains and the superior olive demonstrated virtually no binding sites for ^{3}H-QNB at all. The medial vestibular nucleus was seen to have a high grain concentration after incubation with ^{3}H-QNB while the other nuclei of the vestibular system showed only low levels of grains. The nucleus of the facial nerve also demonstrated a high concentration of autoradiographic grains while the nucleus tractus solitarii and nucleus ambiguus were seen to have intermediate numbers of muscarinic binding sites. The dorsal column projection sites, nucleus gracilis and nucleus cuneatus were also seen to have intermediate numbers of muscarinic receptors. The spinal cord bound a high concentration of ^{3}H-QNB in the substantia gelatinosa of the dorsal horn. A slightly lower number of grains were found in lamina IX and X in the ventral horn of the spinal cord. These autoradiographic localizations of muscarinic cholinergic binding sites will help to localize the muscarinic cholinergic sites of action of the neurotransmitter acetylcholine and may help explain the effects of various anticholinergic drugs.

178.8 GLIAL STORAGE OF ACETYLCHOLINE IN THE FROG FILUM TERMINALE. T. Ritchie, S. Glusman and B. Haber. Marine Biomed. Inst., Dept. Human Biol. Chem. & Genetics & Dept. of Neurology, Univ. Tex. Med. Branch, Galveston, TX. 77550

We have been utilizing the filum terminale (FT) of the frog spinal cord, a structure composed predominately of normal glial cells with only a few peripherally located myelinated axons, to study a number of properties of normal glia. The frog FT is a structure formed during normal development as a result of the degeneration of motoneurons in terminal segments of the tadpole spinal cord during metamorphosis. We have measured the content of acetylcholine in different areas of the frog CNS using a sensitive radiometric assay and found levels in the FT to be roughly 3-10 times higher than in other CNS regions. The enzymatic activity of choline acetyltransferase, the biosynthetic enzyme for ACh, is extremely low in the FT suggesting an absence of cholinergic nerve terminals in the frog FT. These observations would strongly suggest a glial storage of ACh in the frog FT. Observations by Miledi and Slater with the denervated neuromuscular junctions of amphibia show that the reappearance of miniature end-plate potentials following denervation may be related with acquired capabilities of Schwann cells to store and also release ACh in a quantal manner. This is further consistent with the demonstrated presence of ACh in the Schwann cells of the squid giant axon (Villegas, et al.). At the present the source of the ACh in the glia of the FT is obscure, but is unlikely to represent glial synthesis. It is interesting to note that the glia in the FT also contain some serotonin (5HT), and probably also contain significant levels of γ-aminobutyric acid (GABA). The observations of the presence of ACh, GABA and 5HT are supportive evidence for the glial storage of neurotransmitters and are consistent with the presence of high affinity uptake processes for neurotransmitters in both transformed and normal glia.

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178.9 DIETARY CHOLINE MODIFIES THE RESPONSIVENESS OF THE HIGH AFFINITY CHOLINE UPTAKE SYSTEM TO PHARMACOLOGICAL MANIPULATION. B.A. Trommer*, K.L. Parrish and L. Wecker. Department of Pharmacology, Louisiana State Univ. Med. Ctr., New Orleans, LA 70112.

Recent results from our laboratory have indicated that the acute administration of choline to rats prevents both atropine-induced depletion of acetylcholine (ACh) and increased sodium-dependent high affinity choline uptake (HACU) in brain (Sci. 199:86,1978). However, with chronic choline supplementation, the ACh depleting action of atropine was potentiated (Neurosci. Abst.4:436,1978). Therefore, the present study was designed to investigate the effects of dietary (chronic) choline availability on the responsiveness of the HACU system to pharmacological manipulation. Rats were randomly divided into 3 groups and maintained for 28-32 days on: a)choline deficient diet, b)basal choline diet (0.2% choline chloride) or c)choline supplemented diet (2.0% choline chloride). Rats were injected with either saline or atropine sulfate (7.0 mg/kg, i.p.) and sacrificed 30 minutes following injection. The kinetic characteristics (Vmax and Km) of the HACU system were determined in striatal and hippocampal synaptosomes (P_2 fraction) utilizing choline concentrations of 0.1-1.43 uM. In synaptosomes from rats maintained on the basal diet, atropine increased the Vmax of uptake in both striatum and hippocampus to 119% and 124% of controls, respectively. In brain tissue from choline supplemented rats, the atropine-induced increase was potentiated with a Vmax of 131% of control in striatum and 151% of control in hippocampus. In synaptosomes isolated from rats maintained on the choline deficient diet, which were characterized by a dietary-induced elevation in Vmax, atropine administration had no further effect. The affinity of the HACU system was unaltered by either dietary regimen or drug treatment. Contrary to studies with acute choline administration, in which choline blocked the atropine-induced increase in HACU, dietary choline supplementation potentiated the atropine-induced increase in uptake as reflected by an increase in Vmax. Hence, dietary choline supplementation renders the HACU system more sensitive to atropine-induced alterations, in agreement with previous studies on the effects of atropine on the steady-state concentration of ACh (Neurosci. Abst.4:436, 1978). Results support the hypothesis that dietary choline availability modifies the responsiveness of central cholinergic neurons to pharmacological manipulation. The specific mechanisms involved in this drug-diet interaction are currently being investigated in our laboratory. (Supported by NIMH # 33443).

178.10 INDUCTION OF D-SLEEP PHENOMENA WITH THE MUSCARINIC CHOLINERGIC AGONIST BETHANECHOL. MP Goldberg, D. Riew, E. Vivaldi, RW McCarley and JA Hobson. Laboratory of Neurophysiology, Harvard Medical School, 74 Fenwood Road, Boston, MA 02115

Bethanechol (B-methyl carbamyl chloride), a derivative of carbachol, is a cholinergic agonist which is not degraded by cholinesterases. Unlike carbachol, which is a mixed agonist, the action of bethanechol is specific to muscarinic receptors. We now present evidence that the striking effects obtained by microinjection of carbachol into the pontine reticular formation (FTG) of cats can be duplicated using bethanechol.

Bethanechol (2-5 ug) was dissolved in saline or distilled water. The injection volume of 0.5-1.0 µl was pumped over 30 sec through broad-tipped micropipettes (50-200 µm tip diameter) placed stereotaxically or directed by chronically implanted guide tubes. 9 trials were conducted in 4 different animals. In each case, the target was the same region of the FTG as had proven effective for injections of carbachol (AP -3.0, L 2.0, V -7.0 HC).

Polygraphic data (EMG, EEG, EOG) were analyzed for time of occurrence and duration of waking (W), synchronized sleep (S), and desynchronized sleep (D). After four of the injections (one in each animal subject), the cat entered a state indistinguishable from D by electrographic or behavioral criteria. The latency was reduced to less than 1/3 of control values (range 12-30 min vs 60-275 mins) and was not preceded by intervening S sleep in 2 of the 4 cats. The first episode was two to four times longer than is normal (range 10-40 minutes vs 4-10 mins) in the five hours following injection, the amount of D was increased 400% over baseline (range 6-58% vs. 1.5-15%). Effects endured for 3-24 hours. In the five other trials the results were negative, probably because of failure to eject drug from the pipette.

Behavioral observation demonstrated that the bethanechol induced D state was posturally indistinguishable from physiological D. Furthermore, the cats could be awakened by auditory or tactile stimuli. The pupils, which were fissurated in D, promptly dilated when the cats were aroused. During periods of spontaneous or induced waking, the cats were alert, responsive to visual and auditory stimuli and capable of a full range of motor behavior.

We conclude that the cholinergically induced D state may be mediated by muscarinic receptors.

This research was supported by NSF Grant BNS 76-18336.

178.11 A COMPARATIVE STUDY OF CHOLINE AND LECITHIN TREATMENT ON DOPAMINE (DA) SYNTHESIS AND TURNOVER IN THE STRIATUM AND HIPPOCAMPUS OF RATS. Ram B. Rastogi, N.P.V. Nair*, and S. Lal*. Laboratories for Research, Douglas Hospital Research Center, and Dept. of Psychiat. McGill Univ., Montreal, Canada, H4H 1R3.

Studies have shown that cholinergic drugs accelerate both DA synthesis and turnover in the striatum (H. Corrodi et al Life Sci. 6:2557, 1967; F. Javoy et al, J. Pharm. Pharmacol. 27:677, 1975). It has also been demonstrated that administration of the acetylcholine (Ach) precursors such as choline and lecithin elevate brain Ach levels (E.L. Cohen and R.J. Wurtman, Life Sci. 16:1095, 1976; M. Hirsch et al, Brain Res. 125:383, 1977). In the present study we examined the effect of these precursors on DA metabolism in six discrete rat brain areas (striatum, hippocampus, pons, midbrain, cerebral cortex, hypotholamus). Our data demonstrated that a single i.p. injection of choline chloride (120 mg/kg) significantly increased tyrosine hydroxylase (TH) activity in the soluble fractions of striatum and hippocampus by 33% and 51%, respectively. A similar magnitude of increase was seen in the striatum of rats after a single injection of lecithin (200 mg/kg, i.p.) but in hippocampus TH activity was increased by 89% as compared to control values. Administration of both precursors also significantly increased tyrosine levels (the substrate for TH) in striatum and hippocampus. Acute choline treatment produced no change in DA levels in several brain areas except cerebral cortex where it was significantly decreased by 18%. The same treatment significantly increased the concentrations of homovanillic acid both in striatum and hippocampus suggesting that the turnover of DA was increased in these regions. After 7 days treatment with choline chloride TH activity as well as levels of tyrosine and homovanillic acid were increased in striatum and hippocampus however, the magnitudes of change were virtually the same as those seen after a single injection of choline chloride. Our data raise the possibility for the existence of presynaptic inputs from cholinergic neurons on dopaminergic neurons in striatal and hippocampal areas of brain. Experiments are underway to examine if such changes in DA synthesis and turnover persist after long-term treatment with choline and lecithin. (Supported by funds from Douglas Hospital Center).

179.1 TRIGEMINAL OROSENSORY FACTORS AND DIETARY SELF-SELECTION. M. G. Miller. Food and Drug Administ., Washington, DC 20204.

Rats given the opportunity to select the components of their diet maintain a stable protein/carbohydrate (P/C) ratio in their daily intake. To investigate the underlying neural control mechanisms, the animals' oral sensory abilities were manipulated by partial trigeminal deafferentation affecting somatosensation of the lower portion of the oral cavity (anterior 2/3 of the tongue, floor of the mouth and lower teeth). Taste, proprioceptive and motor innervation of tongue and muscles of mastication were spared. The animals had access to two isocaloric diets, a protein (soy bean meal, 44% protein) and a carbohydrate (dextrinized starch) fraction, each containing fat, vitamins and minerals in adequate amounts. Before surgery, the animals selected a mixture of both diets, which amounted to 12% protein intake, and maintained the same growth rate as a non-selection control group which received an isocaloric combination of both fractions (22% protein).

After trigeminal deafferentation, self-selection and non-selection groups exhibited the characteristic deafferentation symptoms of prolonged hypophagia, body weight loss, and delayed recovery of preoperative weight. The dietary self-selection pattern was disturbed throughout the postsurgical period (6 weeks), even when total daily intake reached preoperative levels. Four sequential stages could be distinguished: (1) Protein intake essentially zero, carbohydrate intake severely depressed, body weight decreasing; (2) P/C ratio below preoperative mean, body weight stabilizing at low level; (3) P/C ratio at preoperative level with high between-day variability, body weight increasing; (4) P/C ratio above preoperative level with high between-day variability around the mean of randomly distributed intake (22% protein); body weight increasing. Not all animals entered the last stage.

To ascertain that these changes were not simply a response to body weight loss, an additional control group was reduced in body weight to comparable levels by forced food deprivation. Although on the first day of refeeding, compensatory intake was disproportionately increased for the protein fraction, the C/P ratio rapidly returned to normal levels and showed no increased variability. The disruptive effects of partial trigeminal deafferentation on P/C selection suggest that somatosensory inputs from the oral cavity are important factors for dietary adjustment to metabolic requirements.

179.2 DEHYDRATION-INDUCED ANOREXIA: OLFACTORY CONTRIBUTIONS. J. P. Bruno* and W. G. Hall, Research Section, NC Div. Mental Health, Raleigh, NC 27611 and H. J. Grill, Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Dehydration inhibits feeding behavior in weanling and adult rats. To determine the component of feeding inhibited by dehydration we studied its effects when food was infused into the mouth or when it was presented in shallow cups. Dehydration inhibited feeding from the external environment but did not alter the active swallowing of oral infusions (Table 1).

TABLE 1

Condition	Fluid	Cup Fed (% BW)	Infusion Fed (% infused)
Baseline	Water	.64 ± .12	27.9 ± 5.7
(2-4 hr deprived)	Milk	2.60 ± .70	65.1 ± 5.0
NaCl	Water	3.27 ± .30	72.7 ± 8.6
(1M, 2% BW)	Milk	1.04 ± .21	82.4 ± 6.2

Twenty-day-old subjects in baseline conditions consumed more milk than water in both feeding tests. Dehydrated rats consumed much less milk than water when feeding from a cup. In contrast to the cup-fed group, when infusions were made into the mouth water and milk intakes were comparable following dehydration. Dehydration also inhibited feeding from a cup but not feeding during oral infusions in adult rats. The cup-feeding test requires subjects to locate and approach, as well as to lick and swallow the fluids; as such, it recruits several acts not required by the infusion test. Exteroceptive sensory cues (i.e., olfaction) may thus be more important in the cup-fed group than in the infusion-fed group. The fact that cup-fed dehydrated rats took twice as long to initiate milk consumption (12.6 min) as water consumption (5.4 min) suggests that distal olfactory cues might contribute to the inhibition of milk intake. We tested this hypothesis by offering dehydrated rats water that contained an odor cue with little taste (2% almond extract). Table 2 shows that dehydrated, but not control, weanling rats avoid water scented with almond.

TABLE 2

Condition	Fluid	Intake (% BW)
Baseline	Water	.53 ± .16
(2-4 hr deprived)	Water + Almond	.68 ± .20
NaCl	Water	2.86 ± .36
(1M, 2% BW)	Water + Almond	1.36 ± .71

These results demonstrate that the inhibition exerted by dehydration may act, via olfaction, on only the approach component of feeding while the oral consummatory component remains unaffected.

179.3 GLUCAGON INDUCED SUPPRESSION OF FOOD INTAKE IS ASSOCIATED WITH HEPATIC GLYCOGENOLYSIS WITHOUT LIPOLYSIS OR KETOGENESIS. Nori Geary†, W. Langhans* and E. Scharrer* (SPON: W.T. Lhamon). E.W. Bourne Lab., New York Hosp., White Plains, NY 10605, and Inst. Tierphysiol., Univ. München, D8000 München, FRG.

Meal contingent changes in carbohydrate metabolism may contribute to production of satiety. We have previously shown prandial hyperglycemia to be at least partially caused by transient hepatic glycogenolysis occurring concomitant to ad lib meals in the rat. Further, glucagon injections eliciting similar degrees of hepatic glycogenolysis and hyperglycemia significantly reduce meal size. We now report the effects of similar injections on lipid metabolism. Rats were first administered 180 µg glucagon i.p. as they began to eat after a 12 hr fast. Meal size was reduced in comparison to vehicle injected controls (3.4 ± 0.5 vs. 5.2 ± 0.5 g, $\bar{x}$ ± sem). Then, after two weeks, similar injections were made, and the rats sacrificed 15 min later for liver and aortic blood samples. Metabolite levels in rats sacrificed at meal onset (O), 15 min after vehicle injection (V) or after glucagon injection (G) were:

	O	V	G
Blood glucose (mg/dL)	86	100	115ab
Liver glycogen (%)	1.8	1.7a	0.7ab
Plasma hydroxybutyrate (µM/ml)	0.94	0.18a	0.19a
Plasma free fatty acids (µM/ml)	0.91	0.54a	0.53a

a = different from O value, t-test
b = different from V value, t-test

A glucagon dose which suppressed food intake therefore also elicited rapid hepatic glycogenolysis and exaggerated hyperglycemia without affecting lipogenesis or ketogenesis. We conclude glucagon can influence food intake through its effect on carbohydrate metabolism. Such effects may be signalled to the brain via hepatic vagal afferents.

179.4 GLUCOSE INFUSION BLOCKS ALLOXAN-INDUCED IMPAIRMENT OF GLUCOPRIVIC FEEDING. Sue Ritter and Joan Murnane*. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Alloxan is a toxic drug which can be given systemically to destroy insulin-secreting pancreatic beta cells. Alloxan also reduces the capacity of taste buds to respond to glucose. Several investigators have demonstrated that the effect of alloxan on both pancreatic beta cells and on taste buds can be competitively inhibited by D-glucose, suggesting that gluco-receptors in diverse tissues may have similar properties.

Recently, Woods and McKay reported that centrally-administered alloxan abolishes or greatly attenuates the feeding response to 2-deoxy-D-glucose (2DG) (Science, 202:1209, 1978). These authors suggested that alloxan damaged brain glucoreceptors responsible for 2DG-elicited feeding. In the present experiment we replicated the results of Woods and McKay. In addition, we have demonstrated that alloxan-induced impairment of glucoprivic feeding can be prevented if glucose and alloxan are administered simultaneously.

Adult male Sprague-Dawley rats were given either alloxan (40 µg in 5 µl saline), alloxan and glucose (40 µg alloxan in 5 µl of 3 M glucose) or saline (5 µl) through lateral ventricular cannulae. Body weight and spontaneous food intake did not appear to be altered by any treatment. Feeding and blood glucose responses were measured for 6 hr following subcutaneous injection of 2DG (150 mg/kg), insulin (2 U/kg) and saline. After 2DG injections, controls ate 4.3 ± 0.6 g of pelleted lab chow in the 6 hr test, whereas alloxan-treated rats ate only 1.6 ± 0.5 g ($p < 0.01$). Likewise, after insulin injections, controls ate 5.6 ± 0.3 g of food, while alloxan-treated rats ate only 3.6 ± 0.2 g ($p < 0.001$). In rats that received glucose and alloxan simultaneously, feeding responses were indistinguishable from control in both tests (3.6 ± 0.6 g after 2DG and 5.4 ± 0.6 g after insulin). Blood glucose concentrations did not differ between groups either before or after administration of glucoprivic agents.

Our findings (1) confirm the report that alloxan impairs 2DG-elicited feeding, (2) suggest that the deleterious effects of alloxan may be produced in brain by a mechanism similar to that exerted by alloxan on the pancreatic β-cell and taste bud, and (3) supports the contention that alloxan-induced impairment of glucoprivic feeding is due to a glucose-specific effect on a cellular mechanism.

179.5 INSULIN-INDUCED FEEDING AND INCREASED NOREPINEPHRINE TURNOVER ARE PREVENTED BY INTRAVENOUS GLUCOSE INFUSIONS. Steven I. Bellin and Sue Ritter. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Several investigators have demonstrated enhanced hypothalamic norepinephrine (NE) utilization after administration of glucoprivic agents such as insulin or 2-deoxy-D-glucose. We have shown that the activity of hypothalamic NE neurons appears to be uniquely related to the insulin-induced feeding response rather than to the stressful aspects of the glucoprivic challenge per se (Bellin, S.I. and Ritter, S., Neurosci. Abstr., #688, 1979). The goal of the present experiment was to determine the mechanism by which insulin increases the activity of NE neurons. If these neurons are activated by glucoprivation, then glucose infusions which prevent glucoprivation during hyperinsulinemia should also prevent the insulin-associated increase in NE turnover. This result would be consistent with the view that increased hypothalamic NE activity is causally related to food intake, since other investigations have demonstrated that this treatment also abolishes post-insulin hyperphagia. On the other hand, if hypothalamic NE neurons are activated by insulin itself, then turnover should be enhanced after insulin, even when glucoprivation is prevented.

Forty-eight adult male Sprague-Dawley rats (400-500 gm) were divided into four treatment groups. Continuous intra-atrial infusions of 0.15 M saline or 1.2 M glucose (8 ml/hr) were begun 15 min prior to injection of either saline or insulin (2.5 U/kg s.c.). At 30 min post-injection, infusion rates were reduced to 2.0 ml/hr for the duration of the study. One and one-half hr later, α-methyl-p-tyrosine (AMT, 350 mg/kg, s.c.) was administered to each subject, followed by decapitation sacrifice of 6 animals per group 1/2 and 1 hr post-AMT. Catecholamine concentrations in 4 brain regions were determined by fluorometric analysis. In insulin-treated subjects, glucose infusions abolished the enhanced hypothalamic NE turnover observed in the saline-infused group. NE concentrations 1 hr after AMT were 1.96 ± 0.36 ug/g for glucose-infused rats and 1.37 ± 0.24 ug/g for saline-infused rats ($p < 0.01$). Simultaneous glucose infusions also prevented the hypoglycemia and hyperphagia normally observed after insulin administration. These results suggest that glucoprivation, and not insulin per se, is responsible for enhancing hypothalamic NE neuronal activity. Thus, deficits in either glucose or other energy substrates utilizable by brain may mediate the behavioral and neurochemical consequences of large insulin doses. We are currently testing this hypothesis using infusions of fructose and β-hydroxybutyrate solutions in insulin-treated rats.

179.6 POSTABSORPTIVE GLUCORESTORATION IS ASSOCIATED WITH TERMINATION OF POSTGLUCOPRIVIC FEEDING. Nancy L. Pelzer*, Steven I. Bellin and Sue Ritter (SPON: F.A. Young). Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Recently, Ritter et al. demonstrated that insulin and 2-deoxy-D-glucose (2DG) exert delayed effects on food intake that outlast the glucoprivic episode itself (Ritter, R.C. et al., Am. J. Physiol., 234:E617-E621, 1978). They showed that glucoprivic challenges elicited a significant increase in food intake 6-8 hr after insulin or 2DG, even though blood glucose values indicated that glucoprivation had abated; i.e., postglucoprivically. The amount of food consumed in a 2 hr postglucoprivic feeding test was similar to the amount consumed when food was continuously available during the entire glucoprivic episode. In pursuing these findings, we observed that limited access to food at the nadir of hypoglycemia (1 1/2 - 2 hr after 2.5 U/kg of regular insulin) blocked postglucoprivic feeding. Subsequently, we systematically varied the amount of food that rats were allowed to ingest during that 30 min time period. We found that consumption of as little as 2.5 g of pelleted lab chow was effective in blocking postglucoprivic feeding, even though this amount of food was only 30% of the amount consumed when food was continuously available during glucoprivation. Rats with 30 min access to 2.5 g of food (n = 11) ate 0.3 ± 0.4 g in a 2 hr postglucoprivic feeding test, whereas rats which were not pre-fed (n = 12) ate 6.7 ± 2.6 g of food during the postglucoprivic test. Furthermore, ingestion of glucose (13% solution), but not saccharin (0.1% solution), 1 1/2 - 2 hr after insulin also abolished postglucoprivic feeding. Quantities of food or glucose solution associated with blockade of postglucoprivic feeding were also associated with a rapid and sustained restoration of normoglycemia.

Our results do not explain why rats continue to feed when glucorestoration is allowed to occur spontaneously in the absence of food. Nor can we account for the apparent failure of parenteral glucose administration to abolish postglucoprivic feeding. It is possible that the glucorestoration we observe after ingestion of food is an epiphenomenon and that termination of glucoprivic feeding depends primarily on orogastric stimulation. However, the fact that saccharin consumption did not abolish postglucoprivic feeding suggests that the abolition of this behavior may require the cooperation of both pre- and postabsorptive signals.

179.7 SIMILAR FEEDING PROFILES IN TUMOR-BEARING AND DEXAMETHAXONE-TREATED RATS SUGGEST ENDORPHIN DEPLETION IN CANCER CACHEXIA. M. T. Lowy* and G. K. W. Yim (SPON: S. Sangiah). Dept. of Pharmacology and Toxicology, Purdue University, W. Lafayette, IN 47906.

The feeding patterns of male Sprague-Dawley rats bearing hindlimb Walker-256 carcinosarcomas (W-256/SD) were examined. Three hr daytime rat chow consumption induced by 2-DG (2-deoxy-D-glucose, 400mg/kg) was attenuated in tumor bearing rats (TBR) as compared to control (CON) rats (2.4 ± 0.5 gm vs 7.1 ± 0.8 gm, $p < .001$). Hyperphagia induced by 24 hr food-deprivation (FD) was also decreased in TBR as compared to CON (3.3 ± 0.6 vs 9.3 ± 0.7 gm, $p < .001$). In addition, 12 hr night time food intake was selectively depressed by 25-30% ($p < .01$) in TBR. Since these hyperphagias are naloxone-sensitive, whereas insulin-induced(10U/kg) hyperphagia is intact in naloxone-treated (Lowy et al. Life Sci. 26: 2113, 1980), and in anorexic W-256/SD rats (Morrison, Cancer Res. 33: 526, 1973), it appears that feeding deficits of the W-256/SD rats involve a defective endorphin system. The administration of the glucocorticoid, dexamethasone(400 and 200 μg/kg;24 and 2 hr pretreatment), which inhibits pituitary β-endorphin synthesis (Rossier et al., Proc. Nat. Acad Sci. 77: 666, 1980), produced an identical feeding profile in normal rats (i.e. reduction of 2-DG, FD and night time, but not insulin hyperphagia).

These results suggest that the elevated corticosteroid levels of the W-256/SD rats (Morrison, Phys. Beh. 17: 705, 1976) may have inhibited endorphin synthesis sufficiently to result in endorphin depletion and anorexia in the W-256/SD rats. (Supported in part by Pharmacology-Toxicology Training Grant GM-709504 (MTL) and American Cancer Society Grant IN 172).

179.8 TUMOR ANOREXIA: A ROLE FOR LEARNED FOOD AVERSIONS AND NUTRIENT DEFICIENCES. I. Bernstein* and C. Treneer* (Spon. G. Clark) Dept. Psychol. Univ. of Washington, Seattle, WA 98195

A decline in food intake and body weight is observed in cancer patients and animals with experimental tumors. Although a number of possible central and peripheral mechanisms for this anorexia have been proposed, agreement as to the causes of tumor-produced loss of appetite and weight has not been reached. One possible factor contributing to tumor anorexia is the development of learned food aversions. Tumor-bearing animals may associate some physiological consequence of tumor growth with the available food, develop a learned aversion to that food and reduce their intake of it.

The present studies evaluated this hypothesis by examining the food intake and diet preferences of animals with transplantable tumors. Wistar-Furth (W/Fu) rats with transplantable polyoma-virus induced sarcomas were given continuous access to nutritionally adequate diets. The decline in food intake which accompanied tumor growth was associated with the development of aversions to the specific diet consumed during tumor growth. An immediate and marked elevation in food consumption occurred when a novel diet was introduced. These findings suggested that the development of learned aversions to the specific diet eaten during tumor growth may be a causal factor in the development of tumor anorexia. In this regard tumor-bearing animals are similar to animals suffering from certain nutrient deficiencies.

Since amino acid deficiencies and imbalances are known to produce dramatic depressions in food intake we evaluated whether tumor-induced nutrient deficiencies, specifically deficiencies in specific amino acids, play a role in the development of anorexia and learned food aversions. Plasma samples were obtained from anorexic, tumor-bearing animals and pair-fed controls and amino acid profiles were obtained from these samples. Significant imbalances in the plasma profiles of the tumor-bearing animals were observed, including a marked depression in plasma tryptophan. Subsequent studies have been aimed at determining whether alterations in dietary amino acid composition could correct these imbalances and reduce the anorexia and food aversions seen in tumor-bearing animals.

This work was supported by USPHS Grant CA25419.

179.9 DIURNAL VARIATION IN THE POTENCY OF CHOLECYSTOKININ FOR INHIBITION OF EATING IN RATS IS NOT DEPENDENT UPON CHOLECYSTOKININ'S EFFECT ON GASTRIC EMPTYING. F.S. Kraly. Dept. of Psychology, Colgate University, Hamilton, NY 13346.

Cholecystokinin (octapeptide, CCK-8) is less potent ($p < .002$) for inhibition of eating in the night than in the day: Threshold and ID_{50} at night are roughly twice threshold and ID_{50}, respectively, in the day for rats eating 1 min after i.p. CCK-8 at the midpoint of the night or day phases after 24-hr food deprivation (Kraly, 1980). To assess whether this phenomenon is dependent upon the ability of CCK-8 to alter gastric emptying, gastric emptying of food into the intestine was prevented by sham feeding in the following experiments:

Rats (n=49) with gastric fistula sham fed GIBCO 116EC liquid food at the midpoints of the night or day (12:12 cycle) after 3-hr food deprivation. CCK-8 (i.p., 5-180 U/kg; 1 U $\hat{=}$ .05 mcg) affected sham feeding in a dose-related manner in the night and day, but CCK-8 inhibited sham feeding less at night than during the day ($p < .005$): Threshold for inhibition in the day was 5 U/kg, but was 30 U/kg at night. While 10 U/kg caused 35.4% inhibition in the day, it enhanced sham feeding at night by 20.8% ($p \leq .05$). ID_{50} in the day ($r^2 = .55$) was 15.1 U/kg; ID_{50} at night ($r^2 = .92$) was 64.0 U/kg. The slopes of the regression lines were not different (night vs. day: $p > .10$).

The diurnal variation in the potency of 20% pure cholecystokinin (CCK; Karolinska Institutet) was of a different character: CCK (i.p., 5-180 U/kg) inhibited sham feeding in a dose-related manner in the night and day. While impure CCK failed to systematically inhibit sham feeding less at night than during the day ($p > .20$), ID_{50} in the day ($r^2 = .79$) was 14.4 U/kg, ID_{50} at night ($r^2 = .86$) was 24.7 U/kg, and the slopes of the regression lines were different ($p < .02$). Finally, CCK and CCK-8 were equally potent for inhibiting sham feeding in the day ($p > .20$), but CCK was more potent ($p < .05$) than CCK-8 for inhibiting sham feeding at night.

These results show (1) that the diurnal variation in the potency of cholecystokinin for inhibition of eating is not dependent upon cholecystokinin's effect on gastric emptying and (2) the potency of impure CCK is not equivalent to the potency of CCK-8 for inhibition of sham feeding at night.

179.10 CHOLECYSTOKININ OCTAPEPTIDE ACTS AT AN ABDOMINAL, NOT HYPOTHALAMIC, SITE TO PRODUCE SATIETY IN RATS. K.J. Simansky, C. Jerome* and G.P. Smith. Dept. of Psychiatry, Cornell Univ. Med. College and Bourne Laboratory, New York Hospital, White Plains, NY 10605.

The peripheral administration of the octapeptide of cholecystokinin (CCK-8) produces satiety in rats and other mammals. However, the locus at which this hormone activates satiety mechanisms has been controversial. Bilateral abdominal vagotomy has been reported to either abolish (Lorenz & Goldman, 1978), greatly attenuate (Smith & Cushin, 1978), or have no effect (Anika et al., 1977) on CCK-8 satiety. Similarly, Stern et al. (1976) found that bilateral lesions of the ventromedial hypothalamus (VMH) blocked the satiety effect of a single dose of caerulein--a decapeptide related structurally and in its visceral activity to CCK-8--while Kulkosky et al. (1976) failed to block CCK-8 satiety with VMH lesions.

We investigated this problem further by testing the satiety action of CCK-8 in rats with total abdominal vagotomy (VGX; n=8) or large bilateral hypothalamic lesions placed to include either the VMH (n=8) or another putative central site for the mediation of satiety--the paraventricular nuclei (PVN; n=9) (Eng et al., 1979). VGX rats were also tested with 1.25-160 U/kg i.p. of caerulein. Thirty min intakes of liquid diet were measured in 17-hr food-deprived rats beginning 15 min after injection of peptide or saline. VGX blocked the satiety effect of 80 U/kg of CCK-8. This dose of CCK-8 reduced food intake by 54 ± 4% ($p < .01$) in surgical controls (n=8) while VGX rats ate 15 ± 13% ($p > .10$) more. VGX also greatly diminished the satiety effect of caerulein. Controls demonstrated a significant 34% suppression of food intake after 2.5 U/kg, and 90% decrease after 80 U/kg while only the 160 U/kg dose reduced intake in VGX rats (-26 ± 9%; $p < .05$).

In marked contrast to the effect of vagotomy in blocking the satiety induced by CCK-8 or caerulein, neither VMH nor PVN lesions significantly altered this behavioral action of 10-160 U/kg i.p. of CCK-8. For example, the 80 U/kg dose suppressed intake by 50 ± 15% in controls (n=3) and by 43 ± 5% in VMH-lesioned rats ($p < .01$). Similarly, PVN rats demonstrated a dose-related decrease in food intake after 20, 40, 80, and 160 U/kg, including a 50 ± 8% decrease after 80 U/kg. Thus, destruction of the putative central satiety sites in the area of the VMH and PVN did not disrupt CCK-8-induced satiety.

These results, therefore, strongly suggest that peripherally administered CCK-8 and caerulein act at an abdominal site innervated by the subdiaphragmatic vagus to produce satiety in rats.

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179.11 RATS PREFER REAL FEEDING TO SHAM FEEDING. W. Van Vort* and G.P. Smith. Dept. Psychiatry, Cornell Univ. Med. College and E.W. Bourne Behavioral Research Lab., The New York Hospital, White Plains, NY 10605.

To investigate the relative preference of rats for real feeding and sham feeding, male Sprague-Dawley rats weighing about 350 g were implanted with chronic gastric cannulas (GF). After recovering from surgery, rats were maintained on pellets and water and adapted to a 17 h food deprivation schedule. At the end of the deprivation period, rats were offered sweet milk. On alternate days the rats really ate the milk (GF closed) or sham fed the milk (GF open). For each condition - open or closed - the diet was marked with a specific flavor (almond or vanilla) and offered at a specific spout location at the front of the cage (left or right) for each rat. For example, rat Z would always be offered vanilla on the left on a real feeding day and almond on the right on a sham feeding day. The assignment of flavor and location was randomized. Two groups of 9 rats each were tested separately.

On real feeding days (GF closed), rats (n = 18) ate a large meal (16.5 ± 2 ml) within 10 to 15 min and then displayed the behavioral sequence characteristic of satiety. The milk was removed after the satiety sequence occurred. On sham days (GF open), rats (n = 18) ate almost continuously (102 ± 8 ml) for 60 min and did not display the satiety sequence. Thus, rats ingested approximately six times as much milk during sham feeding as during real feeding. Pellets were given 30 min after the test period and were removed at the beginning of the deprivation period.

Preference testing was conducted under the same deprivation schedule with GF closed. Rats were given simultaneous access to both test diets in their usual location for a 4 min period. Sixteen of 18 rats preferred the diet associated with real feeding. In the first group, 8 of 9 rats ate more of the diet associated with real feeding after 10 sham and 10 real feeding trials. Mean intakes were 7.1 ± 0.5 ml (closed) and 2.4 ± 0.8 ml (open), $p < .005$, paired t test. The second group showed a significant preference after 12 sham and 12 real feeding trials. Again, 8 of 9 rats ate more of the diet associated with real feeding. Mean intakes were 5.6 ± 0.8 ml (closed) and 1.7 ± 0.9 ml (open), $p < .05$.

Thus, under these conditions, rats prefer a real meal that ends in postprandial satiety to a period of sham feeding that is 4 times as long and during which 6 times as much milk is ingested.

Supported by MH 15455 and Career Development Award MH00149.

179.12 VAGOTOMIZED RATS ARE INSENSITIVE TO GASTRIC DISTENTION CUES OF SATIETY. M. F. Gonzalez* and J. A. Deutsch* (SPON: R. M. Boynton). Department of Psychology, University of California, San Diego, CA 92093.

Prior research has established that receptors present in the stomach of the rat are responsible for the production of short-term satiety (Deutsch, J. A., W. G. Young, and T. J. Kalogeris, Science, 201:165, 1978). It has also been demonstrated that there are two classes of receptors involved, one sensitive to gastric volume and the other to the level of nutrient present in the stomach (Deutsch, J. A., M. F. Gonzalez, and W. G. Young, Brain Res. Bull., Suppl. 4, 1980). The present work examines how this information travels from the stomach to the CNS.

Male, Sprague-Dawley rats were kept on a restricted diet of condensed milk and were either vagotomized at the subdiaphragmatic level or subjected to a control laparotomy. Upon recovery from this surgery they were implanted with gastric cannulas and inflatable pyloric cuffs. When the animals were tested after a 15 h period of deprivation with the stomach isolated from the duodenum by means of the cuffs both groups were sensitive to nutritive cues but only the control group was responsive to volume cues of satiety.

When 5 ml of milk were siphoned off the stomach control subjects drank 7.8 ml over baseline level ($t = 5.36$, d.f. = 2, $P < 0.05$), while vagotomized subjects overdrank by 6.5 ml under the same conditions ($t = 3.18$, d.f. = 4, $P < 0.05$). On the other hand, when the animals were counterinjected with normal saline with one half of the volume voluntarily ingested by mouth, control rats exhibited a 29% decrease in their intake ($t = 4.95$, d.f. = 2, $P < 0.05$) while vagotomized rats showed a 10% increase in intake ($t = 0.81$, d.f. = 5, $P > 0.05$). This phenomenon was also observed when saline was counterinjected at a higher one to one ratio.

These results show that nutrient satiety signals that arise from the stomach are not vagally conveyed, but volume satiety signals are.

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180.1 IMMUNOHISTOCHEMICAL IDENTIFICATION OF PARAVENTRICULAR HYPOTHALAMIC NEURONS WHICH PROJECT TO THE MEDULLA OR SPINAL CORD IN THE RAT. P.E. Sawchenko* and L.W. Swanson, Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110

We have developed a method for the simultaneous localization of a retrogradely-transported fluorescent dye (true blue) and antigen to obtain estimates of the relative number, location, and biochemical specificity of cells in the paraventricular nucleus of the hypothalamus (PVH) which project to the medulla and/or spinal cord. Nine rats received a 50 nl injection of 5% true blue in the dorsomedial medulla at the level of the obex, and 10 received two 0.5 µl injections of true blue in upper thoracic segments of the spinal cord. After 7-14 days, the rats were perfused with neutral buffered formalin; multiple series of 20 µm frozen sections were saved and were either left untreated for examination of retrogradely-labeled cells, stained with thionin, or prepared for localization of oxytocin (OXY), vasopressin (VAS), somatostatin (SS) or tyrosine hydroxylase (TH) using an indirect immunofluorescence technique. Preliminary estimates of the number of PVH cells stained with each antiserum, and the number of cells doubly-stained with both true blue and each antiserum are given below. Because some PVH cells project to both the medulla and spinal cord,[1] the percentages given reflect minimum and maximum percentages of antigen-containing somata which may project to one or both loci.

Antigen	Total No. Cells in PVH	No. Double-Labeled after: Spinal Inj.	Medullary Inj.	%
OXY	1858 ± 149	169 ± 18	73 ± 37	9-13
VAS	1530 ± 353	81 ± 36	20 ± 10	5-7
SS	778 ± 135	10 ± 4	10 ± 0	1-3
TH	533 ± 61	19 ± 4	21 ± 1	4-8

OXY- and VAS-containing cells which project to the spinal cord were concentrated in the dorsal, medial and lateral parvocellular regions of the PVH, in the caudal half of the nucleus; OXY- and VAS-containing cells which project to the medulla were most numerous in the medial and lateral parvocellular zones. Small numbers of double labeled SS- and TH-containing cells were detected in various parvocellular regions following either medullary or spinal injections.

PVH neurons with long descending projections are not biochemically homogeneous, with OXY-, VAS-, TH-, and perhaps SS-staining cells each contributing to both projections. Because the total number of doubly-stained neurons identified in these experiments constitute only small percentages of the total number of PVH cells labeled with true blue following spinal or medullary injections, it is likely that neurons containing other putative transmitter substances are involved in these projections. ([1]Swanson, LW & HGJM Kuypers, JCN (in press)

180.2 ACTIVATION OF THE HYPOTHALAMO-NEUROHYPOPHYSEAL SYSTEM: REGIONAL ANALYSIS UTILIZING THE TRITIATED 2-DEOXYGLUCOSE METHOD. D. Wells, J. Buggy, A. J. Beitz, and J. Coleman. Depts. of Physiology, Anatomy, and Psychology. Univ. of South Carolina, Columbia, SC 29208.

2-deoxyglucose is a glucose analogue which competes with glucose for uptake into cells but after phosphorylation cannot be further metabolized and thus remains trapped within cells. By combining radiolabeled 2-deoxyglucose accumulation in neurons during physiological stimulation with autoradiography of brain sections, Sokoloff and colleagues pioneered the technique of functional, in vivo analysis of regional cerebral metabolic activity. One limiting factor in resolution of regional cerebral metabolic activity on X-ray film is the energy of the isotope. Another major concern with the original technique is the high cost of the isotope required to produce adequate labeling of brain tissue for autoradiographic analysis. To improve on the original technique which used ^{14}C labeled 2-DG, we have tried several modifications of this method to study functional changes in the hypothalamo-neurohypophyseal axis. A new X-ray film (LKB ultrofilm) specifically designed for tritium autoradiography was recently introduced. The high sensitivity of this film made it possible to utilize tritium label with the 2-DG tracing technique. The use of ^{3}H-2DG reduced the cost of each experiment by more than half. Furthermore, the lower energy level of tritium versus ^{14}C label improved autoradiograph resolution since the distance beta particles could travel to affect silver grains was decreased. Finally, in situ fixation of the brain tissue by intracardiac perfusion has improved ease of sectioning and tissue quality while further improving resolution of the autoradiographs. When necessary, rats were outfitted the day before an experiment with chronic venous and arterial catheters and probes for electrical or chemical brain stimulation. ^{3}H-2DG was injected iv (167 uci/100 g BW) prior to a 45 mintue exposure period to experimental stimulation. The brain was frozen at -45°C and 15µ sections were collected from the cryostat on prewarmed coverslips. Sections and X-ray film were sandwiched between aluminum plates and exposed for 3 weeks. Four stimulus conditions increased activity in posterior pituitary and preoptic periventricular tissue (AV3V): water deprivation in Brattleboro rats with genetic diabetes insipidus, subcutaneous or intracerebroventricular injections of hypertonic NaCl, and electrical stimulation of preoptic periventricular tissue.

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180.3 DIRECT EFFECTS OF GLUCOSE ON SOMATOSTATIN RELEASE IN VITRO FROM NEURONS IN THE MEDIAL BASAL HYPOTHALAMUS. A. Negro-Vilar and S.R. Ojeda*. Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Neural regulation of energy homeostasis involves the hypothalamus to integrate signals related to metabolic needs. Insulin-sensitive glucoreceptor cells are located in the satiety center, in and around the ventromedial nucleus (VMH). In the rat, one of the responses to hypoglycemia and/or starvation is a lowering of plasma GH levels (Endocrinology 84: 814, 1969). Recent evidence indicates that this GH response may result from enhanced somatostatin (SRIF) release. The present experiments were designed to test whether glucose and/or insulin could directly modify release of SRIF from medial basal hypothalamic (MBH) fragments incubated in vitro. Tissues obtained from adult male rats were incubated in Krebs-Ringer bicarbonate glucose (5.5 mM) buffer, pH 7.4, under an atmosphere of 95% O_2, 5% CO_2. After a 15 min pre-incubation period, medium was changed and replaced by fresh medium for an additional 30 min incubation, during which release of peptides was evaluated. SRIF, LHRH and arginine vasopressin (AVP) were measured by specific RIA's. The results show that lack of glucose in the medium increased SRIF release (3-4 fold) from the MBH significantly. Only minor, non-significant increments in LHRH and AVP release were observed after glucose removal. Addition of glucose to the medium during the incubation at three doses (2, 5.5 and 11 mM) resulted in a step-wise reduction in SRIF release, with the largest dose of glucose reducing release of the peptide to control levels. Incubation of MBH fragments with a non-metabolizable glucose analog, 2-deoxy-glucose (2-DG) increased somatostatin release to values similar to those obtained in the absence of glucose. Moreover, pre-incubation of MBH fragments with 2-DG abolished the inhibitory effect of glucose on somatostatin release. When ME fragments (which contain only nerve terminals and no cell bodies) were incubated with or without glucose, no significant increments were observed in SRIF release, suggesting that the effects of glucose require the presence of neuronal cell bodies, perhaps located in the VMH. Incubation of MBH fragments with insulin (10^{-7} or 10^{-6}M) with or without glucose produced no consistent effects on SRIF release. However, in MBH fragments preincubated with 2-DG, addition of insulin to the KRB-glucose buffer during incubation induced a clear increase in somatostatin release. These results indicate that glucose can directly modulate somatostatin release from medial basal hypothalamic neurons, thereby influencing GH release and perhaps other central actions of somatostatin, and that the effects of insulin may be complex, probably involving glucose-dependent and glucose-independent mechanisms. Supported by NIH HD-09988 and AM-10073.

180.4 TONIC RELEASE OF IMMUNOREACTIVE SOMATOSTATIN FROM RAT HYPOTHALAMIC CELL CULTURES: MODULATION BY γ-AMINOBUTYRIC ACID. D.E. Vaccaro*, R. Gamse*, G. Gamse*, M. DiPace*, S.E. Leeman and T.O. Fox (SPON: C.C. Vito). Dept. of Physiology & Neuropathology & LHRRB, Harvard Med. Sch., and Dept. of Neuroscience, Children's Hosp. Med. Ctr., Boston, MA 02115.

The regulation of the secretion of immunoreactive somatostatin (IR-somatostatin) was studied using cell cultures of embryonic rat hypothalamus (Vaccaro et al., J. Neurobiology, 11, in press, 1980). The cultures were used approximately 2 weeks after plating and contained 1890±160 fmol (n=28) of IR-somatostatin. The IR-somatostatin was characterized by gel permeation and/or reverse-phase chromatography. The major portion of IR-somatostatin co-chromatographed with synthetic somatostatin in each case.

Hypothalamic cell cultures continuously release a percentage of their total content of IR-somatostatin in a "tonic" fashion (1.73±0.13%/3min; n=36). Release of IR-somatostatin was stimulated by exposing the cells to 60mM K^+ (5.0-fold) or veratridine (6.9-fold). Reducing the calcium concentration of the external medium (0.1mM) and adding cobalt (4.5mM) abolished K^+-stimulated release and, in other experiments, reduced the level of tonic release by 76%. When tetrodotoxin (TTX), which blocks the voltage-dependent Na^+ channels, was added to the medium tonic release was lowered 76%.

γ-Aminobutyric acid (50mM GABA) completely inhibited 30mM K^+-stimulated (release) and inhibited tonic release 72%. Bicuculline (50mM), a GABA antagonist, inhibited the action of exogenously administered GABA. When added alone to the cultures, bicuculline promoted a greater than 2-fold increase in IR-somatostatin release.

Previous studies have demonstrated that hypothalamic cell cultures exhibit GABA synthesis and GABA uptake. To study the release of GABA, cultures were preloaded with (^{3}H)GABA. 60mM K^+ stimulated a greater than 2-fold calcium-dependent release of radioactivity. Continuous release of radioactivity was measured. This could be inhibited greater than 30% by either 0.1mM calcium with 4.5mM cobalt or by TTX.

These results are consistent with the suggestion that endogenous GABA may function as a neurotransmitter to regulate the release of somatostatin from hypothalamic cells.

Studies supported by grants HD 10818 (T.O.F.) and AM 16510 (S.E.L.), NRSA (NINCDS to D.E.V.) and performed at the Mental Retardation Res. Ctr. (CHMC).

180.5 AN AUTORADIOGRAPHIC ANALYSIS OF THE EFFERENT CONNECTIONS OF THE SUPRACHIASMATIC NUCLEUS. Mitchell L. Berk and Judith A. Finkelstein. Dept. Anat., N.E. Ohio Univs. Col. Med., Rootstown, Ohio 44272.

The suprachiasmatic nucleus (SCN) of the hypothalamus has been implicated as a generator of circadian rhythms of many biological functions. In the present study, the efferent pathways by which the SCN could produce its effects were investigated with the use of the autoradiographic technique. Iontophoretic injections of ^{3}H-leucine were placed into the SCN of rats, which were allowed to survive for 2-8 days. Emulsion coated slides were exposed for 3-4 months prior to development.

The injections were small and centered in the SCN, but some cells of the adjacent anterior hypothalamic area and anterior periventricular nucleus were also labeled. Many labeled fibers are observed in the periventricular zone at the level of the medial preoptic area, anterior hypothalamic area, and the dorsomedial (DMH) and ventromedial (VMH) hypothalamic nuclei. At the level of the medial preoptic area, these periventricular fibers ascend into the thalamus and densely innervate the periventricular thalamic nucleus. From the periventricular region of the anterior hypothalamus, heavily labeled fibers course dorsolaterally to the ventrolateral border of the paraventricular nucleus. These fibers continue caudally into the periventricular zone adjacent to DMH and appear to send terminals to the medial part of DMH. Some fibers are also present along the periventricular border of the paraventricular nucleus.

The ventromedial part of the anterior hypothalamic area contains many labeled fibers, some of which project laterally, dorsal to the optic chiasm. Posteriorly, labeled fibers are concentrated at the ventrolateral margin of VMH, slightly fewer fibers between VMH and the arcuate nucleus, as well as some fibers dorsal to VMH, thereby encapsulating VMH with SCN fibers. Some fibers are observed to enter the external layer of the median eminence. Further caudally, the fibers at the lateral border of VMH sweep dorsomedially into DMH, join the periventricular fibers and continue dorsally and caudally into the posterior hypothalamic nucleus. These labeled fibers continue dorsally and caudally into the periventricular gray of the midbrain. The fibers ventral to VMH proceed posteriorly and can be traced to the fiber capsule underlying the mammillary complex.

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180.6 EFFECTS OF LESIONS OF THE MEDIAL PREOPTIC NUCLEUS (MPN) ON COPULATION-INDUCED OVULATION. L.V. Rubens* and E. Terasawa (spon. J. Kemnitz). Wisc. Reg. Primate Research Ctr., Univ. of Wisc., Madison, WI 53706

Previously, we have shown that small lesions of the MPN, a periventricular structure immediately caudal to the organum vasculosum lamina terminalis, induce persistent estrus in the rat, are invariably associated with a complete abolition of progesterone-induced LH release in ovariectomized animals, and result in a drastic reduction of hypothalamic content of LHRH. Lesions of the suprachiasmatic nucleus (SCN), on the other hand, do not block the progesterone-induced LH surge, although such lesions sometimes result in persistent estrus. In the present experiment, in order to obtain further evidence of involvement of the MPN in LHRH release, effects of MPN and SCN lesions on copulation-induced ovulation were investigated. Rats were housed under standard laboratory conditions (lights on 0500-1900). Daily vaginal smears were taken, and animals showing regular cycles were used. Bilateral electrolytic lesions were made stereotaxically with tungsten electrodes under pentobarbital anesthesia. In sham animals, electrodes were lowered into the MPN but no current was passed. 5-8 weeks after surgery, animals exhibiting persistent vaginal cornification were placed with sexually active males overnight. Sham lesioned or lesioned animals showing vaginal cyclicity were placed with males on the night of proestrus. Mating was verified by a copulation plug on the cage floor and sperm in the vaginal smear. Animals which did not mate were placed with males for a 2nd night about 1 week later. The morning following mating, laparotomies were performed. If the ampullae were dilated, they were dissected, and the number of ova were counted under a microscope. Lesioned animals were killed and brains prepared for histological determinations. MPN lesions, of approximately 700 μm in diameter, involved midline structures over the anterior half of the optic chiasm, including the entire MPN, while the SCN were spared. SCN lesions, of approximately 900 μm in diameter, involved 80-100% of this nucleus, while sparing the MPN. 7 of 9 persistently estrous rats with MPN lesions mated on the 1st or 2nd trial. None of these animals ovulated. On the other hand, 6 of 11 persistently estrous rats with SCN lesions mated on the 1st or 2nd trial, and 4 ovulated. All 9 cyclic rats with lesions or sham lesions mated, and 8 of these ovulated. In SCN-lesioned rats, the number of ova per ovulating animal was 6.8 ± 2.1, whereas in cyclic rats the number was 11.1 ± 1.1. It is concluded that copulation-induced ovulation is mediated by neurons in the MPN or vicinity. These results provide further support for our previous hypothesis that neurons indispensible for release of LHRH reside in the vicinity of the MPN, and not in the SCN. (Supported by NIH Grants RR-00167 and HD-11355.)

180.7 PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS: ANATOMICAL EVIDENCE OF TEN FUNCTIONALLY DISCRETE SUBDIVISIONS. Edward Tongju Koh* and Juarez A. Ricardo* (SPON: A. Galaburda). Dept. of Psychology, Massachusetts Institute of Technology, Cambridge, Mass. 02139 and Dept. of Anatomy, Harvard Medical School, Boston, Mass. 02115.

The paraventricular nucleus of the rat hypothalamus (pvn) has classically been divided into two parts: magnocellular (mc) and parvocellular (pc). Evidence from many groups, including our own (Brain Res. 153:1, SNS 5:#1524), considered together, indicates that the pvn may be further divided into at least ten discrete neuronal groups, each having a distinct combination of inputs, outputs, and chemistry. Evidence for such a parcellation comes from autoradiographic (ARG), horseradish peroxidase (HRP), and immunocytochemical (PAP) experiments. (See refs. for protocols.)

The ten groups (most uncited findings are our own) are:

I] four mc groups, all projecting to the neurohypophysis; 1) medial, containing oxytocin (OXY), and 2) lateral, containing antidiuretic hormone (ADH) (mcm & mcl/ Brain Res. 108:187, J. Endocr. 67:461), 3) mc posterior fornical and anterior commissural (pfm & acP/ J. comp. Neur. 128:181), both containing OXY &/or ADH; and

II] six pc groups, none containing OXY or ADH; 1) dorsal (pcd) and 2) ventral (pcv), which project to the spinal cord (Exp. Brain Res. 35:315), 3) intermediate (pci) and 4) periventricular (pcp), which project to the median eminence (me/ SNS 5:#754 & #1580), 5) caudal (pcc/ horizontal, fusiform cells), which projects to the parabrachial area (pb) and possibly to me, (Brain Res. 88:403), and 6) pc posterior fornical (pfp), which projects to pb and to the caudal part of the nucleus of the solitary tract (nts).

Afferents to these groups are as follows: caudal nts projects to all pc groups but pcc (most heavily to pci), and possibly to pfm; afferents from pb involve the same six groups plus pcc.

As to transmitters or hormones used by pc groups, the only direct evidence deals with pcp, which, based on PAP staining, contains somatostatin (J.C. King, C. Scouten, & F.L. Snavely, pers. comm.); pci, which, like pcp, projects to me, does not. Since thyrotropin-releasing hormone may be present in the pvn (Science 185:267), it seems possible that it may reside in pci; or pci may contain a corticotropin-releasing factor and affect adrenocorticotropic hormone release (e.g., see Rec. Prog. Horm. Res. 34:357).

The fact that HRP injections in me, nts, and the spinal cord label only pc neurons of pvn conflicts with the notion that OXY- &/or ADH-containing fibers innervating those regions originate in pvn. The HRP method with diaminobenzidine as substrate may not be adequate in these systems; there may be undetected ADH- or OXY-containing pc neurons in pvn; or these fibers may not arise from pvn at all! (Supp. by grants MH 25515 & 5 T05 02220 [USPHS], BNS-7681227 [NSF], & Fellowship Award 04-75/0167 to J.A.R. from the Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil.)

180.8 IMMUNOCYTOCHEMICAL ANALYSES OF THE ACTH COMPONENT OF THE OPIOCORTIN SYSTEM IN NORMAL AND MSG TREATED ANIMALS. S.A. Joseph*, W. Pilcher* and K.M. Knigge (SPON: E. Boyd). Neuroendocrine Unit, University of Rochester School of Medicine, Rochester, NY 14642.

We have previously reported the distribution of the ACTH component of the central opiocortin system on Bouin's-fixed, 50μc Vibratome brain sections, using an antibody generated against synthetic ACTH$^{1-39}$. Perikarya are demonstrated immunocytochemically in the region of the arcuate nucleus, extending caudally to the level of the mammillary region of the hypothalamus. This dense fiber system innervates numerous areas of the hypothalamus. A component can be traced to the limbic system. These fibers project from the hypothalamus to amygdaloid nuclei in two separate bundles. A third efferent component is represented by a large dorsal bundle which issues from the hypothalamus and courses in the dorsal longitudinal fasciculus. It distributes to the midbrain and pontine periaqueductal grey. Neonatal treatment of mouse and rat with monosodium glutamate (MSG) produces a chemical lesion of the arcuate region of the hypothalamus. Animals with complete MSG lesion lack ACTH-immunoreactive perikarya in the bed nucleus as well as the entire fiber distribution of this system.

These data support the conclusion that the ACTH-immunoreactive perikarya in the arcuate region of the hypothalamus represent the bed nucleus of this system.

(Supported by RCDA to S.A.J. HD 00230A, HD 07926, and Program Project NS-15345.)

180.9 ORIGIN OF THE OPIOCORTIN SYSTEM IN RAT BRAIN. K.M. Knigge, S.A. Joseph* and W. Pilcher*. The Neuroendocrine Unit, University of Rochester, Rochester, N.Y. 14642.

An extensive fiber system in rat brain can be immunocytochemically demonstrated using antibodies generated against several peptides of the opiocortin family. Perikarya are seen variably in the mediobasal-arcuate area of the hypothalamus and are suspected to be the cells of origin of this system. This region was destroyed unilaterally from the retrochiasmatic area to the mammillary region using a deafferentation knife with a 1.0x1.2mm wedge-shaped blade. Two weeks later, animals were anesthetized and brains fixed with Bouin's solution by cardiac perfusion. Sections were cut at 50μm on a vibratome. The ACTH component of the opiocortin was revealed by the unlabeled antibody method using a primary antibody generated against synthetic $ACTH_h$ 1-39, conjugated to BSA at the C-terminal with carbodiimide. In all lesioned animals, fibers in hypothalamus, preoptic area, amygdala and dorsal longitudinal fasciculus (dlf) were eliminated almost completely on the side of the lesion. In midbrain and pons, fiber distribution was diminished. The ipsilateral component of the dlf descends with the mesencephalic nucelus of V and distributes to pontine and medullary grey along the floor of the 4th ventricle. The contralateral fibers distribute to tegmental nuclei. These results indicate that the ACTH-immunoreactive cells in the basal hypothalamus represent the bed nucleus of this opiocortin system. Fiber distribution in forebrain appears to be entirely ipsilateral; efferent outflow to the brainstem via the dlf has contralateral and ipsilateral components.
(Supported by RCDA HD00230A to S.A.J. and Program Project NS15345.)

180.10 IMMUNOCYTOCHEMICAL LOCALIZATION OF LHRH IN HYPOTHALAMUS AND PINEAL GLAND USING DIFFERENT FIXATION SOLUTIONS. D.T. Piekut* (SPON: R. Snider). Neuroendocrine Unit, University of Rochester School of Medicine, Rochester, NY 14642.

It has been previously reported in this laboratory that immunoreactive material is visualized in the rat pineal gland using antisera generated against synthetic LHRH and analogs of LHRH. The substance seen immunocytochemically in the pineal gland may have a different structure or antigenic determinants different than the LHRH peptide found in hypothalamus. Effect of different fixation solutions on our immunocytochemical studies is presented. Animals were perfused with Bouin's or Zamboni's solution, brains were cut on a Vibratome at 30μm, and collected in phosphate saline buffer. Sections of hypothalamus or pineal were processed for visualization of LHRH or LHRH-like material using Sternberger B antiserum and the PAP method of immunocytochemistry. Immunoreactive material is demonstrated in sections of the pineal gland that have been fixed in Bouin's solution. Immunostaining is not seen in sections of pineal fixed in Zamboni's solution at pH 7.6, but it is visualized if the pH of Zamboni's solution is buffered to 3.5. More intense fiber and perikarya immunoreactivity is observed in sections of hypothalamus following fixation by Zamboni's solution as compared to Bouin's. When the pH of Zamboni's solution is buffered to basic pH values (8.7), an even greater amount of immunoreactive LHRH is observed; a reduction of LHRH reactive material is apparent in brains fixed with an acidic Zamboni's solution (pH 3.5). These immunocytochemical studies reveal a difference in LHRH immunoreactivity in hypothalamus and pineal gland using antisera generated against different LHRH conjugates, antisera generated against different analogs of LHRH, different fixation solutions, and fixation solutions at altered pH values.
(Supported by NIH grant HD-12956 and Program Project grant NS-15345.)

181.1 LIGHT MICROSCOPIC AUTORADIOGRAPHIC DIFFERENTIATION OF MU AND DELTA OPIATE RECEPTORS IN RAT BRAIN. R.R. Goodman*, S.H. Snyder, M.J. Kuhar and W.S. Young, III. Dept. of Pharmacology & Experimental Therapeutics, and Psychiatry & the Behavioral Sciences, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Evidence supporting the existence of multiple opiate receptors has accumulated from binding studies and effects on smooth muscle (Kosterlitz, H.W., Lord, J.A.H., Paterson, S.J. & Waterfield, A.A., Br. J. Pharmacol., 179:333-342, 1980). The mu or morphine-like receptor has higher affinity for opiates than for enkephalins, while the opposite is true for the delta or enkephalin-like receptor. With techniques for in vitro light microscopic autoradiography of neurotransmitter receptors (Young, W.S., III, & Kuhar, M.J. Brain Res., 179:255-270, 1979), we have demonstrated a differential distribution of mu opiate receptors (labeled by [^{125}I][D-Ala^2, MePhe^4, Met(O)^5-ol]-enkephalin) and delta opiate receptors (labeled by [^{125}I][D-Ala^2, D-Leu^5]-enkephalin). The mu receptors display discrete localizations that in many regions are distinct from the more diffusely distributed delta receptors. High concentrations of mu receptors in the fourth layer of the cortex (an important area for the integration of sensory input), certain thalamic nuclei and the periaqueductal gray are all consistent with a role in antinociception. Delta receptors are more diffusely, localized with high concentrations in the second, third, fifth and sixth layers of the cortex, the striatum, the amygdala, the nucleus accumbens and the olfactory tubercle. These localizations are consistent with the evidence that delta agonists are sedating, seizure provoking and related to general behavior and reward. These autoradiographic localizations resemble regional variations in mu and delta binding (Chang, K-J., Cooper, B.R., Hazum, E. & Cuatrecasas, P. Mol. Pharmacol., 16:91-104, 1979).

(Supported by USPHS grants DA00266, MH00053, and GM7309).

181.2 ULTRASTRUCTURAL LOCALIZATION OF LEUCINE ENKEPHALIN IN THE SUPERFICIAL DORSAL HORN OF THE CAT. Ellyn J. Glazer and Allan I. Basbaum, Department of Anatomy, University of California, San Francisco, CA 94143.

The mechanism by which spinal endorphins modulate nociceptive transmission is unclear. Both pre- and postsynaptic models of opiate mediated inhibition of spinal nociceptors have been proposed. In the cat enkephalin-containing processes are densely distributed in laminae I and II of the superficial dorsal horn. Lamina I also contains numerous enkephalin cell bodies; fewer cell bodies appear in lamina II. To determine the synaptic circuitry through which enkephalin modulates spinal nociceptive input, the ultrastructural localization of leucine enkephalin immunoreactive processes was studied in the cat superficial dorsal horn. Cats were perfused with 4% para/.2% glut in 0.1M phosphate buffer. Sections through C1 and the trigeminal nucleus caudalis were sectioned transversely on a Vibratome, processed for enkephalin immunoreactivity by the PAP method, osmicated and flat embedded in Epon-Araldite. Control sections were incubated in specific enkephalin antisera preabsorbed with an excess of leucine enkephalin. The most commonly labeled profile was a small unmyelinated process which appeared in bundles containing predominantly unlabeled profiles. It was not possible to morphologically identify these processes as dendrites or unmyelinated axons. Enkephalin axon terminals in lamina I and II contained either small, round or a mixed population of small, round and large vesicles. The reaction product surrounded the small vesicles and filled the lumen of the large vesicles; the large vesicles in several terminals were peripherally displaced. Labeled axon terminals were associated with synaptic glomeruli. These complexes consist of a centrally located primary afferent terminal surrounded by axonal and dendritic profiles. Although enkephalin axons did not directly contact the primary afferent they were associated with non-vesicle-containing dendrites postsynaptic to the central terminal. This arrangement is consistent with an indirect opiate modulation of primary afferent terminals or a direct opiate inhibition of second order nociceptors. Outside the glomeruli enkephalin terminals were associated with dendrites, perikarya and axons. It could be determined that enkephalin axons were presynaptic to both dendrites and soma, but the polarity of axo-axonic relationships could not be established. There was, however, a frequent association of an enkephalin axon terminal with an unlabeled, flat vesicle containing profile. The latter was presynaptic to a dendrite. Since GAD immunoreactivity in the superficial dorsal horn has been demonstrated in profiles containing flat vesicles this arrangement may reflect a GABAergic-enkephalinergic interaction.

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181.3 DISTRIBUTION OF ACTH- AND ENDORPHIN-RELATED PEPTIDES IN BOVINE BRAIN. J. Lépine*, A. Dupont* and F. Labrie* (SPON: F. Garcin Department of Molecular Endocrinology, CHUL, Quebec G1V 4G2, Canada.

Study of the distribution of β-LPH, β-endorphin, ACTH and their related peptides in anterior and intermediate pituitary lobes have shown the presence of the same peptides but in different proportions in the two tissues. We now report the distribution of the same peptides in different areas of bovine brain. Thirty structures of bovine brain frozen immediately after dissection at the slaughterhouse were extracted with 2 N acetic acid and lyophilized before measurement with specific RIAs for $ACTH_{1-24}$, β-endorphin, γ-LPH_{1-47}, γ-endorphin and α-MSH. The area preoptica, the habenula including the paraventricular nucleus of thalamus, and the septum are the most concentrated extrahypothalamic regions in β-endorphin immunoreactivity. The finding that ACTH and γ-LPH_{1-47} immunoreactivity in these structures parallel the distribution of β-endorphin suggests that neurons from these areas produce the biologically active peptides by cleavage of the precursor molecule. Study of the distribution of the endorphins, ACTH and α-MSH by gel filtration in extracts from the median eminence, hypothalamus and area preoptica shows that β-endorphin is the main opioid peptide present in the regions studied while its presumed precursor, β-LPH, is also present but in lower concentrations. Two main forms of ACTH are present in these regions: $ACTH_{1-39}$ and a low molecular weight immunoreactive material eluting ahead of α-MSH. This unidentified material is seen in equivalent concentrations in the area preoptica as well as in the posterior and median hypothalamus. However, $ACTH_{1-39}$ is the main form of ACTH in the median eminence and the anterior hypothalamus. Finally, γ-LPH immunoreactivity resolves into three peaks in all the structures studied: the first one corresponds to β-LPH, the second peak coelutes with γ-LPH while the last one elutes at a position slightly faster than the β-endorphin marker. In conclusion, these results show that neurons from the anterior, median and posterior hypothalamus, as well as from the median eminence, the area preoptica, the septum and the habenula, including the paraventricular nucleus of thalamus, contains β-LPH, ACTH, γ-endorphin and α-MSH in different concentrations. While the proportion of β-LPH, γ-LPH, β-endorphin and γ-endorphin immunoreactivity is the same in all brain areas, the distribution of $ACTH_{1-39}$ and lower molecular form of ACTH immunoreactivity shows wide variations. The present data indicate a differential processing of the 31K precursor molecule in various bovine brain structures.

181.4 OPIATE RECEPTOR REGULATION OF THE NIGROSTRIATAL PATHWAY. Paul L. Wood. Merck Frosst Lab., Dept. of Pharmacology, P.O. Box 1005, Pointe Claire-Dorval, Quebec, H9R 4P8.

A simple but sensitive and specific assay for the simultaneous measurement of striatal dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), dopamine (DA) and 3-methoxytyramine (3-MT) was established and used to monitor opiate actions on nigrostriatal function. Mu, delta and epsilon receptor agonists were found to produce dose dependent elevations of striatal DOPAC and HVA indicating the presence of these receptors on nigrostriatal neurons. In addition, a species difference in the coupling of opiate receptor activation and DA release was observed. In the mouse, both intraneuronal metabolism and DA release were elevated after parenteral morphine while in the rat no such parallelism was observed. In contrast to mu, delta and epsilon receptor agonists, kappa receptor agonists did not alter dopamine metabolism in the striatum suggesting that these receptors are not involved in the regulation of these neurons. Agonist/antagonist analgesics also elevated DA metabolites at low doses; however, these actions were reversed at higher doses where the antagonistic activity of these agents prevails. Sigma receptor agonists also altered striatal DA metabolism but these actions were not reversed by naloxone, arguing against an opiate receptor mechanism.

In summary, our studies indicate that multiple opiate receptors are involved in the regulation of activity in the nigrostriatal pathway but that kappa receptors are not present on these neurons.

181.5 NON-COMPETITIVE INHIBITION OF THE BINDING OF ^{3}H-NALOXONE BY LEUCINE ENKEPHALIN - REVERSAL BY MORPHINE. Richard Rothman* and Thomas C. Westfall (SPON: V. Fischer). Departments of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22908 and St. Louis University School of Medicine, St. Louis, Missouri 63104.

In the experiments to be described, the specific binding of ^{3}H-naloxone to rat brain membranes was determined in ice-cold 10 mM NH_4Ac, pH 7.7, at 4°C in the presence of 400 mM NaCl. Saturation binding experiments with ^{3}H-naloxone demonstrated that this ligand labels a single class of binding sites (K_d = 1.7 nM, R_t = 464 fmol/mg protein). By displacement of a fixed concentration of ^{3}H-naloxone, the Ki's of met-enkephalin, leu-enkephalin, etorphine, and morphine were calculated to be 190 nM, 1000 nM, 6.6 nM, and 100 nM, respectively. Scatchard analysis of the binding of ^{3}H-naloxone in the absence and presence of 30 nM etorphine demonstrated that in addition to a four-fold increase in the K_d, from which a Ki of 9 nM was calculated, there also occurred a 20% decrease in the number of binding sites. When this was taken into account, the true Ki of etorphine was calculated to be 14 nM. Similar experiments with 10 µM leu-enkephalin and 1 µM met-enkephalin demonstrated that these drugs were non-competitive inhibitors of the binding of ^{3}H-naloxone with true Ki's of 13 µM and 2.5 µM, respectively. In contrast, 200 nM morphine was a competitive inhibitor with a Ki of 60 nM, and, in addition, reversed the non-competitive inhibition caused by 500 nM leu-enkephalin. (Supported in part by USPHS-NINCDS 16215 and NIGMS 57055.)

181.6 CHARACTERIZATION OF GUANINE NUCLEOTIDE INTERACTIONS WITH OPIATE RECEPTORS IN RAT BRAIN. Steven R. Childers and Margaret Marsh* Dept. of Pharmacology, Univ. of Florida Coll. Med., Gainesville, Fla. 32610.

Recent evidence indicates that guanine nucleotides, in particular GTP, play an important role in regulating the binding of agonists to opiate receptors by selectively reducing opiate agonist and not antagonist binding to rat brain membranes. The mechanism of the GTP effect principally involves increased dissociation rates of agonists from receptor sites. In order to explore the relationship between the opiate receptor active site and the GTP regulatory site, rat brain membranes were incubated with various reagents to determine whether one site could be separated from the other. Protein modifying reagents such as N-ethylmaleimide (NEM) and iodoacetamide, and enzymes such as trypsin and phospholipase A, were effective in inhibiting ^{3}H-opiate agonist binding. At the same concentrations, these agents also inhibited the GTP effect on ^{3}H-agonist binding, reducing both the IC_{50} and maximum effect of GTP in inhibiting ^{3}H-dihydromorphine and ^{3}H-d-Ala enkephalin binding. Incubation of rat brain membranes with 250 µM NEM reduced ^{3}H-d-Ala enkephalin binding by 60%, while reducing the effect of 50 µM GTP on binding by approximately 80%. In order to selectively protect the opiate site from the actions of NEM, membranes were incubated with 0.2 µM d-Ala enk before NEM treatment, followed by extensive washing to remove both reagent and opiate. Protection with opiates resulted in only a 40% loss of binding with NEM; however, the GTP effect was completely eliminated up to 100 µM GTP. This "non-regulated" preparation of receptors exhibited over 90% of normal antagonist binding, normal IC_{50} values for unlabeled opiates, and normal sensitivity to sodium. These receptors were not affected by manganese, GTP or GMP-PNP in steady-state experiments. The GTP effect could be partially restored by incubation of membranes with 100 µM GMP-PNP prior to NEM treatment: while ^{3}H-d-Ala enk binding was still reduced by 60%, the effect of GTP was reduced only about 10-20% by NEM. Pre-treatment with both GMP-PNP and d-Ala enk parially restored both binding and GTP regulation. These experiments indicate that GTP regulates opiate receptor sites by binding to a protein which is physically distinct from the opiate receptor itself, and which may mediate the divalent cation effects on receptor interactions.

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181.7 DIBUTYRYL CYCLIC AMP DECREASES SPECIFIC [^{3}H]-DIHYDROMORPHINE BINDING TO RAT MIDBRAIN HOMOGENATE. (D.A. Hosford, P.M. Iuvone* and H.J. Haigler, Dept. of Pharmacology, Emory Univ., Atlanta, GA 30322)

The intracerebral or intravenous administration of dibutyryl cyclic adenosine 3',5'-monophosphate (cAMP) reversed morphine-induced analgesia in mice (Ho et al., J. Pharmacol. Exp. Ther. 185, 336, 1973). In the rat mesencephalic reticular formation, morphine blocked the increase in firing evoked by a nociceptive stimulus (Haigler, Life Sci. 19, 841, 1976); this blockade by morphine was reversed by the microiontophoretic administration of dibutyryl cAMP (Hosford and Haigler, Neurosci. Abst. 5, 560, 1979). To determine if dibutyryl cAMP blocked the effect of morphine by interfering with its binding to membrane receptors, we tested the effects of dibutyryl cAMP, butyrate, and 3-isobutyl-1-methylxanthine (IBMX: a phosphodiesterase inhibitor) on the specific binding of [^{3}H]-dihydromorphine to brain homogenates prepared from rat midbrain.

Male Sprague-Dawley rats were decapitated and the brains were removed within 2 min. The midbrain was homogenized in ice-cold 50 mM Tris buffer (pH 7.4, 37°C); the homogenate was diluted and incubated for 30 min at 37°C with 2 nM [^{3}H]-dihydromorphine. Except for controls, one of the following was added to each of the homogenates: dibutyryl cAMP (100 µM), IBMX (100 µM) or butyrate (100 µM). All incubations were carried out in the presence or absence of levorphanol. Specific binding was calculated as the difference between binding in the presence and absence of levorphanol. Both dibutyryl cAMP and IBMX significantly decreased specific [^{3}H]-dihydromorphine binding. Butyrate had no effect on binding. Adenosine 5'-monophosphate (5'-AMP) did not decrease [^{3}H]-dihydromorphine binding to rat brain homogenate (Blume, Proc. Natl. Acad. Sci. 75, 1713, 1978). The decrease in specific [^{3}H]-dihydromorphine binding by dibutryryl cAMP, but not by its metabolites, butyrate and 5'-AMP, implies that the effect is caused by the cyclic AMP analogue. The mechanism underlying this effect remains to be determined. (Supported in part by NIH grant T32-GM-07415-03 [D.A.H.], NIH grant 5S07-RR05364 [P.M.I.], and NIDA grant 1-R01-DA-01344-04 [H.J.H.])

181.8 INHIBITION OF OPIATE RECEPTOR BINDING BY ADENOSINE 5'-DIPHOSPHATE David A. Brase, Dept. of Pharmacology, Eastern Virginia Medical School, Norfolk, VA 23501.

Studies by other investigators (Blume, *Life Sci. 22*: 1483, 1978; Childers & Snyder, *Life Sci. 23*: 759, 1978) have shown that opiate receptor binding, particularly of agonists in the presence of 100 mM NaCl, is reduced by GDP and GTP, but not by adenine nucleotides up to a concentration of 100 µM. Since some adenine nucleotides exist in cells in higher concentrations, their effects on specific (^{3}H)etorphine and (^{3}H)diprenorphine binding were studied, by preincubation of the adenosine compounds with rat brain homogenates in 50 mM Tris-HCl buffer (pH 7.4) ± 10 µM naloxone, followed by incubation with (^{3}H)-ligand (1 nM) for 20 min at 37°C. Incubations were terminated by placing the vials on ice, followed 5 min later by filtration through GF/C filters and two 8-ml washes with cold buffer. With a 5-min preincubation, 1 mM adenosine, AMP or cyclic AMP had no effect, but ADP and ATP caused about 40% and 20% inhibition of binding, respectively, of both etorphine and diprenorphine, either in the absence or presence of 100 mM NaCl. A 5-min preincubation with 1 mM ADP did not inhibit binding to a washed particulate fraction of rat brain. The inhibitory effect of ADP could be restored by adding the 40,000xg supernatant fraction from the homogenates back to the washed particulate fraction. The supernatant fraction, itself, was inhibitory to both agonist and antagonist binding, in the absence or presence of 100 mM NaCl. The inhibitory effect of the supernatant fraction was enhanced by boiling, not affected by trypsin, partially destroyed by potato apyrase, and completely removed from boiled supernatant by activated charcoal.

In homogenates, the inhibition by ADP increased with preincubation time (0-20 min) and with concentration (0.125-1.0 mM). At 1 mM, 2-deoxyADP and 1,N^6-ethenoADP mimicked ADP, whereas α,β-methyleneADP and β-thioADP completely blocked, and ATP partially blocked the inhibitory effect of ADP. Other adenosine-containing compounds that were tested at 1 mM, but failed to mimic or block ADP included: adenosine, AMP, ADP-glucose, ADP-ribose, NAD, and P^1,P^2-diadenosine 5'-pyrophosphate. Addition of α,β-methyleneADP just before (^{3}H)diprenorphine, after a 20-min preincubation with ADP, failed to reverse the inhibition of binding by ADP.

These studies indicate the possibility that ADP interacts with an unidentified soluble factor to produce an inhibitor of opiate receptor binding. It is tempting to speculate that this inhibitor is endogenous and may play a role in the regulation of opiate receptor function. Studies are in progress to further characterize the supernatant inhibitor and to compare its effects with those of ADP and guanine nucleotides. (Supported in part by NIH Biomedical Research Development Grant RR09028-03)

181.9 OPIATE AND PHENCYCLIDINE RECEPTORS IN RAT AND MONKEY BRAIN. Roberta M. Palmour,* Elizabeth Zobell* and Frank R. Ervin. Dept. Genetics, U. California, Berkeley and Dept. Psychiatry, McGill University, Montreal, Quebec, Canada H3A 1A1.

The existence of multiple brain opiate receptor subpopulations is substantiated by pharmacological studies, bioassay observations and direct binding data. At least 5 classes of opiate receptors--μ, σ, κ, δ, ε--have been proposed, but biochemical distinctions of the different classes of binding sites are incomplete. Using a rapid filtration assay, we have defined kinetic parameters and regional distributions of opiate and phencyclidine (PCP) specific receptors in rat and African green monkey brain. ^{3}H-etorphine (E) was used as a "generalized" opiate agonist and ^{3}H-naloxone as an antagonist. Saturation analysis reveals approximately equal numbers of agonist and antagonist binding sites in whole brain. Agonist binding sites are highest in limbic areas (hippocampus, amygdala, cingulate), moderate in striatum, thalamus and cortex and very low in cerebellum. Displacement of low concentrations (∿ 0.1 K_d) of ^{3}H-E by unlabelled enkephalin (D), endorphin (βE), N-allylnormetazocine (S), N-ethylketocyclazocine (K) yielded biphasic displacement plots and 2-component Hill plots, while displacements with E, M or N gave monocomponent plots. Biphasic plots were more pronounced in limbic and striatal regions than in whole brain. Displacement of low concentrations of ^{3}H-K, ^{3}H-DHM and ^{125}I-βE with homologous and analogous competitors allowed partial distinction of κ, μ and ε opiate receptors. Again effects were more pronounced in limbic and striatal regions.

In vervet monkey brain, ^{3}H-PCP binding is specific and saturable, having a K_d of 1.5 x 10^{-7} M and B_{max} of 350 fmol/mg protein. The regional order of binding is hippocampus > thalamus > cingulate/septum/amygdala > striatum/cortex. Specifically bound PCP is displaced by low concentrations of PCP, PCE and TCP, by moderate conceptrations of S, cyclazocine and ketamine, and by higher concentrations of K, naloxone, quipazine and amphetamine in rat brain membrane preparations. Cross-displacements suggest that ^{3}H-PCP does not label the σ receptor; definitive demonstration awaits availability of ^{3}H-S.

In a green monkey social group, low-dose chronic PCP (0.15 mg/kg/day) reduced affiliative and increased aggressive behavior in both treated and untreated animals. At higher doses, a subset of animals appears to show differential behavioral and neurological sensitivity to PCP. A potential receptor basis for this phenomenon is under study.

We thank NIDA for drugs and Dr. M. McGuire, Behavioral Sciences Fdn., St. Kitts for animals.

181.10 CHARACTERIZATION OF [^{3}H]MET- AND [^{3}H]LEU-ENKEPHALIN BINDING IN CAT CNS: EFFECTS OF LUMBAR RHIZOTOMY. G. J. Wastek, T. L. Yaksh and E. Richelson. Depts. of Psychiatry, Pharmacology and Neurosurgery, Mayo Foundation, Rochester, MN 55901.

[^{3}H]Met- and [^{3}H]leu-enkephalin binding were measured in various regions of cat brain and spinal cord using a microcentrifugation assay at 0°C. The $t_{½}$ association of these [^{3}H]opioids to both cat brain and spinal cord was ∿ 10 min for [^{3}H]met- and ∿ 17 min for [^{3}H]leu-enkephalin and the binding of both ligands equilibrated by 90 min. The association rate constants (k_1) for both [^{3}H]opioids at 0°C was ∿ 1-2 x $10^6 M^{-1}$ min^{-1}. The $t_{½}$ dissociation of these ligands from both brain and spinal cord at 0°C was ∿ 240 min giving dissociation rate constants (k_{-1}) of ∿ 4-6 x 10^{-4} min^{-1}. The dissociation constant (K_D) calculated from the ratio of these two kinetic constants for both [^{3}H] ligands was 0.3 nM. Scatchard analyses of saturation isotherms, using both [^{3}H]opioids in cat brain homogenates, revealed one population of binding sites with maximum binding (B_{max}) between 500-700 pmol/g prot. and K_Ds of 2-4 nM. Similar studies in the cat spinal cord revealed one population of binding sites for both [^{3}H]opioids with B_{max}s of ∿ 200 pmol/g prot. and K_Ds of 1-4 nM. Na^+(100 mM) reduced [^{3}H]opioid binding in both the brain and spinal cord to approximately one-sixth of control values for each ligand. Both met- and leu-enkephalin were more potent in inhibiting the [^{3}H]opioid binding than were naloxone or morphine (the least potent). The distributions of [^{3}H]met- and leu-enkephalin binding in the cat brain were as follows, with met- being uniformly higher than leu-enkephalin binding in all regions examined: septum, hypothalamus, hippocampus, thalamus, PAG, caudate > medulla, superior and inferior colliculi, MRF, pons > frontal, parietal, temporal and occipital cortices, cerebellar vermis and sciatic nerve. [^{3}H]Opioid binding varied between 200-300 pmol/g prot. in the dorsal and ventral regions of the cervical, thoracic and lumbar segments of the cord, with met- being uniformly higher than leu-enkephalin binding in all regions examined. Lumbar rhizotomy significantly decreased the binding of both [^{3}H]opioids in the lumbar dorsal region of the cord. These findings confirm and extend those of Fields et al. (Nature 284:351, 1980) in showing that opioids act directly on the terminals of small diameter primary afferents to selectively inhibit nociceptive input to dorsal horn neurons. (This work supported by a Sandoz Fndn. Fellowship to G.J.W., DA 02110 to T.Y. and DA 1490 to E.R.)

181.11 OPIATE RECEPTOR CHARACTERISTICS IN ETHANOL-TREATED MICE. P. L. Hoffman* and B. Tabakoff. Dept. Physiol. & Biophys., Univ. of Ill. Med. Ctr. and West Side V.A. Med. Ctr., Chicago, IL 60612.

Chronic ethanol treatment alters the function of several components of the striato-nigral feedback loop which acts to control dopamine (DA) synthesis. Thus, both DA agonists and antagonists are less effective in altering DA synthesis in ethanol-treated mice. Enkephalin-containing neurons have also been postulated to control DA metabolism in the striatum and our prior studies demonstrated that the striatal DA neurons of ethanol-treated animals are significantly less responsive to submaximal doses of morphine, in terms of DA metabolism, as compared to controls. We therefore examined the affinity and number of striatal opiate receptors in ethanol-treated animals. Male C57Bl mice were exposed chronically to a liquid diet containing 7% (v/v) ethanol and controls received a diet containing equicaloric amounts of sucrose. After seven days, the ethanol-consuming animals were given the control diet (withdrawal). The animals were shown to be tolerant to and physically dependent on ethanol. Opiate receptor function was examined at 24 hours after withdrawal, when withdrawal symptoms were no longer evident, by determining ^{3}H-dihydromorphine binding to striatal membranes. Scatchard analysis revealed a high- and low-affinity binding site, with K_D's of 0.47±0.07 nM and 2.52±0.48 nM, respectively, in control animals. Each portion of the Scatchard plot was corrected for the contribution of the other portion by computer analysis. The K_D of the high-affinity site in the ethanol-treated animals was 0.82±0.09 nM, and was significantly higher ($P<0.05$) than that in controls. No significant changes occurred in the B_{max} values for the high-affinity site. Determination of association and dissociation rate constants revealed that the decreased affinity of the high-affinity morphine-binding site in the ethanol-treated animals was largely due to a decreased association rate constant. We are currently examining the effects of Na^+ on morphine binding in control and ethanol-treated animals, since high Na^+ concentrations have also been found to affect the affinity of the opiate receptor for agonists. Furthermore, the possible relationship of changes in opiate receptor affinity to ethanol tolerance or dependence is being assessed by following the time course of the development and dissipation of the affinity change. Chronic ethanol exposure has been postulated to alter the physical properties of neuronal membranes, including lipid composition. Our results are in line with the hypothesis that such membrane changes may alter the function of the lipid-containing morphine receptor.

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181.12 PHARMACOLOGICAL PROFILE OF THE FUMARAMIDO METHYL ESTER DERIVATIVE (βFNA) OF NALTREXONE. Susan J. Ward*, P. S. Portoghese* and A. E. Takemori* (SPON: Alice Larson). Depts. of Pharmacol. and Med. Chem., U. of Minn. Minneapolis, Minnesota 55455.

βFNA has recently been shown to possess reversible agonist and irreversible antagonist actions on the electrically stimulated guinea-pig ileal longitudinal muscle preparation. The pA_2 for the antagonism of βFNA's agonist actions by naloxone (NLX) was 8.06, similar to that for nalorphine-naloxone interactions. In contrast to any other opiate antagonist, βFNA selectively antagonised the agonist actions of morphine (M) yet was ineffective against the agonist actions of nalorphine. Since morphine-like and nalorphine-like compounds are considered to interact preferentially with μ and k receptors respectively, this data would suggest that βFNA possesses irreversible antagonist actions at the μ receptor and reversible agonist actions at the k receptor in the guinea-pig ileum preparation. The in vivo profile of βFNA supports this hypothesis.

βFNA, like other mixed agonist-antagonist compounds, did not demonstrate any agonist activity on the mouse tail flick test following peripheral administration. Some agonist action could be detected following icv administration (ED_{50}:4.8 nmol/kg) and this was antagonised by NLX. Following ip injection, βFNA readily antagonised the analgesic effects of both M and ethylketazocine (EK) (a pure 'k' agonist) and this antagonist action was apparent up to 4 days following a single administration. This ability of βFNA to antagonise EK-induced analgesia on the tail flick test is not inconsistent with the suggestion that βFNA possesses antagonist action only at the μ receptor since the agonist actions of EK in this test are probably mediated primarily by μ receptor interaction.

In the acetic acid writhing test, a less stringent analgesic assay in which mixed agonist-antagonist compounds are active, βFNA demonstrated an agonist action (ED_{50}:7.3 μmol/kg) that was antagonised by NLX. βFNA antagonised the analgesic actions of M in this test, but enhanced the analgesic actions of EK.

The unique importance of βFNA's selectivity of agonist and antagonist action lies in the irreversibility of only one of these actions. As such βFNA may prove to be an invaluable tool in the elucidation of receptor types in opioid binding assays. (Supported by U.S. Public Health Service Grants DA 00289 and DA 01533 from the National Institute of Drug Abuse.)

182.1 FEEDING RESPONSES TO 2-DEOXY-D-GLUCOSE AND INSULIN AFTER DESTRUCTION OF THE AMYGDALOID COMPLEX IN THE RAT. Paula J. Geiselman, Michael G. Tordoff*, Carlos V. Grijalva, Stephen W. Kiefer, and Donald Novin. Brain Research Institute and Department of Psychology, UCLA, Los Angeles, CA 90024.

The lateral hypothalamus (LH) and the amygdaloid complex are functionally related in the control of food intake. The sequelae to LH destruction, however, have been studied more extensively. Rats sustaining LH damage no longer show enhancement of food intake in response to either 2-deoxy-D-glucose (2DG) or insulin. Comparable metabolic challenges have not been studied following amygdaloid destruction. The present experiment was, therefore, conducted to investigate the possible role of the amygdala in the mediation of glucostatic feeding.

Twelve male albino rats sustained electrolytic destruction of the amygdaloid complex, and an additional 12 rats were sham operated. Following recovery, free-feeding animals received intraperitoneal injections of 2DG (100, 200, and 400 mg/kg), subcutaneous injections of insulin (10 U/kg), or equal volumes of isotonic saline by similar routes. All tests were begun at light onset. Food was measured hourly for three hours and again at 24 hours postinjection.

Following injections of 2DG, control animals showed an increase in food intake during the first hour as a function of dosage. By the second hour, the lowest dose of 2DG (100 mg/kg) was no longer effective; but the other two doses (200 and 400 mg/kg) continued to increase food intake. By the third hour, the effects of 2DG had largely subsided in control animals. Rats with amygdaloid damage were unresponsive to 2DG until the third hour, at which time they showed an increase in food intake in response to the two larger doses only. During the 4th through 24th hours, both groups of animals showed alteration in food intake as an inverse function of dosage of 2DG. In response to insulin, however, there were no differences between the two groups: Both control and lesioned animals increased food intake throughout the initial three hours postinjection of insulin and decreased food intake during the remaining portion of the 24-hour period.

This study demonstrates that amygdaloid destruction has a dissociative effect on 2DG- and insulin-induced feeding behavior. These results are in contrast to LH damage, which alters both 2DG- and insulin-induced feeding.

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182.2 EFFECTS OF VAGAL TRANSECTIONS ON PLASMA INSULIN AND GLUCOSE LEVELS IN NORMAL AND VENTROMEDIAL HYPOTHALAMIC LESIONED RATS. Bruce M. King, Glenn R. Phelps,* and Lawrence A. Frohman.* Department of Psychology, University of New Orleans, LA 70122, and Division of Endocrinology and Metabolism and Department of Medicine, Michael Reese Hospital, and University of Chicago, IL 60616.

Animals with ventromedial hypothalamic (VMH) lesions display marked elevations in plasma insulin levels both after a fast (Goldman, Bernardis, and Frohman, 1974; Hales and Kennedy, 1964; Han and Frohman, 1970) and in response to an oral glucose load (Steffens, 1970; Steffens, Mogenson, and Stevenson, 1972). In order to assess the role of vagally-mediated hyperinsulinemia in hypothalamic obesity, plasma insulin and glucose levels were assayed in vagotomized (n = 12) and sham-vagotomized (n = 10) female rats after a 6-hour fast and after a measured glucose meal (6 ml sweetened milk) both prior to and 10-14 days following VMH lesions (150 days after vagotomy). The vagotomized and sham-vagotomized animals displayed mean weight gains of 104.3 and 112.2 g, respectively, in the first 10 days after VMH lesions ($t = 0.88$, $p > .30$), but fasting insulin levels were significantly elevated only in sham-vagotomized animals ($p < .005$). Fasting glucose levels were not affected by vagotomy. VMH lesions enhanced the insulin response to oral glucose in both vagotomized and sham-vagotomized animals ($p < .005$). The glucose and insulin response to oral glucose was also markedly elevated by vagotomy ($p < .001$), both prior to and following VMH lesions. As vagotomy reduces gastric retention and increases intestinal transit of liquid loads (Ralph & Sawchenko, 1978; Snowdon, 1970), this effect was probably the result of more rapid absorption of glucose from the intestine. It is concluded that postabsorptive insulin hyperresponsiveness to an oral glucose load in VMH-lesioned animals is independent of vagal mediation, and that while fasting hyperinsulinemia is under vagal control, its elimination does not prevent the development of obesity.

182.3 TACTILE CONTROL OF FEEDING IN THE MALLARD (ANAS PLATYRHYNCHOS L.) H. Berkhoudt*(SPON: H.P. Zeigler). Dept. of Morphol., Zool. Lab., Leiden, The Netherlands 2300 RA.

Previous work has shown that the ingestive behavior of the mallard involves a continuous flow of inputs from mechano-receptors in the oral region. These inputs are used to control the organization of oromotor activity during the different phases of eating and drinking.

The central projections of oral afferents were determined by multi-unit analyses of central trigeminal structures. These studies provided somatotopic maps of the main sensory trigeminal nucleus and a second order trigeminal telencephalic relay, the nucleus basalis. Cinematographic analyses have suggested predictions as to the sequencing of input from different parts of the buccal region during pecking, straining and drinking. These hypotheses were tested by correlating beak movements, muscle activity and neural activity during ingestive behavior. Beak movements were monitored using strain gauges and EMGs recorded from serpihyoid muscle (tongue retractor muscle). Electroneurograms were obtained using chronic multi-unit electrodes implanted in somatotopically differentiated (beak and tongue) regions of the nucleus basalis.

During drinking, mechanoreceptor activity continued throughout the entire act (often for more than two sec.), reaching its highest level during water intake and gradually decreasing prior to swallowing. Mechanoreceptor activity during drinking was not correlated either with beak or tongue movements. The level of mechano-receptor activity during straining was low but correlated better with beak closure/tongue protraction. During pecking there was a clear-cut correlation between the different positions of the food items within the oral region and the occurring activity in different somatotopic regions of nucleus basalis. Multi-unit activity in these regions also correlated well with the occurrence of beak closure/tongue protraction movements. These results confirmed predictions as to the role of mechanoreceptors based upon morphological analyses of their topography and distribution.

182.4 POST-DYNAMIC PHASE OF FATTENING IN RATS WITH VENTROMEDIAL HYPOTHALAMIC LESIONS. J. D. Hallonquist and J. S. Brandes. Dept. of Psychiatry, Mount Sinai Hospital, Toronto, Ontario, Canada M5G 1X5.

Lesions of the ventromedial hypothalamus (VMH) of adult rats result in immediate hyperphagia accompanied by a rapid, negatively-accelerated increase in body weight reflecting increased fat stores. Following this 4 to 10 week "dynamic" phase, a permanent "static" phase is commonly reported in the literature. This phase is described repeatedly as consisting of normal food intake and an elevated body weight beyond which there is no further increase except that exhibited by normal rats of the same age.

In contrast to this description of a "static" phase, we report the absence of a post-dynamic plateau in body weight in female rats with large electrolytic lesions in the VMH area. Instead, beginning by the 11th. postoperative week, such rats showed a steady increase in body weight at a mean rate more than double that of operated control rats of the same age (1.6 vs. 0.7 g/day, $P < .001$). In several cases the post-dynamic phase weight gain amounted to 500 g. Experimental manipulation of the body weight of individual rats by food restriction during the post-dynamic phase suggests that in addition to an immediate elevation of a "set-point" for body weight that produces the dynamic phase, the lesions also induce an additional, gradual elevation of the set-point with age (i.e., a climbing set-point).

The characterization of this gradually developing obesity in rats with VMH-area lesions is especially important considering that most human idiopathic obesity also develops gradually. As a model for human obesity, this long-term effect of VMH area lesions may be more important than the immediate effects. Because normal rats increase fat stores slightly with age, the long-term effect of VMH area lesions may also reflect the acceleration of a normal process.

182.5 PITUITARY BETA-ENDORPHIN LEVELS AND NALOXONE EFFECTS ON FOOD INTAKE IN SEVERAL OBESITY SYNDROMES. M. W. Gunion* and R. H. Peters* (SPON: G. Ellison). Dept. of Psychology, Iowa State Univ., Ames, Iowa 50011.

Beta-endorphin (BE) has been implicated in some genetic obesities. Genetically obese mice (ob/ob) and rats (fa/fa) have elevated pituitary BE levels, and show enhanced suppression of food intake after injection of the opiate antagonist naloxone (Margules et al., Science, 202:988-991, 1978). In the present experiments the role of BE in several non-genetic obesity syndromes was examined. Adult female hooded rats received ventromedial hypothalamic lesions (VMH), dorsolateral tegmental lesions (DLT), parasagittal hypothalamic knife cuts (KC), intraventricular 5,7-dihydroxytryptamine (serotonergic neurotoxin; 5,7-DHT), ovariectomy (OVX), or control surgery. The effect of naloxone (0.5, 1.8, 6.8, or 25.0 mg/kg ip) on food intake was measured during daily four-hour food access periods while all animals were kept at control body weights. Intake was significantly decreased by 1.8 mg/kg (6%, $p<.05$), 6.8 mg/kg (13%, $p<.01$), and 25.0 mg/kg (20%, $p<.01$). The groups did not differ in this dose-dependent suppression.

Half of each group continued on this diet and feeding regimen after naloxone testing. The remaining half of each group was maintained on ad libitum wet mash (70% water) until all rats were sacrificed 4-6 weeks after naloxone testing. VMH and KC became very obese, and OVX and DLT moderately obese on wet mash (68%, 68%, 22%, and 16% above control, respectively; all $p<.01$). Pituitary beta-endorphin-like immunoreactivity (BELI) was decreased by 61% ($p<.01$) in both lean and obese KC rats (23.1 pg/mg), but was unaltered in other groups (mean of 59.8 pg/mg). Obesity had no effect on BELI. In a second experiment, female rats were made obese (69% above control; $p<.0001$) by maintenance for 6 months on palatable foods (sweetened milk, sweetened chow, and syrup-peanut butter slurry). These obese rats had pituitary BELI levels 36% ($p<.02$) above controls.

Feeding mechanisms involving opioid peptides do not appear to be of etiological significance in the syndromes examined. Changes in pituitary BE levels do not appear to be directly related to obesity. The enhanced naloxone suppression of food intake previously found in some genetically obese rodents may be due to obesity alone.

182.6 STRAIN DIFFERENCES IN BEHAVIOR EVOKED BY ELECTRICAL STIMULATION OF THE LATERAL HYPOTHALAMUS. G. Mittleman & E.S. Valenstein, Depts. Psych. & Neurosci. Lab., Univ. Michigan, Ann Arbor, Mich. 48109.

Electrical stimulation of the lateral hypothalamus (ESLH) has been shown to produce individual response differences that cannot be attributed to the neuroanatomical locus of the electrode (Valenstein, Cox & Kakolewski, Psych. Rev., 77:16-31, 1970; Wise, Physiol. & Behav., 6:569-572, 1971. It has been proposed that the response to ESLH may be significantly influenced by behavioral characteristics of the individual animal (Valenstein, Brain, Behav. & Evol. 2:295-316, 1969. The possibility that strain differences may underlie individual responses to ESLH has been raised by reports that hypothalamic self-stimulation rate is correlated with genetic background in rats (Lieblich, Cohen & Beiles, Physiol. & Behav., 21:843-849; Lipp, Brain Res. Bull., 4:553-559, 1979) and mice (Cazala, Cazals & Cardo, Brain Res., 81:159-167, 1974). Moreover, preliminary observations in this laboratory indicated that the incidence of evoking stimulus-bound (S-B) eating and drinking by ESLH was much higher in animals from a Long-Evans (Simonsen Lab, Gilroy, CA & Charles River, Wilmington, Mass.) strain than from a Sprague Dawley (Holtzman Co., Madison, WI) albino strain although these animals did not differ in daily food or water consumption or body weight. The purpose of this experiment was to systematically investigate these differences in behavior evoked by ESLH in Sprague Dawley (S-D) and Long Evans (L-E) rats.

Bipolar electrodes were implanted bilaterally in the lateral hypothalamus of 51 mature S-D male rats and 49 (L-E) male rats. All animals were tested for S-B eating and drinking on 7 separate sessions using an ascending series of stimulus intensities. Those reliably exhibiting eating or drinking in response to ESLH were classified as positive animals, the others as negative. Positive animals were given threshold tests (Bachus & Valenstein, Physiol. & Behav., 23:421-426, 1979) to determine the minimum current capable of evoking S-B behavior.

Results indicated that 56% of the L-E rats showed S-B eating or drinking while only 22% of the Holtzman rats were positive ($\overline{X}^2=10.93$, $p<.001$). Analysis of variance indicated that strain, but not electrode placement or their interaction, was the only significant determinant of the frequency of S-B behavior. Moreover, even though the probability of success was relatively low in the S-D strain, all 10 animals designated as positive at one electrode exhibited S-B behavior at the second electrode site. There were no significant differences in the current threshold for evoking S-B behavior in the positive animals from the two strains. The results support the hypothesis that characteristics of animals associated with strain differences can be a significant determinant of the behavior evoked by ESLH.

182.7 PERIPHERAL LOCUS FOR EPINEPHRINE AND AMPHETAMINE INDUCED ANOREXIA. Michael G. Tordoff,* Donald Novin and Mauricio Russek. Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA90024.

To test whether epinephrine and amphetamine produce their anorexic effects by activation of a peripheral satiety mechanism, each drug was administered to rats with selective vagotomies.

Groups of six rats were subjected to one of the four following procedures: 1. Total subdiaphragmatic vagotomy (TSV), 2. Destruction of the esophageal-hepatic branch of the vagus (EHV) 3. Esophageal-hepatic spared vagotomy (EHSV) which entailed destruction of both vagal esophageal trunks distal to the esophageal-hepatic branch, and 4. A sham operation as a control (SHM).

Commencing 24 days postsurgery and separated by 4 days, intraperitoneal injections of epinephrine chloride (0, 20, 40, 80, 160 µg/kg) were administered in a counterbalanced design. Tests began at the onset of the dark period of the vivarium light:dark cycle (0500:1700) with food and water intakes being recorded 1, 2, 3, and 24 hr later. In SHM and EHV animals, epinephrine administration resulted in a severe dose-related suppression of feeding in the first two postinjection hours. TSV and EHSV groups showed a similar, but significantly less severe, suppression which was clearest at the two highest doses.

Seven days after completion of epinephrine testing, all rats received in counterbalanced order 0, 0.1, 0.2, and 0.4 mg/kg amphetamine sulfate with injections separated by 3 days and consumption measured 0.5, 1, 2, 3, and 24 hr postinjection. As with epinephrine injections, TSV and EHSV groups showed a significantly milder dose-related decrease in food intake than the SHM or EHV groups. This was most apparent in the first postinjection hour after the 0.4 mg/kg dose. No effects of epinephrine or amphetamine were discernable on food intake later than 2 hr postinjection or on water intake at any time.

We interpret these results as evidence that epinephrine and amphetamine stimulate peripheral glucoreceptors, probably by induction of hepatic glycogenolysis. As total destruction of the vagus attenuated, but did not eliminate, the anorexia produced by epinephrine and amphetamine, we conclude that some, but not all, afferents from hepatic glucoreceptors travel via the vagus. The finding that EHV failed to attenuate drug-induced anorexia implies that these fibers are unimportant in glucostatic regulation.

(Supported by NS07687)

182.8 OROSENSORY MECHANISMS AND THE NEURAL CONTROL OF INGESTIVE BEHAVIOR IN THE RAT. M. Jacquin* & H.P. Zeigler, Biopsychology Dept., Hunter Col., CUNY, NY, NY 10021.

To clarify the roles of trigeminal orosensory and gustatory inputs in the neural control of ingestive behavior detailed analyses were carried out in rats subjected to trigeminal orosensory deafferentation (inf., and ant. sup. alveolar; lingual, sphenopalatine nerves) or gustatory deafferentation (chorda tympani, glossopharyngeal, pharyngeal branch of vagus).

Trigeminal deafferentation is followed by a syndrome of behavioral and morphological effects whose magnitude varies with the number of orosensory nerves sectioned. These effects include: (1) Incisor overgrowth and abrasions of the palate, lower lip and tongue; (2) A reduction in responsiveness to perioral stimuli which normally elicit biting, mouth opening and tongue extrusion; (3) Disruption of the sequential organization of eating and drinking and a reduction in consummatory efficiency; (4) A reduction in responsiveness to food and water; (5) A reduction in food intake whose magnitude and persistence vary with the texture of the available diets; (6) A reduction in water intake. The resumption of intake involves the development of alternative modes of feeding behavior (e.g., "scooping") or contorted drinking postures. After trigeminal deafferentation, intake remains insufficient to compensate for weight lost after surgery and the rats remain below control weight levels for prolonged periods. Our analyses indicate that the reduced intake seen after trigeminal deafferentation is not accounted for either by morphological changes in the oral region or decreases in consummatory efficiency but reflect a disruption of neurosensory processes mediating hunger and thirst. Preliminary data suggests that recovery is due to a combination of nerve regeneration and behavioral compensation.

Gustatory deafferentation is followed by a reduction in food intake whose magnitude and persistence vary with the taste of the available diets. Water intake is significantly reduced and weight is regulated below control levels. The effects of gustatory deafferentation are quantitatively and qualitatively different from those seen after trigeminal orosensory deafferentation suggesting a reevaluation of the role of these two sensory systems in the neural control of ingestive behavior.

182.9 INVESTIGATION OF SEROTONERGIC INFLUENCE ON CANCER-INDUCED ANOREXIA M. von Meyenfeldt, W. T. Chance, J. H. James* and J. E. Fischer*. Dept. Surgery, Univ. Cincinnati Med. Ctr., Cincinnati, OH 45267.

Cancer is often paralleled by anorexia and cachexia, which prohibit the use of aggressive and possibly-curative therapies. Our previous research has demonstrated the onset of anorexia 6 days after the injection (im.) of 5 X 10^4 Walker 256 carcinosarcoma cells in 60 to 80 g, female, Sprague-Dawley rats. This anorexia has also been correlated with increased plasma free tryptophan (Trp), brain Trp and brain serotonin (5-HT) turnover. Therefore, the role of CNS 5-HT in mediating cancer anorexia was investigated in 2 experiments employing intraventricular (ivt.) injections of the Trp-hydroxylase inhibitor, p-chlorophenylalanine (PCPA) in tumor-bearing (TB) rats. Two mg of DL-PCPA methyl ester HCl was administered (ivt., 8 ul) bilaterally, 2 days after the im. injection of W. 256 cells (5 X 10^4, n = 8) or saline (NTB, n = 8). Two additional groups (n = 8) received ivt. saline, 2 days after these im. injections. Food intake and body weight were monitored across the next 7 days of ad lib. feeding. The PCPA-TB group ate significantly more than did the S-TB rats on days 5 - 7 following the tumor treatment, with no differences being observed between PCPA-TB and NTB groups across these 3 days. Nine days after the injection of the tumor cells, the rats were sacrificed by decapitation. The brains were removed and blood collected for the fluorometric determination of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and Trp. These biochemical analyses revealed decreased brain 5-HT and 5-HIAA in the PCPA-NTB group, while significant increases in 5-HIAA and brain Trp were observed in the S-TB group. Although plasma total Trp was decreased in both TB groups, free Trp was significantly elevated. In order to assess biochemical changes at a point that more closely coincides with the effect of PCPA, we next investigated the effects of ivt. PCPA treatment on both brain indoleamines and catecholamines (CA) 6 days after the tumor treatment. Two days following the im. injection of saline (n = 16) or 1 X 10^5 W. 256 cells (n = 16), PCPA (2 mg) was bilaterally-administered (ivt.) to half of each group of rats (120 - 160 g). The rats were sacrificed 4 days later, with brain hemisections frozen for the fluorometric determination of 5-HT and 5-HIAA and of CA by high pressure liquid chromatography. S-TB rats exhibited significantly decreased eating 5 days after tumor treatment. By day 6, PCPA-TB rats ate more than did S-TB rats, and there was no difference between groups PCPA-TB and S-NTB. Biochemical analyses indicated significant decreases in 5-HT in both PCPA-treated groups and 5-HIAA in the PCPA-NTB group. Analysis of CA indicated no depletions of norepinephrine or dopamine across any groups. Thus, these data implicate CNS 5-HT derangements in the mediation of cancer-induced anorexia.

182.10 STUDIES OF THE SITE OF ACTION OF NALOXONE IN SUPPRESSING FOOD AND WATER INTAKE IN RATS. Jeffrey G. Jones* and Judith A. Richter (SPON: J.I. Nurnberger), Depts. Pharmacology & Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46223.

Naloxone causes a decrease in certain appetitive behaviors of the rat (Holtzman, J. Pharm. Exp. Ther. 181, 51, 1974). The site of action of naloxone in producing these effects is not known. This abstract summarizes several experiments done in an attempt to elucidate where and how naloxone produces these effects.

Bilateral injections of 15 µg/rat of naloxone into the lateral ventricles of cannulated, food and water deprived rats caused a significant ($p<.05$) decrease in food intake when compared to saline injected controls. Water intake in naloxone-treated animals did not differ significantly from that of saline-treated controls during the one hour tes t period. The total dose of naloxone given centrally, 15 µg, did not produce a change in eating or drinking if given peripherally. This suggests that naloxone exerts its effect on food intake at a central site.

In a second experiment, the effect of i.p. administered naloxone on food and water intake in rats with either a subdiaphragmatic vagotomy (vag) or a sham vagotomy (sham) was evaluated. A dose-related and significant suppression of water intake was seen after treatment with naloxone in doses of 1, 3, and 10 mg/kg in vag and sham rats. Although a significant suppression of food intake was seen in the sham rats, no suppression of food intake was seen in the vag rats at any dose of naloxone tested. This implies that the vagal nerve is necessary for mediation of naloxone's effect on eating.

A third experiment was done to test whether the vagal efferent fibers or the vagal afferent fibers were necessary for mediation of naloxone's effect. In rats pretreated with methyl atropine (5 mg/kg i.p.), naloxone (3 mg/kg i.p.) was equivalent to saline in that it did not decrease food intake during the one hour test period. The naloxone treated group did show a significant decrease in water intake.

These results suggest that the suppression of food intake by naloxone has a central site of action which is mediated by vagal efferents. The results also suggest that naloxone's effect on water intake is mediated by a different mechanism than that involved with food intake.

(Naloxone was generously supplied by Endo Laboratories).

182.11 PERIPHERAL MEDIATION OF OPIATE EFFECTS ON FEEDING IN RATS. G. K. W. Yim, M. T. Lowy*, M. P. Holsapple*, and M. B. Nichols*. Dept. of Pharmacology and Toxicology, Sch. of Pharmacy, Purdue Univ., W Laf IN

The reduction, by naloxone (Lowy et al., Life Sci. 26: 2113, 1980) and by dexamethasone (Lowy and Yim, The Pharmacologist 22: 1980), of 2-DG, food-deprivation (FD), and night feeding suggests opiate involvement in these hyperphagias. These studies were aimed at identifying the type(s) and location of the receptors involved in opiate-mediated feeding.

Three hr daytime rat chow consumption of non-deprived rats were elevated to 3.9 times control intake by 8 mg/kg sc of the µ receptor agonist, morphine sulfate. Feeding was increased to 4.9 times control by 4.0 mg/kg sc of the κ receptor agonist, ketocyclazocine. Meperidine (0.5-32 mg/kg) did not increase feeding. Levorphanol (1.0 mg/kg) elevated food intake to 2.1 times control, while its optical isomer, dextrorphanol (1.0 mg/kg) was ineffective. Loperamide, the peripherally effective opiate agonist which presumably does not pass the blood brain barrier, caused a 2.7 times increase in food intake. Naloxone (0.25 mg/kg sc) caused a 70% reduction of feeding induced by loperamide (10 mg/kg). Single doses of the opiate agonists did not increase 24 hr food intake. Instead, decreases were noted following high doses of some of the agonists. Six hr water intake was elevated to 4.7 times control by 16 mg/kg morphine; to 4.1 times control by 4.0 mg/kg ketocyclazocine; and to 6.7 times control by 1.0 mg/kg levorphanol. Dextrorphanol (0.33-3.0 mg/kg); meperidine (0.5-32 mg/kg); and loperamide (0.33-32 mg/kg) did not increase 6 hr water intake.

The injection of 100 and 400 µg naloxone into the lateral ventricles only decreased one hr FD-induced eating by 41 and 46% respectively. The sc injection of 250 µg of naloxone decreased 1 hr FD eating to the same extent (43%).

These results suggest that opiate mediated feeding involves stereospecific activation of peripheral opiate receptors of the µ and/or κ type. (Supported in part by Pharmacology-Toxicology Training Grant GM-70904, a Purdue Research Foundation Fellowship (to MPH) and an American Cancer Society Grant IN 172).

182.12 SATIETY IS PRODUCED BY GASTRIC NUTRIENT CONTENT. J. A. Deutsch* and M. F. Gonzalez* (SPON: J. S. O'Brien). Department of Psychology, University of California, San Diego, CA 92093.

We have reported previously that satiety signals arise from the stomach and not the duodenum (Deutsch, J. A., W. G. Young, and T. J. Kalogeris, Science, 201:165, 1978). We have also demonstrated that two sets of signals contribute to this regulation. The nature of one of these signals has been shown to be the sheer volume in the stomach that operates at high levels of intake (Deutsch, J. A., M. F. Gonzalez, and W. G. Young, Brain Res. Bull., Suppl. 4, 1980). Here we show that the second signal operates at lower levels of intake and that it is generated by the content of nutrient present in the stomach.

Eleven rats (male, Sprague-Dawley) were implanted with gastric tubes and inflatable pyloric cuffs, and trained to drink a calorically dense liquid diet (Nutrico Standard Research Diet, ICN Pharmaceuticals, Inc.) when 15 h deprived. The mean intake after 15 min was 4.86 ml ± 0.30 ml (SEM). When the animals were counterinjected with normal saline with one half of the volume voluntarily taken by mouth the amount consumed was 4.91 ml ± 0.64 ml (SEM) which was not significantly different from the control level ($t = 0.45$, d.f. = 10, $P > 0.05$). However, when 5 ml were siphoned out of each of the subjects' stomachs, intake was elevated to a mean value of 8.50 ml ± 0.56 ml (SEM) ($t = 6.01$, d.f. = 10, $P < 0.01$). These results show that stomach volume is not a determining factor in the production of satiety at this level of intake.

In a second experiment we permitted seven of the rats to drink normally for 10 min. The pyloric cuffs were then inflated and intake was recorded 15 min later. When the rats were run under control conditions, they consumed a mean of 0.57 ml ± 0.30 ml (SEM) during this period. After a mean volume of 4.14 ml was siphoned off, this value was significantly increased to 3.64 ml ± 0.76 ml (SEM) ($t = 5.3$, d.f. = 6, $P < 0.01$). These results show that at levels where volume is not a factor, the stomach alone (and not the duodenum or the oropharyngeal cavity) generates messages concerning nutrient content that produce satiety.

This research was funded by NSF grant BNS 78-01605 to J. A. Deutsch.

182.13 INTERACTION OF HEPATIC AND CEREBRAL RECEPTORS IN THE CONTROL OF FEEDING STIMULATED AFTER RECOVERY FROM ACUTE GLUCOPRIVATION IN RATS. J. Granneman* and M. I. Friedman Dept. of Psychol., Univ. of Mass., Amherst, MA. 01003

Injections of insulin and 2-deoxy-D-glucose (2DG) stimulate feeding when food is witheld until after recovery of normoglycemia, 6 hours post-injection. Because the acute energy emergency produced by these injections has presumably abated by the time rats are given access to food, we investigated the possibility that enduring alterations in peripheral metabolism might trigger feeding under these conditions. Feeding after recovery from hypoglycemia is associated with depletion of liver glycogen. Intravenous infusion of fructose, a hexose that does not enter the brain, during hypoglycemia inhibited feeding after recovery from hypoglycemia in intact, but not hepatic-vagotomized rats. Further, food intake is highly correlated with liver glycogen under these conditions. These results suggest that fructose may inhibit feeding after recovery from hypoglycemia by antagonizing insulin-induced changes in hepatic metabolism. Hepatic production of glucose, however, is not necessary for delayed access feeding because adrenal demedullation, which abolishes 2DG-induced hyperglycemia and reduces insulin-induced depletion of glycogen, does not reduce feeding. Finally, intraventricular injections of 2DG stimulate food intake in intact and demedullated rats, suggesting that receptors in the brain may be sufficient to initiate feeding in this paradigm.

In summary, the results of these experiments suggest that receptors in the brain may trigger feeding in the delayed access paradigm, and receptors in the liver may inhibit this response. Finally, hepatic production of glucose, or depletion of glycogen per se, are not necessary to trigger feeding after recovery of normoglycemia. (NIH Grant AM-20022)

182.14 FEEDING RESPONSES IN RATS FOLLOWING APPLICATIONS OF MORPHINE TO THE VENTROMEDIAL HYPOTHALAMUS. F.S. Tepperman*, M. Hirst and C.W. Gowdey* Dept. of Pharmacology, The University of Western Ontario, London, Canada.

We have shown that after s.c. injections of several narcotic analgesics into rats, a period of behavioral depression occurred, followed by "stimulated" feeding activity[1], both phases being antagonized by naloxone. It was of interest to determine whether morphine injected into the ventromedial hypothalamus (VMH) would alter food intake. Cannulae were implanted stereotaxically into the VMH of adult male Sprague-Dawley rats which were maintained in a temperature- and light-controlled room for 7 days before infusions (at 1300 hr) of saline, or 4, 8 or 16 nMol morphine or 10, 20 or 40 nMol norepinephrine (NE) in 0.5 µl sterile saline. Food consumption and hourly core temperatures were measured for 3 hr. Drugs were given re a Latin square design on every 5th day with a saline control intervening (day 3). Food consumption was increased significantly after morphine cf. saline, and the amounts eaten were similar to those after NE, but morphine-stimulated feeding was slower in onset and persisted longer. Some morphine-treated rats showed transient behavioral depression preceding the feeding and all seemed more apprehensive during the feeding phase than NE rats. Core temperatures increased after morphine (more than after NE), the rise often persisting for the 3 hr observation period. In another experiment, both 1 and 2 nMol morphine increased core temperature, but only 2 nMol increased feeding. Naloxone (4 mg/kg s.c.) given 30 min before 8 nMol morphine reduced the "stimulated" feeding. Procaine (110 nMol) into VMH had no apparent effect on temperature or feeding. This study suggests that morphine elicits feeding by an action on opiate receptors in the VMH.

(Supported by the Medical Research Council of Canada, grant MA-7278.)

1. Thornhill, Hirst, Gowdey. Can. J. Physiol. Pharm. 57: 1028, 1979.

182.15 LATERAL HYPOTHALAMIC LESION INDUCED ADIPSIA PREVENTED BY A PREOPERATIVE RESTRICTED WATERING. Timothy Schallert. Department of Psychology, University of Texas at Austin, Austin, TX 78712.

Prior to receiving small lesions of the lateral hypothalamic area, rats were adapted to a schedule of daily 30-minute access to water for a period of several weeks, during which time they lost little or no body weight. A group was permitted ad lib access to water and just prior to surgery, was given a slightly reduced amount of food to match the weight loss of rats in the restricted watering group. Another group of rats was adapted to a schedule of daily 1-hour access to food for a period of several weeks, but had ad lib access to water. The body weight of an additional control group was reduced all at once just prior to surgery to match the slight loss observed in rats on the special feeding regimen.

After surgery, rats in the brain damaged control groups were aphagic and adipsic. In striking contrast, the rats adapted to a restricted watering schedule showed no adipsia; indeed, they drank water vigorously and chronically. The rats adapted to a restricted feeding schedule were adipsic but not aphagic. The results suggest that preoperative behavioral and physiological conditioning play a large role in recovery of function after brain damage.

182.16 CHRONIC CEREBROVENTRICULAR INFUSIONS OF ANGIOTENSIN II STIMULATE EXCESSIVE DRINKING BUT NOT SALT APPETITE IN DOGS. C. J. Brown*, T. N. Thrasher*, L. C. Keil* and D. J. Ramsay. Department of Physiology, University of California, School of Medicine, San Francisco, CA 94143 and Ames Research Center, NASA, Moffett Field, CA 94035

A salt appetite can be demonstrated in dogs following dietary sodium restriction. A group of eight dogs were given a choice of water or 0.3 M NaCl, a sodium concentration which was aversive to that population of dogs when sodium replete. The dogs were fed a low sodium diet for 5 days and given furosemide (20 mg im) on each of the first 3 days. This procedure decreased plasma sodium concentration from 142 ± 1 to 132 ± 2 mequiv/l and increased plasma renin activity from 3.5 ± 0.6 to 24.5 ± 1.9 ng/ml/3h by day five. On the 5th day the dogs consumed 406 ± 110 ml 0.3 M NaCl and 261 ± 73 ml water in a 4-h testing period.

A number of maneuvers designed to replicate the raised plasma angiotensin II and mineralocorticoid levels characteristic of sodium depletion were tested on salt appetite in sodium-replete dogs. Intravenous infusion of angiotensin II (20 ng/kg/min) in normal dogs, and in dogs pretreated for 5 days with either DOCA (30 mg/day) or aldosterone (5 mg/day) drank water but no salt. Similarly, peripheral administration of renin, acutely or chronically, failed to induce salt appetite.

A vigorous salt appetite has been shown to follow chronic, central administration of angiotensin II in rats. Dogs were prepared with chronic third ventricular cannulas. Angiotensin II (1 µg/kg/h at 1 µl/h) was infused for 7 days using osmotic minipumps (Alzet) and the dogs were offered a choice of water and either 0.3 or 0.45 M NaCl. A maintained, greatly increased water intake was observed for the seven day period. The dogs drank volumes of water between 50 and 100% of their body weights per day. This treatment reduced plasma sodium concentration between 20-25 mequiv/l and suppressed plasma renin activity and plasma vasopressin. Thus although central administration of angiotensin II acutely stimulates vasopressin secretion, it is not maintained, presumably due to dilution and expansion of the extracellular fluid. There was no evidence of salt appetite when compared to vehicle-infused controls.

Thus sodium-depleted dogs show a salt-appetite. This cannot be reproduced by either central or peripheral administration of angiotensin II to the salt-replete animal.

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182.17 INSULIN INDUCES INCREASED MILK INTAKE BY SUCKLING RATS IN ABSENCE OF GLUCOPRIVATION. Christina L. Williams and Elliott M. Blass. Dept. Psychol., The Johns Hopkins Univ., Baltimore, MD 21218.

In adult animals insulin-induced glucoprivation is associated with an increase in food intake. Recently, R.C. Ritter et al. (Am. J. Physiol., 234:E617, 1978) have demonstrated that if food is returned to adult rats 6 hr after regular insulin administration, intake is elevated even though normoglycemia has been restored. Utilizing new techniques for studying milk intake during suckling and independent feeding in 5- to 20-day-old rats, we investigated the effects of insulin on these two forms of ingestive behavior 2 hr (during glucoprivation) and 6 1/2 hr (when normoglycemia was restored) after insulin treatment.

Consistent with earlier reports (Houpt et al., Am. J. Physiol. 225:58, 1973; Lytle et al., J. Comp. Physiol. Psychol., 77:388, 1971) we found that 8 hr food and suckling deprived 5- to 20-day-old rats did not increase their milk intake when the dam was returned 2 hr after insulin administration (.5-1.0 U/kg, s.c.); a time when pups' blood glucose levels were low. However, when the dam was returned to 8 hr deprived pups 6 1/2 hr after insulin administration, their milk intake was increased by 35-45% over saline-treated controls. This occurred when pups sucked milk from the nipples of an anesthetized dam that was periodically (once every 4 min for 40 min) injected i.v. with oxytocin (.002 U/inj.). Twenty-day-old pups treated with insulin and tested 6 hr later with another suckling test protocol (pups receiving milk through a tongue cannula while they sucked from their dam's nipples) actually consumed more milk ($\bar{X}$=10.0±.6% b.wt.)than saline-treated controls ($\bar{X}$=7.5±.5% b.wt.)before they reached satiety and stopped suckling. Although insulin treatment increased the rate and total volume of milk intake in suckling rats, the treatment had no effect on pups' latencies to attach to their mothers' nipples.

In contrast, insulin did not increase milk intake when pups ingested food independently of the dam. Regardless of whether insulin was given 2 or 6 1/2 hr prior to milk presentation (either through a tongue cannula or in a bowl) intake did not increase.

These data suggest: 1) Insulin can modulate the intake of young rat pups when milk is ingested by sucking, 2) This modulation only occurs after normoglycemia has been restored, and 3) Since insulin only alters the milk intake of pups when they are suckling and not when they are feeding independently of the dam, the physiological controls of these two ingestive behaviors may be different.

182.18 LATERAL HYPOTHALAMIC (LH) LESIONS PRODUCE EXTENSIVE BLOOD BRAIN BARRIER (BBB) DAMAGE AND CEREBRAL EDEMA. Peter H. Cooper[1], Donald Novin[1,2], and Larry L. Butcher[1,2]; Department of Psychology[1] and Brain Research Institute[2], University of California, Los Angeles; CA, 90024; U.S.A.

A large literature exists demonstrating that brain lesions disrupting the BBB allow for extravasation of serum proteins and for the development of cerebral edema (e.g., Olsson & Hossman, Acta Neuropath. 16: 103, 1970). Evans Blue dye (EB) has been found to be a particularly good marker for the extent of cerebral edema, and horseradish peroxidase (HRP, Sigma Type II) has been used similarly to assess BBB damage. Because of possible relevance for studies of eating and drinking, unilateral LH lesions were made electrolytically in rats. At various times after the ablations, either HRP (50 mg/kg) or EB (40 mg/kg) was administered intravenously. The brains were subsequently examined histologically for BBB involvement.

Two days after lesion induction, there was massive leakage of EB and HRP into the brain parenchyma surrounding the ablations, in some cases approximately 4 mm from the border of cavitation. Apparent BBB damage was seen dorsally in the ventrobasal thalamus, laterally in the internal capsule and in some cases in the adjacent cortex, rostrally in the ventral striatum, and caudally in the anterior substantia nigra and adjacent structures. Three to five weeks after LH ablations, as the volume of the electrolytic lesions decreased (Wolf & DiCara, Exptl. Neurol. 23: 529, 1969), the presence of EB and HRP in the tissue surrounding the cavitation was markedly reduced compared to that seen at shorter intervals, suggesting repair of the BBB.

Cerebral edema may contribute to the ingestive deficits of the LH syndrome by functionally impairing tissue not physically destroyed by the lesions. Repair of the BBB and reduction of edema may be a factor in recovery of function after brain damage. Preliminary evidence indicates that intracerebral administration of 6-hydroxydopamine also produces cerebral edema. As with electrolytic LH lesions, such edema may contribute to the total constellation of pathologic involvement after lesions produced by aqueous solutions of cytotoxins. [Support: USPHS NS 10928 to L.L.B., NS 7687 to D.N. and MH 15795 to P.H.C.]

182.19 QUANTITATIVE BEHAVIORAL ANALYSIS OF NEUROPEPTIDES WHICH SUPPRESS FOOD INTAKE. J. Gibbs, L. Gray*, C.F. Martin*, W.T. Lhamon and J.A. Stuckey*. Cornell University Medical College and Bourne Laboratory, The New York Hospital, New York, NY 10605.

A growing list of brain-gut peptides has been reported to suppress food intake in rats after systemic administration. In order to determine if the suppressive effect of each peptide was due to a specific action on feeding behavior, we devised a method for reliably rating all behaviors emitted by rats at a test meal. The behavior of each rat was observed during and only during a 0.6 sec 'window' which was signalled by the occurrence of a low-volume background tone played regularly once every two sec by a prerecorded tape. During the 1.4 sec interval between each successive tone, one of 18 defined behaviors was recorded for later analysis; if no behavior could be instantaneously identified, none was recorded. Inter-rater reliability was examined by determining the rate of agreement between three individuals observing 12 rats eating a test meal: Correlations between scores by the three observer pairs were 0.91, 0.91 and 0.92. Test-test reliability was examined by determining the rate of agreement between scores by one individual observing 11 rats eating test meals on consecutive days: There were no statistically significant differences in scores across days.

Twelve adult male Sprague-Dawley rats were deprived of food but not water from 1000 to 1300 h. At 1300 h they were injected intraperitoneally with one dose of one peptide dissolved in 0.15M NaCl, or equivolumetric 0.15M NaCl as a control, and immediately offered liquid food (25% EC116, GIBCO) for 60 min. Cumulative food intakes were recorded at intervals during the test; behavior was rated as outlined above. Substance P (SP; 1-200 μgkg^{-1}), neurotensin (NT; 1-100 μgkg^{-1}), thyrotropin-releasing hormone (TRH; 10 μgkg^{-1}) and bombesin (BBS; 8 μgkg^{-1}) each produced potent suppressions of food intake; somatostatin (1-10 μgkg^{-1}) and pancreatic polypeptide (50-100 μgkg^{-1}) did not. Suppressions of food intake by SP, NT and TRH were always accompanied by abnormal behaviors (e.g. coarse wheezing, arching of back, 'treading' of forepaws after SP and NT) or disruption of the normal distribution of behaviors (e.g. after TRH, the percentage of behaviors represented by apparent sleep shifted from 71±3% to 24±6%, while grooming shifted from 4±1% to 24±3%. No abnormal behaviors or significant change in distribution of behaviors accompanied the suppression of feeding after BBS. These results: (1) support a satiety role for BBS, but not for SP, NT and TRH, and (2) indicate that a systematic behavioral assessment is required to evaluate putative satiety signals. (Supported by NIAMDD grant AM17240 and an Irma T. Hirschl Career Scientist Award.)

182.20 MIF-I SUPPRESSES DEPRIVATION-INDUCED WATER CONSUMPTION IN RATS. R. D. Olson, A. J. Kastin, G. A. Olson, B. M. King, T. K. von Almen*, M. C. Berzas*, M. L. Ibanez, and D. H. Coy*. Dept. of Psychology, University of New Orleans, New Orleans, LA 70122

To study the effects of several neuropeptides on fluid intake across five levels of incentive motivation, rats were deprived of water and given 8 grams of food for 12 hours prior to testing. Animals were given a 0.1 mg/kg i.p. injection of either MIF-I, (D-Ala^2, F$_5$Phe^4)-Met-enkephalin-NH$_2$, D-Phe^4-Met, enkephalin, dynorphin, naloxone, or the diluent vehicle, placed in their home cage for 10 min., and then given ad lib. access to the test solution in a repeated measures design with solutions counterbalanced over five days. The solutions included 20% sucrose, 10% sucrose, water, 0.01% quinine, and 0.02% quinine. Water consumption was measured every hour for 4 hours.

A mixed analysis of variance yielded significant results for all main effects and the peptides by water and hours by water interactions. For the 4-hr. test period, naloxone and D-Phe^4-Met-enkephalin produced reliable increases in water consumption while MIF-I produced a reliable decrease. These differences were obtained only with sucrose solutions, which were consumed in reliably greater amounts than the other solutions. Most fluid was ingested in the first hour for the sucrose and control fluids but no differences existed for the quinine solutions. The data clearly indicate that peptides modulate fluid consumption at positive levels of incentive motivation.

In addition to the highly reliable decrease produced by MIF-I, the increase produced by naloxone was of interest as we had some previous data to suggest that MIF-I and naloxone might act in a comparable fashion. To reconcile these findings, 0.1, 1.0, and 10.0 mg/kg of naloxone and MIF-I were administered as before but to independent groups of rats and intake measures were taken every 30 min. These data replicate and extend the above findings by showing that during the first 30-min. both naloxone and MIF-I suppress intake in a dose-dependent fashion, with MIF-I being more effective at each dose. The 0.1 mg/kg naloxone group increased consumption over time, however, and achieved a total consumption greater than that observed in the control animals. Perhaps the low dose generates a rebound effect during the 4-hr. testing period while a comparable phenomenon might occur with higher doses more than 4 hours after the injection. The two higher doses of naloxone and all doses of MIF-I produced marked suppression during the entire 4 hours. It appears that at very low doses naloxone increases consumption but at more commonly tested higher doses it has a suppressant effect. The very similar results produced by naloxone and MIF-I suggest that these substances might have a common course of action. Finally, in this experimental paradigm, MIF-I acted like naloxone.

182.21 PARALLEL CHANGES IN CONSUMMATORY BEHAVIOR AND PREABSORPTIVE INSULIN RELEASE FOLLOWING TASTE-LiCl ASSOCIATION. Kent Berridge*and Harvey J. Grill
Dept. Psychol., Univ. of Pennsylvania, Philadelphia, Pa. 19104

Oral infusions of glucose (2.53 M) concomitantly elicit an observable ingestive response and a preabsorptive insulin release (PIR). Plasma insulin levels begin to rise during the first min. and reach a mean peak of 1.5 ng/ml over baseline by the 4th minute. Conversely, oral quinine evokes behavioral rejection and a much smaller PIR (0.1 ng/ml). Following a single pairing of novel glucose and LiCl i.p., glucose no longer elicits an ingestive response but instead produces a quinine-like rejection. Our purpose was to examine whether the association of glucose with LiCl would inhibit the reflex PIR to glucose. Twenty-nine male rats (300-400g) were implanted with chronic oral and jugular cannulae. Two groups of four rats each were given 1 min. oral infusions of glucose and 0.2 ml blood samples were taken at -3,0,1,2,3,4,6 and 8 min.; the behavior of the rats was simultaneously videotaped. Following the infusion rats were given 0.15 M LiCl i.p. (1 ml/100g). This procedure was repeated on the next two days, except that LiCl was omitted on the last day. Duplicate plasma glucose (10 μl) assays and radioimmunoassays for insulin (20 μl) were performed. Presentation of oral glucose to naive rats (n=4) produced a normal PIR and elicited behavioral ingestion. A single pairing of oral glucose with LiCl eliminated the PIR to oral glucose and changed the behavioral response to vigorous rejection. These altered autonomic and somatic responses resemble those given by naive rats to oral quinine (.003 M; n=5). The glucose finding was replicated in a second experiment (n=4). These results cannot be explained by blood glucose dependent changes in insulin levels. Control experiments also exclude explanations invoking direct effects of LiCl on pancreatic function (n=6) or response habituation due to repeated exposures to oral glucose (n=4). These results demonstrate that the PIR to taste is susceptible to modification by experience and suggest a parallel relationship between autonomic and behavioral responses to tastes. (Supported by NIH grant AM-21397-03 and by the Diabetes Center, University of Pennsylvania.)

182.22 CHANGES IN TRIGEMINAL MOTOR NEURONAL EXCITABILITY CAUSED BY GLOBUS PALLIDUS LESIONS. F.J. Denaro*, J.S. Schneider and T.I. Lidsky (SPON: J.S.Stamm). SUNY,Stony Brook, N.Y.11794

The trigeminal system, because it controls jaw movement, is intrinsic to eating and drinking behaviors. These behaviors are severely disrupted by globus pallidus (GP) lesions. Although it is known that such damage produces a variety of sensorimotor problems, it is not clear the extent to which the trigeminal system is affected. The present experiment was intended to provide some of this information by assessing trigeminal functioning before and after GP lesions.

The excitability of trigeminal jaw closure motor neurons was measured in chronically prepared rats. Stimulating electrodes were implanted in the trigeminal mesencephalic nucleus (Mes 5). Mes 5 contains the cell bodies of 1A afferents which monosynaptically excite jaw elevator motor neurons. The threshold of the jaw closure reflex which results from electrical stimulation of Mes 5 served as an index of motor neuronal excitability. Reflex thresholds were calculated before and after the production of GP lesions. These lesions caused a dramatic drop in threshold indicating that these motor neurons became hyperexcitable. This effect lasted at least several weeks and persisted well after the lesion-induced aphagia and adipsia had dissipated.

Previously, we have shown that GP stimulation modulates the processing of sensory information originating from facial and perioral tissues (Labuszewski and Lidsky, Exp. Neurol., 1979, 65: 471-477). Numerous investigators have demonstrated that sensory information from these areas can have potent influences upon the excitability of trigeminal motor neurons (e.g. Sessle, Exp. Neurol., 1977, 54: 323-339). Taken together, these two findings make plausible the possibility that the GP lesion-induced increase in trigeminal motor neuronal excitability is due in part to altered influences by facial and perioral somatosensory inputs. To test this possibility, the effect of blocking somatosensory influences upon the jaw closure reflex was tested in normal and GP lesioned rats. Local anesthetization of the face caused no or a slight elevation of threshold in intact rats. In contrast, local anesthetization caused a striking increase of threshold in GP-lesioned rats.

These data indicate that the GP influences those components of the trigeminal system which control jaw movement. Moreover, one way in which this influence is exerted is via regulation of a somatosensory stimulus' access to motor neurons.

182.23 CONTINUOUS ENDORPHIN BLOCKADE DECREASES FEEDING AND DRINKING. Vicki J. Merriman,* & Larry D. Reid, Dept. of Psych., Rensselaer Polytechnic Institute, Troy, New York 12181.

This study follows earlier work of the effects of opioid antagonists on motivated behaviors. Naltrexone hydrochloride (NTX), for example, reduces intake of water among rats while hardly modifying responding for intracranial stimulation (ICS) (Cruz-Morales, Merriman, & Reid, Eastern Psychol. Assoc. Meeting, 1980; Maickel, Braude, & Zabik, Neuropharm., 1977). In this study rats were chronically administered NTX (across 6 days) either by periodic injections or by way of subcutaneously (sc) implanted osmotic minipumps for continuous delivery of small quantities of drug (Alza Corp.) while daily intakes of food and water were measured.

Five groups, of 8 male Sprague-Dawley rats each, were given a different drug regime. Four doses of NTX were delivered via the minipumps, with doses computed as 0.0, 0.068, 0.27, and 1.08 mg/day. The 5th drug condition was a daily sc-injection of NTX, 4.0 mg/kg/day, a dose equivalent in mg/kg to the highest minipump-dose. Subjects were weighed and food and water measurements were taken once a day. After 3 days of baseline, all subjects were anesthetized with ether and underwent a sham or actual implantation of a minipump. After 6 drug days, the pumps were removed, followed by 3 days of post drug baseline. Throughout the study, patterns of drinking were monitored using a drinkometer circuit.

Food intake was decreased by about 2, 10, 18, and 22% compared to predrug baseline for the 4 minipump-doses (0 to 1.08 mg/day) and <1% for the sc-dose, respectively. Water intake was decreased by <1, 2, 15, and 15% respectively, with the minipump-doses and <1% with the sc-dose. NTX of .27 and 1.08 mg/day reliably decreased both food and water intake ($ps < .05$), while the 0 minipump- and sc-doses produced insignificant decreases. Therefore, small doses, continuously delivered, successfully decreased consummatory behavior, while an equal or larger dose given as an acute injection did not.

These data fit into a growing body of evidence indicating that different antagonists of morphine-endorphin modify different kinds of motivated behavior. For example, NTX and naloxone reduce feeding and drinking (NTX in remarkably small doses) while large doses of NTX do not reliably modify pressing for ICS. Naloxone decreases and Win 44,441 increase pressing for ICS. Nalorphine reduces deprivation-induced drinking but does not reduce ad lib. intake of sugar-water. It is inferred that different opioid systems are links in the various motivational schema.

182.24 LESIONS OF THE SUBFORNICAL ORGAN INHIBIT ANGIOTENSIN-INDUCED DRINKING IN THE DOG. M. H. Reed*, T. N. Thrasher*, J. B. Simpson, L. C. Keil* and D. J. Ramsay. Department of Physiology, University of California, School of Medicine, San Francisco, CA 94143 and Ames Research Center, NASA, Moffett Field, CA 94035

The sub-fornical organ (SFO) has been implicated in many of the centrally mediated actions of circulating angiotensin in the brain. In particular, injections of angiotensin II directly into the SFO cause drinking, vasopressin secretion and an increase in blood pressure in rats. In the present study, a technique was devised to make lesions of the SFO in dogs. The effect of such lesions on drinking and vasopressin secretion brought about by angiotensin and hypertonic saline was studied.

Lesions were made with the dog placed in a stereotaxic apparatus. A 20 gauge cannula was placed in the III ventricle and 0.5 ml contrast medium injected. From the X-ray, precise coordinates for placing the lesioning electrode in the SFO were calculated. The position of the lesioning electrode was checked with a second X-ray and altered if necessary. After all observations had been made, the brain was fixed in formalin by perfusion, removed and later stained for Nissle substance. In the initial series of 12 dogs, complete destruction of the SFO was attained in 5 dogs.

The drinking response to angiotensin II (20 ng/kg/min, i.v.) was reduced from 222 $\pm$ 65 ml to 27 $\pm$ 24 ml over a 30 min test period following SFO lesions. When retested one month later, the drinking response to angiotensin II was still absent (20 $\pm$ 20 ml). Thus, the effect of the lesion was sustained for at least one month and the drinking response to angiotensin showed no tendency to recover. In contrast, drinking responses to 0.85 M NaCl were not affected by the lesion (315 $\pm$ 92 before and 269 $\pm$ 70 ml after the lesion). However the latency to drink was significantly lengthened from 10 $\pm$ 3 to 22 $\pm$ 3 min. Whether this increase in latency indicates that the SFO is involved in drinking responses to osmotic stimulation is uncertain.

The effect of SFO lesions on vasopressin responses to angiotensin and 0.85 M NaCl is more variable. This is due in part to the inhibitory effects of drinking itself on vasopressin secretion. However, it appears that complete lesions of the SFO suppress the capacity of brain to respond to these agents.

In summary, in the dog the drinking response to circulating angiotensin, but not to hypertonic saline, depends on the integrity of the SFO.

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182.25 SPECIFIC INCREASE IN CARBOHYDRATE CONSUMPTION AFTER NOREPINEPHRINE (NE) INJECTION INTO THE PARAVENTRICULAR NUCLEUS (PVN). J.R. Tretter* and S.F. Leibowitz. Rockefeller Univ., New York, NY 10021. (SPON: R. L. Thompson)

While the phenomenon of increased feeding after PVN noradrenergic stimulation is believed to reflect a physiologically active system, little is known about the specific role this system has in the feeding process. The present study examined a possible function of NE in controlling the animals' elective consumption of particular nutrients. With the use of a self-selection feeding paradigm, results revealed that noradrenergic stimulation of the PVN, while increasing total food intake, also produces a specific enhancement in the animal's preference for carbohydrate, thus implicating NE is the process of balancing dietary nutrients.

Rats (350 g) with PVN cannulas were maintained and tested on one of several sets of 2 diets. These diets, simultaneously available, consisted of either: 1) lab chow powder mixed with various proportions of sucrose versus fat (Crisco or corn oil used with mineral oil to make diets isocaloric), or 2) pure protein (casein), starch (dextrin or corn starch), or sugar (sugar or dextrose), or various combinations of these constituents, mixed with corn oil, vitamins, minerals, L-cystine, and in a few cases cellulose to equate caloric density. These diets were tested under two different paradigms: a) The 2 diets were available ad lib; the drug or vehicle control tests, lasting 1 hr, were conducted in the day when a small amount (1-4 g) of spontaneous eating occurred. b)The food was restricted to a 6-hr period during the day. The drugs or vehicles were injected 1 to 3 times throughout this period, and food intake was measured hourly.

The basic phenomenon, that PVN noradrenergic stimulation specifically increases preference for carbohydrate (sweet or non-sweet), received preliminary support from initial experiments showing that NE elicited consumption of sugar cubes and 20% sucrose water but not saccharin water, and that NE increased preference for sugar cubes over lab chow pellets. The two main and more extensive experiments, using self-selection feeding paradigms and diets described above, yielded comparable results, although the 6-hr feeding schedule permitted more careful and continuous minitoring of the animals' total daily food intake pattern and generally revealed more stable effects. With all the various diets tested, PVN injection of NE consistently increased the rats' elective consumption of carbohydrate relative to other dietary constituents, and this occurred whether the carbohydrate was sweet or non-sweet. No differential effects on intake of protein or fat were observed. These results are consistent with those obtained with the α-adrenergic agonist clonidine (Fahrbach et al., this meeting), and preliminary experiments suggest a similar increased carbohydrate preference after injection of tricyclic antidepressants.

182.26 LOCAL INJECTION OF MORPHINE OR AN OPIATE PEPTIDE INTO THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS ELICITS FEEDING S. McLean* and B. G. Hoebel. Prog. in Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08540.

Bilateral injections of morphine (2ug) or D-ala^2-met^5-enkephalinamide (DALA) (2ug, 4ug, and 8ug in 0.3ul) into the paraventricular nucleus of the hypothalamus elicited feeding in satiated rats. The 2ug dose of morphine produced a mean intake of 2.5 gm. of food pellets in the second hour after injection, compared to a mean of 0.5 gm. during the same time period after saline control injection. DALA increased food intake in a dose dependent manner. Morphine elicited feeding with a latency of 60-90 minutes; DALA initiated feeding within 30-60 minutes. Morphine's effect appeared to be receptor mediated because local injection of naloxone, 40 minutes following morphine, attenuated the feeding induced by morphine. It is concluded that exogenous opioids injected into the paraventricular nucleus can induce food intake with a long latency.

183.1 ACTION OF CYCLIC AMP ON APLYSIA NEURON R15. S. N. Treistman. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We have previously reported that a long-lasting synaptic hyperpolarization produced in cell R15 after stimulation of the branchial nerve is augmented in the presence of the phosphodiesterase inhibitor, theophylline. Also, intracellular injection of guanylylimidodiphosphate (GMP-PNP), an activator of adenylate cyclase, causes a long-lasting hyperpolarization in R15, further suggesting that the synaptic event may be mediated by cyclic AMP. In this report, the biophysical effects of branchial nerve stimulation and cyclic AMP elevation are compared, in an attempt to further test the possibility that cAMP mediates the synaptic event, and to explore the conductance mechanisms that underlie the nucleotide action.

Maximal stimulation of the branchial nerve silenced R15 and the quasi-steadystate I-V plot indicated an increased slope conductance in response to hyperpolarizing pulses plus reduction or abolition of the negative slope resistance region (NSR) normally present in R15. By varying stimulus parameters, it was possible to activate inputs which acted primarily to decrease NSR, increase slope conductance, or both.

Cyclic AMP was elevated by four different routes: 1) perfusion with cAMP derivatives, 2) intracellular injection of cAMP derivatives, 3) intracellular injection of cAMP after perfusion with the phosphodiesterase inhibitor RO-1724, and 4) intracellular injection of guanylylimidodiphosphate (GMP-PNP). All caused burst reduction or silence in R15, and the I-V plot in all cases showed an increased slope conductance in response to hyperpolarizing pulses. The NSR was generally not reduced, except after GMP-PNP injection, where a reduction of NSR often occurred. Thus, cyclic nucleotide action in R15 appears to result mainly from an increased conductance, presumably to potassium ions, and it is this component of the synaptic response which is most likely to be mediated by cAMP in R15. The nucleotide-induced response in R15 is not due to a spike-related event such as an increased Ca^{2+}-mediated K^+-conductance since GMP-PNP injection into a clamped cell resulted in a silent cell when the clamp was removed, even though no action potentials had occurred.

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183.2 ACTIVATION OF ADENYLATE CYCLASE BY A SUBSTANCE OF PROTEINOUS NATURE FOUND IN RAT SPINAL CORD. S. T. Bissen* and T. Ueda. Mental Health Research Institute and Dept. of Pharmacology, The Univ. of Michigan, Ann Arbor, MI 48109

We have recently described that the synapse-specific proteins, Proteins Ia and Ib, and adenylate cyclase are enriched in cervical and midlumbar segments of the rat spinal cord (Ueda, Stratford, and Larson, 1980; J. Neurochem., in press). The adenylate cyclase activity in the cell-free system of the spinal cord was not affected appreciably by the putative neurotransmitters dopamine, norepinephrine, serotonin, and histamine (0.1 mM), when assayed under the incubation conditions which allowed dopamine to stimulate adenylate cyclase in caudate nucleus (Kebabian, Petzold, and Greengard, 1972; Proc. Nat. Acad. Sci. 69, 2145). We now report that acid extracts of the spinal cord of matured rats contain a factor(s) of proteinous nature that can stimulate as much as 8-10 fold adenylate cyclase in the cell-free preparation of the cord. The stimulatory action of the factor(s) does not require exogenously added GTP or Ca++. The ability of the factor(s) to stimulate the adenylate cyclase is lost upon treatment with proteolytic enzymes but not with lipolytic or nucleolytic enzymes. Moreover, the activating substance is not dialyzable and is thermolabile under acidic and neutral conditions, suggesting that the substance is not a small peptide. The purification and further characterization of the substance are now in progress.

Supported by NIH Grant NS 15113.

183.3 ISOPROTERENOL EFFECTS ON CORTICAL ADENYLATE CYCLASE IN CA^{++}_1-SATURATED MEMBRANES. Scott R. Whittemore*, Yigal H. Ehrlich†, and Edith D. Hendley, Dept. of Physiol. & Biophys. and †Dept. of Psychiatry & Biochem., Univ. of Vermont College of Medicine, Burlington, VT. 05405.

Neurohormonal effects on adenylate cyclase (AC) have been examined in the absence of endogenous Ca^{++} since von Hungen and Roberts (Nature New Biology, 242:58, 1974) demonstrated enhanced epinephrine stimulated AC in the presence of EGTA. However, the interpretation of these findings in relation to physiological function is complicated as Ca^{++} is present intracellularly in micromolar concentrations. This study was initiated to investigate the interactions of neurotransmitters and Ca^{++} ions in mechanisms regulating AC activity. The effects of Ca^{++} on cortical AC have been reported to be dependent on calmodulin (CaM). Therefore, we have prepared fractions containing synaptic membranes from rat cerebral cortex, by osmotically shocking synaptosomes in the presence of 50μM $CaCl_2$. Using this procedure it has been shown that the membranes support endogenous Ca^{++}/CaM-dependent protein phosphorylation without addition of exogenous CaM (Ehrlich et al. J. Neurochem. vol. 5 (in press) 1980, Life Sci. 26:1965, 1980. Thus, these membranes contain bound CaM. Other experiments were carried out with excess EGTA (2mM) in the homogenization and osmotic-shock buffers. Basal AC activity produced 78 picomoles cAMP/min/mg protein. GTP (10μM), (-)-isoproterenol (ISO) (100μM), and ISO plus GTP stimulated basal activity 30%, 20%, and 100% respectively. Basal activity in membranes used with Ca^{++} buffer was 3-5 fold greater than that seen with EGTA prepared membranes and GTP always resulted in increased AC activity, as expected from the report of Brostrom et al. (Arch. Biochem. Biophys., 191:341, 1978). Addition of 100μM ISO produced small (13.9±5.4%) but consistent ($p<.05$, n=7) decreases in AC activity. This decrease in AC activity induced by ISO in Ca^{++} saturated membranes occurred in the absence of exogenously added GTP. AC activity in these membranes in the presence of GTP and ISO was always greater than basal activity. However, the effects of ISO in the presence of added GTP were variable. Out of 7 experiments, ISO had no effects in two, produced an increase in AC activity in two experiments, and a decrease in GTP stimulated AC in the remaining three. These results serve to demonstrate the complexity of interactions between various factors that affect adenylate cyclase activity in the presence of physiological concentrations of Ca^{++} ions, and indicate the need for further and more detailed characterization of this system. These experiments are in progress. Supported by Grants DA02747 from NIDA and PHS RO1-25811.

183.4 THE EFFECT OF CALMODULIN ON THE RESPONSE OF RAT CAUDATE TO CALCIUM AND DOPAMINE. M.T.Piascik*, R.J.Hitzemann* and J.D.Potter*. (SPON: G. Eckstein). Dept. of Pharmacol. and Cell Biophysics and Dept. of Psychiatry, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267

Recently it has been proposed that calmodulin may participate in dopamine receptor supersensitivity in the rat straitum (Gnegy et al., J.P.E.T. 202, 558, 1977) by making the striatal adenylate cyclase more sensitive to dopamine (Gnegy and Treisman, Fed. Proc. 39, 516, 1980). In the present study we have examined the effects of purified calmodulin on the basal and dopamine stimulated adenyl cyclase found in rat striatal microsomes. Cortical microsomes were used as a control. Both brain regions contained a basal adenylate cyclase which responded in a biphasic manner to changes in free Ca^{2+} concentration, $[Ca^{2+}]$, (half maximal stimulation=0.2μM $[Ca^{2+}]$, half maximal inhibition=0.8μM $[Ca^{2+}]$).However, the basal striatal adenylate cyclase was stimulated only two fold while the basal cortical adenylate cyclase was stimulated seven fold. These data suggest that the striatum contains an adenylate cyclase which,relative to cortex,is less responsive to Ca^{2+}.The lower Ca^{2+} dependent stimulation was not due to loss of calmodulin during preparation since addition of excess calmodulin (60μg/mg protein) resulted in only a 2.5 fold stimulation of basal adenylate cyclase. Although the adenyl cyclase of the striatum is relatively unresponsive to Ca^{2+} and calmodulin, the work of Gnegy et al. suggests that these modulators should play an important role in regulating the cyclase response to dopamine. Our data suggest otherwise. At 0.2μM $[Ca^{2+}]$, the dopamine EC_{50} was not changed by the addition of excess (64μg/mg striatal membrane) calmodulin. Depletion of calmodulin from these membranes by washing with 0.1 mM EGTA resulted in a significant loss in the sensitivity of the striatal cyclase to Ca^{2+} but did not change basal activity. Furthermore, the EC_{50} to dopamine was not changed in these membranes (EC_{50}=5μM) when compared to control (EC_{50}=6μM). In conclusion our data do not support the concept that calmodulin regulates the sensitivity of the striatal adenyl cyclase to dopamine. Thus it is unlikely that a change in calmodulin levels is responsible for the supersensitivity of the dopamine stimulated adenyl cyclase. Supported by grants from the NIH (HL22619-IIIA,E; HL07382, MH25487, NS16061),and the American Heart Association (78-1167).

183.5 MUSCARINIC POTENTIATION OF DOPAMINE STIMULATED CYCLIC AMP ACCUMULATION IN RAT RETINA J.H. Brown and M.G. Rietow*, Division of Pharmacology, University of Calif. San Diego, La Jolla, CA 92093

Muscarinic agonists are among a group of agents previously thought to have no effect on adenylate cyclase, but more recently demonstrated to cause inhibition, particularly of hormone-stimulated adenylate cyclase. We have examinied potential interactions between dopamine (DA) and the choline ester, carbachol (CARB) on cyclic AMP accumulation in the retina. Intact retinas from adult rats were incubated in oxygenated Krebs Henseleit buffer pH 7.4 at 35°C, in the presence of 100 μM phosphodiesterase inhibitor, isobutylmethylxanthine, and then exposed to hormones for 2 min, frozen and assayed for cyclic AMP by competitive protein binding. DA (50 μM) increased the cyclic AMP concentration from 17 to 45 pmol/mg prot. CARB (30 μM) caused a smaller but significant increase in cyclic AMP to 27 pmol/mg protein. When the two agents were tested in combination they were found to be synergistic. Thus while additivity would predict that CARB plus DA produce a 30% greater response than DA alone, the response to DA was instead almost doubled in the presence of CARB. Two pieces of data indicate that this effect of CARB is mediated via muscarinic receptors: it was completely antagonized by atropine (10 μM) and it was mimicked by the muscarinic agonist oxotremorine (OXO). OXO (10 μM), like CARB, caused marked potentiation of the response to DA. Retinas depolarized by incubation in 57 mM K^+ medium also showed increased responsiveness to DA. This suggested the possibility that muscarinic effects on adenylate cyclase were mediated indirectly via release of another neurohormone. However, CARB still increased cyclic AMP and synergised with DA in retinas incubated in calcium-free, EGTA containing medium for 20 min, making a calcium dependent release unlikely. In brain slices adenosine (ADO) is released by depolarization and potentiates the effects of amines on cyclic AMP formation. ADO (100 μM) did not mimic the effects of CARB on basal or DA stimulated cyclic AMP formation in the retina. The data suggests that the acetylcholine containing cells of the retina may function to modulate aspects of dopaminergic neurotransmission mediated through adenylate cyclase. The molecular mechanisms that underly the dopaminergic-cholinergic interactions shown here remain to be elucidated.

This work was supported by USPHS grant HL 24441 from the NIH.

183.6 EFFECTS OF MUSCARINIC AND NICOTINIC AGONISTS AND ANTAGONISTS ON BRAIN REGIONAL CYCLIC NUCLEOTIDES. J.L. Meyerhoff, G.J. Kant, R.H. Lenox, L.L. Pennington* and D.R. Collins*. Dept. of Med. Neurosciences, Walter Reed Army Inst. of Res., Wash., DC 20012 and Dept. of Psychiatry, Univ. of Vermont, Burlington, VT 05405

We have previously reported that cholinergic agonists elevate cyclic AMP and cyclic GMP in several rat brain regions in vivo (R.H. Lenox, G.J. Kant, and J.L. Meyerhoff, Neurosci. Absts. 5: 1373, 1979). We have now compared the effects of muscarinic versus nicotinic agonists and also examined the effect of pretreatment with muscarinic or nicotinic blockers.

Male albino rats of the WRC strain, weighing between 250-300 grams were maintained in a light-cycled chamber. Two groups of rats were pretreated with methylatropine nitrate (0.5 mg/kg, ip) and later injected with either oxotremorine (2.0 mg/kg, ip) or saline. Another two groups were pretreated with atropine sulfate (60 mg/kg, ip) and later also injected with either oxotremorine or saline. In a second study, two groups were pretreated with saline injections followed by either an injection of nicotine (1.2 mg/kg, ip) or another saline injection. Another two groups were pretreated with mecamylamine (1.0 mg/kg, ip) followed by an injection of either nicotine or saline. Pretreatment always occurred 40 min prior to sacrifice and the second injection was given 10 min prior to sacrifice, which was accomplished by exposure to microwave irradiation (2.5 kw, 2450 MHz, 5 sec).

Following sacrifice and decapitation, trunk blood was collected for radioimmunoassay for prolactin and 21 brain regions were dissected for radioimmunoassay for cyclic AMP and cyclic GMP. Oxotremorine caused marked increases in cyclic AMP in the pituitary as well as in the hypothalamus, interpeduncular region and substantia nigra all of which were attenuated by pretreatment with atropine. Nicotine markedly elevated cyclic AMP in the pituitary and the interpeduncular region and caused significant increases in plasma prolactin as well. The nicotine-induced changes were attenuated by mecamylamine pretreatment.

The regional pattern of increase in cyclic GMP levels in the animals receiving oxotremorine is similar to the response following locomotor activity (Meyerhoff et al., Life Sci., 1979) consistent with the behavioral observation of tremor. The increases in cyclic GMP in the septal region, however, were not seen following locomotor activity alone. The tremors and the brain cyclic GMP increases were attenuated by pretreatment with atropine.

Nicotine lowered cyclic GMP in the cerebellum and decreased locomotor activity. These changes were attenuated by pretreatment with mecamylamine. Muscarinic and nicotinic agonists have marked effects on cyclic nucleotide levels in several regions of rat brain and these effects are attenuated by pretreatment with appropriate antagonists.

183.7 LOSS AND RESTORATION OF SENSITIVITY TO CATECHOLAMINES IN CULTURED BHK CELLS: INFLUENCE OF DANSYLCADAVERINE. A. Reggiani*, F. Vernaleone*, and G. A. Robison. Dept. of Pharmacology, University of Texas Medical School, Houston, Texas 77025

The adenine prelabeling technique has been used to further study the accumulation of cyclic AMP (cAMP) in BHK cells in response to isoproterenol (ISO) and PGE_1. Cyclic AMP rises and then falls in these cells in response to either agonist; the decline is due to hydrolysis by phosphodiesterase (PDE) and to the development of tachyphylaxis, i.e., a state of reduced responsivity to stimulation by agonists. Although ISO and PGE_1 clearly compete with each other insofar as the activation of adenylyl cyclase (AC) is concerned, PGE_1 has no influence on the rate at which tachyphylaxis develops to ISO. The evidence suggests that receptors must be activated in order for AC to be activated, but that the active receptors are inactivated at a rate independent of the extent to which they interact with the AC system.

When cells are exposed to 10^{-6}M ISO for 2 hours, and the ISO then removed, the maximum response to ISO is only 15% of that of control cells. After incubating in the absence of ISO for several hours, the response increases up to about 60% of control but no further, suggesting that about half of the inactivated receptors had been irreversibly lost (as indicated also by a comparable reduction in the maximum specific binding of ^{125}IHYP). We had previously found that when tachyphylaxis was induced in the presence of cycloheximide or actinomycin D, and the agonist then removed, responsiveness to ISO returned to 100% of control within 1 hour, suggesting a possible involvement of protein synthesis in the maintenance of tachyphylaxis. We have now found that dansylcadaverine (DC), which has no significant effect on protein synthesis but which is a potent inhibitor of transglutaminase and hence of endocytosis (Nature 238:162, 1980), produces a similar effect, i.e., DC does not prevent the initial development of tachyphylaxis, but does permit the complete restoration of responsivity to ISO after the ISO and DC have been removed. We conclude that the long-term loss of responsivity to agonists in these cells occurs in two stages, first the initial development of tachyphylaxis (conversion of active to inactive receptors) followed by the slower endocytotic uptake of inactive receptors, and protein synthesis does not seem to be involved in either process. We also conclude from studies of cells grown under different conditions that endocytotic activity depends more upon cell density (it is greater at high density) than on the rate at which the cells are growing. Supported by grants from the USPHS (GM 27378) and the Burroughs Wellcome Fund.

183.8 ENHANCED CYCLIC AMP ACCUMULATION IN RAT SPINAL CORD TISSUE SLICES FOLLOWING 6-HYDROXYDOPAMINE. D.J.Jones, L.F.McKenna* and P.M.Mason* Departments of Anesthesiology and Pharmacology, The University of Texas Health Science Center, San Antonio, TX 78284

Previous studies from this laboratory have demonstrated agonist specificity for cyclic AMP accumulation in rat spinal cord tissue slices (Jones and McKenna, Neuropharmacology, 1980). The presence of catecholamine containing neurons in spinal cord and their degeneration by 6-hydroxydopamine (6-OHDA) suggests that adrenergic denervation supersensitivity might be associated with the adenylate cyclase-cyclic AMP response in this tissue. The purpose of this study was to determine if there occurs an enhanced cyclic AMP response to catecholamines in spinal cord following 6-OHDA.

Three, seven, ten or 14 days prior to sacrifice rats were etherized and injected intracisternally with either a single or two, 0.20 mg doses of 6-OHDA in 0.01 ml of 0.1% ascorbate. Sham animals recieved vehicle only. Following sacrifice, spinal cords were removed and sliced on a McIlwain tissue chopper (300 μm) with subsequent incubation for one hour in oxygenated Krebs-Ringer bicarbonate buffer. Following this period, the slices were placed into incubation flasks for 15 min at which time various agonists were added. At the end of a 10 min incubation period tissue protein was denatured and cyclic AMP measured by radioimmunoassay.

One week following injection of 0.20 mg (x2) of 6-OHDA spinal cord norepinephrine (NE) levels were depleted over 95% from 619 ± 72 to 32 ± 10 ng/gm tissue. Depletion was also evident at 14 days. Consonant with depletion at 7 days and 14 days was an enhanced, concentration-dependent accumulation of cyclic AMP in response to NE. The enhanced response was reflected in an increase in maximal response only, without a change in the EC_{50}. In regional studies, enhanced accumulation of cyclic AMP in response to NE was evident as early as 3 days in the cervical cord. However lumbar spinal cord required at least 10 days for this response to develop suggesting a time course dependent on diffusion of 6-OHDA to the lumbar level. Enhanced cyclic AMP accumulation was also evident with iso proterenol. In addition the enhanced response to both NE and isoproterenol was potentiated in the presence of the phosphodiesterase inhibitor RO 20-1724. Pretreatment of animals with intracisternal 6-OHDA did not alter the response of spinal cord tissue slices to adenosine. This data suggests that adrenergic denervation supersensitvity, as evidenced by enhanced cyclic AMP accumulation, is evident in spinal cord tissue slices following 6-OHDA.
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183.9 ISOPROTERENOL-INDUCED CYCLIC AMP INCREASES IN VIVO IN PINEAL AND OTHER RAT BRAIN REGIONS. G. Jean Kant, Vernice E. Bates, Robert H. Lenox and James L. Meyerhoff. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20012 and Dept. of Psychiatry, Univ. of Vermont College of Medicine, Burlington VT 05405

Central β-receptors appear to be linked to cyclic AMP generating systems as determined primarily in in vitro studies. Tissue slices respond to isoproterenol (a β-agonist) with increased levels of cyclic AMP and this increase can be blocked by prior incubation with the β-antagonist propanolol. Recently the pineal β-receptor has been investigated in vivo and pineal cyclic AMP has been shown to increase after administration of isoproterenol. Since β receptors are found in many rat brain regions, we measured cyclic AMP levels in 10 brain regions of rats sacrificed by microwave irradiation 10 min after challenge with isoproterenol. The sensitivity of the pineal β-receptor has been shown to increase in rats maintained in continuous light and therefore in one experiment a group of rats was kept in continuous light for 3 weeks prior to isoproterenol challenge. All other rats were maintained on a 12 hr light-dark cycle (0600-1800). All rats were habituated to saline injection, handling, and to being placed in a plastic tube (similar to one used to immobilize the rats briefly during microwave sacrifice).

Rats were injected with saline or isoproterenol (10 mg/Kg i.p.), and sacrificed 10 min later by microwave irradiation at 2450 MHz using 2.5 Kw for approximately 5 sec. The heads were cooled on dry ice; the brain regions were dissected, weighed and sonicated in 50 mM sodium acetate buffer pH 6.2. The sonicates were centrifuged and the supernatants were stored at -70°C until assayed for cyclic AMP by radioimmunoassay. Isoproterenol significantly increased levels of cyclic AMP in the light-dark cycled rats in pineal, pituitary, cortex, hypothalamus, midbrain, frontal cortex, brainstem, and cerebellum.

In the animals kept in continuous light there was an enhanced cyclic AMP response to isoproterenol, suggesting the presence of supersensitivity.

183.10 IN VIVO CYCLIC AMP LEVELS IN RAT BRAIN REGIONS FOLLOWING KINDLING. Vernice E. Bates, James L. Meyerhoff, G. Jean Kant and Robert H. Lenox. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20012 and Dept. of Psychiatry, Univ. of Vermont College of Medicine, Burlington, VT 05405

Kindling consists of repetitive intermittent low intensity electrical stimulation of subcortical structures, especially the amygdala. This results in progressive changes in electrical activity and behavior and eventually culminates in a generalized seizure in response to an electrical stimulus which previously had produced no effect.

Seizures induced by maximal electroshock or pentylenetetrazol result in increased brain levels of cyclic AMP (cAMP) immediately following a seizure. Iontophoretically applied cAMP has an inhibitory effect on CNS neurons and it has been suggested that the cAMP rise following seizures has an antiepileptic effect in the brain. However, the precise role of cAMP in these acute seizure episodes is not clear and their role in chronic seizure models such as kindling has not been evaluated. We were interested in determining whether regional levels of cAMP measured in vivo would be altered following amygdaloid kindling.

Bipolar amygdaloid electrodes were implanted unilaterally into 12 male Sprague-Dawley rats. Seven days later, daily electrical stimulation was begun in 6 experimental animals. Stimulation consisted of 1 second of 60 Hz biphasic square wave pulses at 400 μA using a Grass S88 constant current stimulator. Six sham kindled animals with unilateral amygdaloid electrodes were handled similarly but did not receive electrical stimulation. Animals were considered fully kindled when they had 3 consecutive days of stage 5 seizures. This occurred following a mean of 12 ± .5 stimulations. Following the last seizure, amygdaloid electrodes were removed from both kindled and sham kindled animals. Three days later all animals were sacrificed by microwave irradiation at 2.5 kilowatts at a frequency of 2450 ± 20 MHz. After sacrifice, brains were dissected and cAMP was assayed by a modification of the radioimmunoassay of Steiner et al. Regions assayed included amygdala, hippocampus, striatum, substantia nigra, midbrain, brainstem, cerebellum, thalamus, cingulate gyrus, pyriform cortex and frontal cortex.

Cyclic AMP levels were significantly decreased in the stimulated amygdala when compared to sham kindled animals ($p<.01$). No differences were found in other brain regions. These findings suggest a role for cAMP mechanisms in amygdaloid kindled seizures.

183.11 CYCLIC GMP BIOCHEMISTRY IN HIPPOCAMPUS: COMPARISON OF RAT AND GUINEA PIG. P.E. Kilbride*, D.A. Kinscherf*, A.C. Blank* and J.A. Ferrendelli (SPON: T. Lysz). Div. of Clin. Neuropharm. and Depts. of Neurol. and Pharm., Washington Univ. Med. Sch., St. Louis, MO. 63110.

Over the past several years there have been numerous investigations of cyclic nucleotides in nervous tissues. Most of those concerned with mammalian brain have examined whole brain or cerebral cortex, cerebellum and/or striatum, and there have been fewer studies of other regions of CNS. Since hippocampus is now becoming an area of brain of increasing interest and study, we have examined cyclic nucleotide biochemistry in this structure to prepare for and facilitate future investigations. This report summarizes our results of studies on cyclic GMP biochemistry in rat and guinea pig hippocampus.

Levels of cyclic GMP in rat and guinea pig hippocampi rapidly frozen, in situ, were 0.41 ± 0.08 and 0.71 ± 0.12 pmoles/mg prot, respectively. In animals frozen during metrazol-induced convulsions, cyclic GMP levels increased 4-fold to 2.74 ± 0.47 in guinea pig hippocampus, but only 2.5-fold to 0.98 ± 0.09 in rat. Incubated slices of rat and guinea pig hippocampi had levels of cyclic GMP similar to basal levels, in vivo, 0.49 ± 0.03 and 1.06 ± 0.24 pmoles/mg prot, respectively. Exposure of tissue slices to 64 mM K^+ elevated cyclic GMP levels 20-fold in guinea pig hippocampus, but only 2-fold in rat.

In an attempt to explain the marked difference between rat and guinea pig, we measured cyclic GMP phosphodiesterase (PDE) and guanylate cyclase activities in hippocampi from the two animals. Both had similar cyclic GMP-PDE activity, 38 ± 2 and 40 ± 2 nmoles GMP formed/mg prot/min in the rat and guinea pig, respectively. Guanylate cyclase activity in particulate fractions of hippocampal homogenates was also similar to the two animals and was approximately 14 pmoles cyclic GMP formed/mg prot/min. However, guanylate cyclase activity in soluble fractions from guinea pigs was twice as great as that in rat, 114 vs. 63 pmoles cyclic GMP formed/mg prot/min. The apparent Km·GTP for this enzyme was about the same (ca. 25 μM) in the two animals.

These results demonstrate that cyclic GMP biochemistry in CNS may differ markedly among animal species. The striking difference in cyclic GMP regulation between rat and guinea pig hippocampus may be, partially, a result of lower soluble guanylate cyclase activity in rat tissues.

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183.12 IDENTIFICATION AND CHARACTERIZATION OF cAMP AND CALCIUM DEPENDENT PHOSPHOPROTEINS IN RAT BRAIN SYNAPTIC MEMBRANES. L. P. Kleine*, R. G. Sorensen* and H. R. Mahler. Dept. of Chemistry, Indiana University, Bloomington, IN 47405

We have previously reported on the phosphorylation of substrate proteins in synaptic membranes (SM) from rat brain by endogenous cAMP and Ca^{+2}-dependent protein kinases [De Blas, Wang, Sorensen and Mahler (1979) J. Neurochem. 33, 647-659]. Both systems affect a variety of substrates in the 50,000 to 60,000 dalton range. Also, there exist at least four components in this region that bind 8-azido-[^{32}P]cAMP. We have therefore, initiated a detailed analysis of these phosphorylatable and cAMP binding components in SM. Our aim is to elucidate possible identities between phosphoproteins and cAMP binding components and between Ca^{+2}- and cAMP-dependent phosphoproteins.

We have identified and characterized these cAMP and Ca^{+2}-dependent phosphoproteins by means of two-dimensional techniques as well as by peptide mapping of ^{32}P-labeled proteins using [^{32}P]-γ-ATP and 8-azido-[^{32}P]cAMP as donors. The possible relationships of these proteins to the regulatory subunits of type I and II protein kinases were explored by comparison with authentic regulatory subunits from muscle and brain. Inhibitory effects of ACTH (~10^{-5} M) and its fragment peptides [Zwiers, Schotman and Gispen (1980) J. Neurochem., in press] have been studied in collaboration with H. Zwiers and W.H. Gispen, State University of Utrecht, The Netherlands. (Supported by Research Grant NS 08309 from the NIH.)

183.13 A CYCLIC NUCLEOTIDE-INDEPENDENT PROTEIN KINASE FROM RAT BRAIN.
R. F. Alderson* and P. Y. Sze (SPON: B. E. Ginsburg). Dept. of Biobehavioral Sciences, Univ. of Connecticut, Storrs, CT 06268.

Two soluble forms of protein kinase from rat brain (all tissue anterior to the cerebellum) have been separated by DEAE-cellulose chromatography with a linear NaCl gradient (50 mM Tris acetate, pH 7.4 - 1 mM DTT-2 mM EDTA), using histone as the substrate. The first peak elutes at 0.08 M NaCl, and the second peak elutes at 0.2 M salt. This elution profile remains the same whether midbrain, striatum, or cerebral cortex is used. Peak II protein kinase is totally dissociable by cAMP. Peak I contains a protein kinase that is not dissociated or stimulated by cAMP and is not inhibited by the endogenous inhibitor of cAMP-dependent protein kinase. The peak I protein kinase has been further fractionated on ATP-agarose and hydroxyapatite columns. The highly purified enzyme exhibits the following characteristics in addition to its lack of response to cAMP: (1) binding to ATP-agarose in the absence of cAMP; (2) incapable of binding ^{3}H-cAMP; (3) not stimulated by cGMP; (4) with a substrate preference in the order protamine > casein > phosvitin > whole histone; (5) requiring an optimal Mg^{+2} concentration at 10 mM. These properties are consistent with the criteria for a cyclic nucleotide-independent protein kinase. Its M.W. has been estimated as 90,000 from gel filtration. When tested on midbrain tryptophan hydroxylase, this kinase results in the activation of the midbrain enzyme in the presence of ATP and Mg^{+2}.

From the DEAE-cellulose elution profile, the protein kinase characterized in this study appears to be similar to the "protamine kinase" described by Inoue et al. from rat whole brain which is also cAMP-independent (Inoue et al., BBRC, 228, 50, 1973). In bovine cerebellum, another soluble cAMP-independent protein kinase with M.W. 64,000 has been identified which elutes at 0.2 M NaCl from DEAE-cellulose and is detectable only in the presence of high Mg^{+2} concentrations (Takai et al., JBC, 252, 7603, 1977). This protein kinase may be present in brain regions other than the cerebellum (Raese et al., Commun. Psychopharmacol., 3, 295, 1979). Thus, at least two soluble cyclic nucleotide-independent protein kinases may exist in brain tissue. The relative distribution and function of the two protein kinases in various brain regions remain to be determined.

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184.1 BEHAVIORAL TOLERANCE TO THE STIMULANT EFFECTS OF DIETARY L-TYROSINE. G. B. Freeman*, J. R. Ieni*, J. Soblosky*, and J. B. Thurmond. Neuropsychopharm. Program, Univ. of Louisville, Louisville, Kentucky 40292

Behavioral and brain neurochemical changes were assessed in mice maintained on L-tyrosine supplemented 12% protein diets for 2, 5, 10, or 14 days. Male CF-1 mice were fed a semi-synthetic 12% casein diet for 1 week, then switched to a diet modified by the addition of 4% L-tyrosine supplement. Measures of territorial aggressive behavior and locomotor activity were obtained before and after the dietary supplement was administered. Compared to control animals maintained on 12% casein, resident mice fed the L-tyrosine supplement displayed an increase in the number of attacks on intruders and shorter attack latencies.

The strongest stimulating effect of L-tyrosine on aggressive behavior was observed after 2 days on the dietary supplement. The ability of L-tyrosine to induce changes in behavior was considerably diminished following longer periods of maintenance on the diet, suggesting the development of behavioral tolerance. No unusual changes in weight gain were noted as a function of maintenance on the diets and no ill effects were observed.

Other groups of mice maintained on 12% casein (controls), or administered the L-tyrosine supplement, were sacrificed for analyses of brain tyrosine, norepinephrine, dopamine, serotonin, and certain metabolites of these monoamines. In contrast to the reduced behavioral effects after 14 days, neurochemical changes induced by addition of L-tyrosine to the diet were sustained over the two-week period.

184.2 DIFFERENCES IN (i) BEHAVIOUR, (ii) BRAIN AMINO ACID DISTRIBUTION AND (iii) REVERSIBILITY BY THIAMINE BETWEEN THIAMINE-DEFICIENT AND PYRITHIAMINE-TREATED RATS. Edith Hamel*, Roger F. Butterworth, François B. Jolicoeur* and André Barbeau. Clinical Research Institute of Montreal, Montreal, Quebec, Canada.

Male Sprague-Dawley rats (200-225 g) were fed a thiamine-deficient diet or treated with the central thiamine antagonist pyrithiamine. Thiamine-deficient rats were subjected to a series of neurobehavioural tests including a detailed analysis of gait as previously described (Can. J. Neurol. Sci., 6: 209, 1979), as was that of the appropriate pair-fed and ad libitum controls. Thiamine-deficient rats lost their righting and weight shift reflexes and their gait was found to be ataxic. These abnormalities were also present in the PTh-treated rats; however, these rats also lost their traction and forelimb extension reflexes and were severely cataleptic. Administration of thiamine (diet + injections of 20 µg/100 g body wt.) promptly reversed all the neurological abnormalities of the thiamine-deficient group but was without effect on the symptomatic PTh-treated rats.

Amino acid concentrations were determined in many regions of the CNS of the symptomatic thiamine-deficient and PTh-treated rats together with appropriate controls as already described by us (J. Neurochem., 33: 575, 1979). Glutamate, Aspartate, GABA and Glycine were significantly decreased in the cerebellum, brain stem and spinal cord of the thiamine-deficient group. Aspartate, Glutamate and GABA concentrations were diminished in the cerebral cortex and brain stem of the PTh-treated rats, the cerebellum and spinal cord being spared. Glycine levels were unchanged by PTh treatment.

These results clearly show that chronic thiamine-deficiency induced by dietary deprivation of the vitamin and that produced by treatment with the central thiamine antagonist pyrithiamine exert their CNS effects by different mechanisms, as shown by the differences in behaviour, regional vulnerability, amino acid changes and reversibility of symptoms by thiamine administration.

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184.3 COMPARISON OF THE EFFECTS ON BODY TEMPERATURE OF THE RABBIT OF ALANINE WITH THAT OF SERINE, GLYCINE AND TAURINE. J.M. Lipton and J.R. Glyn* (SPON: W.G. Clark). Depts. of Physiology and Neurology, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

Serine, glycine and taurine cause dose-related hypothermia and delay the onset of temperature increases induced by leukocytic pyrogen (LP) or prostaglandin E_2 (PGE_2). Alanine, another small neutral amino acid, is transported by similar mechanisms and shares neuronal inhibitory properties with serine, glycine and taurine (Cutler, R.W.P. & Coull, B.M., In: Taurine and Neurological Disorders, 1978, p. 95; Curtis, D.R., Hosli, L. & Johnston, G.A.R., Nature, Lond. 215:1502, 1968). Since alanine also lowers body temperature in the rat (Sgaragli, G.P. & Pavan, F., Neuropharmacology 11:45, 1972), the present experiments compared the effects of alanine on body temperature with those produced by the other amino acids. Intracerebroventricular (icv) injections of alanine (0.5-2.0 mg) caused no change in body temperature at an ambient temperature (T_a) of 30°C; a small decrease at T_a = 23°C; and a greater, dose-related decrease at T_a = 10°C. Alanine was additive with glycine and taurine in producing hypothermia at T_a = 10°C. With serine, alanine was slightly subadditive, like previous results in which serine appeared to be subadditive with glycine and taurine. 2 mg icv alanine significantly reduced both LP fever and PGE_2 hyperthermia. Therefore alanine, glycine and taurine appear to act at similar sites in the central thermoregulatory pathway, with serine possibly acting at other sites as well. (Supported by National Institutes of Neurological and Communications Disorders and Stroke Grant NS 10046.)

184.4 HALOTHANE INDUCED RELEASE OF GABA-T FROM RAT BRAIN SYNAPTOSOMES. S-C. Cheng and E.A. Brunner*. Dept. Anesthesia, Northwestern Univ. Med. Sch., Chicago, IL 60611. Volatile anesthetic agents inhibited GABA disposal($^{14}CO_2$ from [1-^{14}C]GABA) with intact synaptosomes but not with solubilized synaptosomal GABA-T (Cheng and Brunner, Biochem. Pharmac., 28:105, 1979). A direct correlation between inhibition of GABA disposal and of Complex I of the electron transport chain (Miller et al., Anesth. Analg. 53:132, 1974) has not been established. Volatile anesthetics also released proteins from beef heart mitochondria (Richter et al., Biochim. Biophys. Acta, 543:106, 1978). One of them was aspartate aminotransferase. We question if a similar release of GABA-T might occur from synaptosomes. Rat forebrain synaptosomes were prepared by fractionating the crude mitochondria between 0.9-1.2M sucrose and resuspending the synaptosomes in 0.32M sucrose. Aliquots were incubated for 1 hr at 30° in the absence (C) or presence (H) of halothane. After incubation, C and H suspensions were centrifuged to yield clear supernatants (C_S and H_S) and sediments (C_P and H_P). The sediments were resuspended again in 0.32M sucrose. Samples from each of these were tested for GABA-T activity in an assay previously described. Halothane was introduced in two ways: (1) it was vaporized into N_2 in a Copper Kettle and diluted with 40%O_2:60%N_2, and (2) liquid halothane was added to the center-well of a calibrated 25ml flask. The halothane concentration in the gas phase above the synaptosomes was calculated assuming a synaptosome/gas partition coefficient of 2.0. This value was derived from the total volume of the flask, the volume and protein concentration of the synaptosomes and the protein/wet weight ratio and the halothane partition coefficient of brain tissue (Ikeda, Anesthesiology, 37:87, 1972). The specific activity of GABA-T of the initial synaptosomal suspension was 0.72 µmole/hr/mg protein. Both C and H fractions lost some GABA-T activity at various halothane concentrations. In paired experiments, H and H_P lost more activity than C and C_P respectively. The additional losses were dose-related. Activities of H_S and C_S were highly variable. The presence of GABA-T activity in these supernatants confirmed the loss of protein from the synaptosomes and may reflect leakage of protein or rupture of synaptosomes. The amount of protein released was very small and this could account for the instability of released GABA-T in H_S and C_S. The loss in GABA-T activity in H_P, when compared to C_P, was dose-related and showed a 10% effect in the range of ED_{50} for halothane. This might account for the correlation of ED_{50} for halothane to ID_{10} for GABA disposal (Cheng, in GABA - Biochemistry and CNS Function, p. 161, 1979).

184.5 VALPROATE INTERACTIONS WITH GABA INHIBITION IN HYPOTHALAMIC TISSUE CULTURES. H.M.Geller. Dept. of Pharmacology, CMDNJ-Rutgers Medical School, Piscataway, NJ 08854

Explant tissue cultures of newborn rat tuberal hypothalamus have been utilized in order to examine whether valproate (dipropyl acetate) acts to potentiate GABAergic inhibition. Cultures were prepared from newborn rats and maintained in roller tubes until 3-4 weeks of age. At this age, many neurons are spontaneously electrically active and respond to locally applied GABA with a depression in firing rate. Additionally, putative GABAergic (picrotoxin-and bicuculline-sensitive) inhibition can be evoked with focal electrical stimulation. In these experiments, the activity of 37 neurons in 17 cultures was recorded extracellularly with 3 M NaCl filled micropipettes. Action potentials were amplified, discriminated and fed to a computer for on-line histogram construction. Inhibitions were evoked electrically (0.1 msec pulses, 10-30 V) or with repeated pulsed iontophoretic application of GABA (5-10 sec, 1-50 nanoamperes). Peri-event histograms were used to quantify drug-or electrically-induced inhibition and to quantify changes in inhibition produced by perfusion of sodium valproate (0.1-4 mM). Potentiation of GABA inhibition by valproate was observed in only 8 neurons (22%) while a reduction of inhibition was observed in 12 (32%); the remaining 17 showed no change or irreversible alterations in firing rate. Thus, these data utilizing hypothalamic cultures do not support the hypothesis that valproate actions are mediated by an acute potentiation of GABA. Alternately, valproate actions may be regionally specific, with the hypothalamus being relatively unaffected. Further experiments utilizing other brain regions are necessary to assess this possibility. Supported by NIH NS 15468 and NSF BNS 79-14003.

184.6 FURTHER STUDIES ON THE BINDING OF β-ALANINE TO SYNAPTOSOME-ENRICHED FRACTIONS OF RAT CENTRAL NERVOUS SYSTEM. L. M. Orensanz* and Gloria Corrochano* (SPON: F. V. DeFeudis). Dept. de Investigación, Centro Especial Ramón y Cajal, Madrid-34, Spain.

Using techniques which permit biochemical identification of neurotransmitter receptors in the mammalian brain we have previously demonstrated a sodium-independent specific binding of β-alanine to synaptosome-enriched fractions of rat brain. The present experiments were undertaken to study in more detail the characteristics of this sodium-independent β-alanine binding.

Experiments were conducted in rat tissue containing brain stem plus spinal cord. Specific [^{3}H]β-alanine binding, that is, that obtained by subtracting from the total bound radioactivity the amount not displaced by high concentrations of unlabeled β-alanine, was higher in crude synaptosomal fractions than in frozen/thawed synaptosomal fractions or synaptic membranes. Bound radioactivity was analyzed by thin layer chromatography and was shown to migrate as authentic β-alanine. Binding rose linearly when increasing amounts of tissue were added to the incubation medium. In the 2-160 nM concentration range binding occurred by a double affinity mechanism, the highest affinity having a binding constant, K_D, of 5×10^{-8} M and binding capacity, B_{max}, of 380 f-moles per mg protein. Compounds capable of inhibiting the uptake of β-alanine in rat spinal cord slices, at 2.5×10^{-4} M, did not affect the specific binding of 5 nM [^{3}H]β-alanine, while specific binding was abolished by compounds which have no effect on the uptake, such as glycine or DL-2,3-diaminopropionic acid. In addition, specific binding was not detectable to similarly prepared fractions from liver and spleen.

The present results may indicate that specific receptors may be implicated in the binding of β-alanine to synaptosome-enriched fractions of rat brain stem and spinal cord. The observed iontophoretic depressant action of β-alanine in these regions may be linked to interaction with these receptors.

184.7 ENDOGENOUS INHIBITORS OF GABA-AGONIST BINDING FROM SYNAPTOSOMAL FRACTIONS OF RAT BRAIN. L. M. Yunger. Dept. Biol. Res., Smith Kline & French Labs., Philadelphia, PA 19101

Pretreating synaptic plasma membranes with 0.2 to 0.05% Triton X-100 produced a 5- to 50-fold increase in GABA agonist binding, depending on the brain area and ligand used. This increase appears to be due to release of endogenous inhibitors which mask the high affinity GABA agonist binding sites. These inhibitors are not equally distributed in brain; for example, the potency of inhibitors in the buffer eluate from hippocampal synaptic plasma membranes is almost 7 times that from cerebellar membranes. Since at high concentrations these endogenous inhibitors can block low affinity GABA agonist binding as well, this may account for the very low GABA agonist binding observed in fresh hippocampal homogenates (44 fmol ^{3}H-muscimol bound/mg protein) compared to fresh cerebellar homogenates (490 fmol ^{3}H-muscimol bound/mg protein). We have partially characterized inhibitors of GABA agonist binding (1) which are eluted into the hypotonic medium when synaptosomes are lysed (H_2O fraction) and (2) which are eluted into the first buffer wash of the synaptic plasma membranes (buffer fraction). Inhibitors from both fractions are heat stable, and, once heated, are stable at 4°C for at least 7 days. Approximately 95% of activity is lost from the H_2O fraction and 60% of activity is lost from the buffer fraction on dialysis (3500 to 12000 pore size). Most of the dialyzable activity in the H_2O fraction appears to be GABA. The inhibition of GABA agonist binding produced by either fresh or dialyzed H_2O and buffer fractions was not affected by pretreating the membranes with 10 μM diazepam. At present, it is not known if the non-dialyzable inhibitors correspond to those which have been described in the literature.

184.8 HOMOCYSTEINE INDUCED CONVULSIONS: ENHANCEMENT BY VITAMIN B_6 AND INHIBITION BY HYDRAZINE. R.W. Hurd*, E.J. Hammond*, B.J. Wilder. (Spon: A.J. Dunn) Department of Neuroscience, University of Florida, and Neurology Service, VA Medical Center, Gainesville, FL 32610.

Intraperitoneal injections of homocysteine in animals have been shown to produce tonic-clonic convulsions and electrographic seizures which resemble seizures in humans. Some patients with homocystinuria also have seizures. The actual mechanism of seizure induction in both humans and experimental animals are, however, obscure.

We studied the effects of pretreatment with the B_6 vitamers, pyridoxal phosphate, pyridoxine (50 mg/kg) and the B_6 antagonist hydrazine sulfate (50 mg/kg) in mice receiving injections of homocysteine thiolactone (5.5 mM/kg). Both pyridoxal phosphate and pyridoxine significantly decreased the latency ($P < .01$) and increased the severity, lethality, and duration of homocysteine induced convulsions. Hydrazine, which is normally a convulsant, prevented the tonic component of the homocysteine convulsions ($P < .001$) and increased the latency to the clonic component ($P < .01$). These data indicate that a vitamin B_6 dependent step is critically involved in the metabolic changes which precede homocysteine seizures.

Supported by the Epilepsy Research Foundation of Florida.

184.9 A COMPARISON OF THE EXCITATORY EFFECTS OF L-ASPARTATE AND L-GLUTAMATE ON PURKINJE CELLS IN VITRO IN THE CEREBELLAR CORTEX OF GUINEA PIG. Sandra L. Morzorati, William J. McBride and Robert C.A. Frederickson, Departments of Psychiatry and Biochemistry, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46223.

Neurochemical evidence (Nadi et al., J. Neurochem. 28: 661, 1977) has shown that the level of aspartate decreases in the cerebellum of rat following a single injection of 3-acetylpyridine, which destroys the inferior olivary nuclei and thus the cerebellar climbing fibers. From this data, it was proposed that aspartate may be the excitatory transmitter released from climbing fibers onto Purkinje cells. This hypothesis was supported by the fact that the microiontophoretic application of L-aspartate produced an increase in the firing rate of all Purkinje cells tested in vivo in rat (Morzorati et al., Society for Neuroscience Abstracts, 4: 448, 1978).

A number of advantages have been cited for using the thin slice preparation over the intact animal preparation. Thus we have adopted the in vitro technique in which to study the sensitivity of Purkinje cells to L-aspartate (Asp) and L-glutamate (Glu), the transmitter possibly released from granule cells. Thin sections of the guinea pig cerebellar vermis (300-500 µm) were sliced parallel to the long axis of the folia using a tissue chopper and placed in a perfusion chamber. Multibarrelled micropipettes served both to record extracellularly and to eject the amino acids. Preliminary results showed that the microiontophoretic application of Asp and Glu produced an increase in the spontaneous firing rate of all Purkinje cells tested. The excitatory effects of both amino acids were dose dependent and characterized by a rapid onset. The 'doses' (current x ejection time) necessary to produce half maximal responses to Asp and Glu on a single cell were obtained from linearized dose-response relationships. Subsequent potency ratios showed Purkinje cells to be more sensitive to Glu than Asp. These same dose-response relationships were generally found to be non-parallel, implying different mechanisms of action (and thus different receptor sites) for the two amino acids. These data indicate that the thin cerebellar slice is a useful tool in our hands, and that Asp may be an excitatory transmitter in the mammalian cerebellum. (Supported in part by PHS Grants NS 13925 and MH 00203 and a grant from Eli Lilly and Co.).

184.10 EXCITATORY AMINO ACID STIMULATION OF CEREBELLAR CYCLIC GMP IN THE RAT. P.J. Roberts* and G.A. Foster* (SPON: W.G. Van der Kloot). Dept. of Physiol. and Pharmacol., Sch. Biochem. and Physiol. Sci., Southampton Univ., Southampton, SO9 3TU, England.

The postsynaptic actions of several types of neurotransmitter are mediated, or modulated through the cyclic nucleotides, cyclic AMP and cyclic GMP (cGMP). The cerebellum contains high concentrations of cGMP, and recently it has been demonstrated that during a short period of postnatal development, glutamate is able to elicit very large increases in cGMP in rat cerebellar slices. In this study, we have investigated the cerebellar cGMP response in tissue slices (0.5 x 0.5 mm) from 8-day old female rats. After a 90 min pre-incubation, slices were exposed to agonists for 5 min and cGMP concentrations determined by radioimmunoassay. L-glutamate produced a dose-related increase in cGMP with the half-maximal response occurring at approximately 1 mM. With respect to other agonists, a rank order of potency was observed similar to that for excitation of mammalian spinal neurons, i.e. NMDA > 4-fluoroglutamate > DL-homocysteate > (±)-ibotenate > L-glutamate = L-aspartate = kainate > dihydrokainate. The proposed excitatory amino acid antagonist, L-glutamate diethylester inhibited the stimulation by glutamate, aspartate and NMDA, while D-α aminoadipate and DL-α-aminosuberate were inactive. The stimulation of cGMP by kainate did not attain the same maximal response as that with L-glutamate, suggesting partial agonism or different sites of action. When L-glutamate (1 mM) was combined with kainate however, a marked synergism was observed with an apparent increase in sensitivity to kainate of approx. 35 fold. This finding may have implications for the neurotoxic actions of kainate.

184.11 HIGH-AFFINITY BINDING OF L-[^{3}H]GLUTAMATE TO HIPPOCAMPAL SYNAPTIC MEMBRANES IN THE ABSENCE OF SODIUM. Linda L. Werling* and J. Victor Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Considerable evidence suggests that glutamate serves as the transmitter of several excitatory projections to the rat hippocampal formation, in particular the perforant path fibers and commissural-ipsilateral fibers that arise from CA3 pyramidal cells. We have therefore attempted to identify binding sites that might correspond to physiologically relevant glutamate receptors by use of hippocampal synaptic membranes. Freshly-prepared synaptic membranes were washed 3-5 times with H_2O to remove endogenous glutamate and were incubated with L-[^{3}H]glutamate (45Ci/mmol) in a 1.4 ml volume of tris-HCl buffer. Non-specific binding was determined from parallel incubations with 0.1 mM non-radioactive glutamate and accounted for less than 20% of the total binding. Preliminary studies with 20 nM [^{3}H]glutamate revealed a sharp pH optimum around 6.5 and an optimal incubation temperature of 38°C. At 4°, 25° or 38°C, specific binding reached a plateau in less than 5 min, but when the incubation was continued for 20 min or longer a substantial and progressive increase in specific binding was obtained. Preincubation of the synaptic membranes for 30-60 min at 38°C in the absence of glutamate increased specific binding at 5 min of incubation, but preincubation at 4°C did not. These findings suggest that specific binding can be increased by two processes, one temperature-dependent and the other substrate-dependent.

When synaptic membranes were incubated for 5 min with a range of L-[^{3}H]glutamate concentrations (1-1000 nM), specific binding was found to be saturable. A biphasic Scatchard plot was obtained, suggesting the possibility of two distinct populations of binding sites. Apparent K_D values were: 13 nM and 580 nM, with corresponding B_{max} values of 4.2 pmol/mg protein and 70 pmol/mg protein. Hill plots gave two lines with slopes of about 1.2, supporting the existence of positive co-operativity. In displacement studies no striking pharmacological differences have emerged between the two presumptive sites. Both are relatively stereospecific for L-glutamate and interact with a number of excitatory amino acids and excitatory amino acid antagonists, but neither N-methyl-DL-aspartate nor kainate at a concentration of 0.1 mM displaces L-[^{3}H]glutamate from either site. Our results are consistent with a role for these synaptic membrane binding sites in glutamatergic transmission. (Supported by NIH grant NS 16064 and NSF grant BNS 78-13051.)

184.12 THE LATERAL OLFACTORY TRACT TRANSMITTER MAY BE NEITHER GLUTAMATE NOR ASPARTATE. D. J. Braitman, N. Hori*, C. R. Auker and D. O. Carpenter. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Aspartate (Asp) and glutamate (Glu) are principal candidates for the excitatory neurotransmitter released by the lateral olfactory tract (LOT) in prepyriform cortex of the rat. Both are present in this region and are released on stimulation of the LOT. However, identity of action of the natural transmitter with exogenous Glu and/or Asp has not yet been demonstrated.

In a prior study (Fed. Proc. 39:281, 1980) we found that bath perfusion of 2-amino-4-phosphonobutyric acid (APB), a presumed specific Glu antagonist, markedly decreased the amplitude of LOT-stimulated field potentials in rat prepyriform cortex slices. We used a similar preparation attempting to establish the identity of action of the LOT transmitter and either Glu or Asp. In the current study we recorded single unit activity from the pyramidal cell layer of prepyriform cortex. We attempted to prove identity of action by blocking, with APB, both the LOT-stimulated activity and the activity evoked by ionophoretic application of various amino acids including Asp and Glu.

Tangential slices (200-400 u thick) of rat prepyriform cortex, including the LOT, were cut by hand and preincubated at 35°C in modified oxygenated Kreb's-Ringer solution. Individual slices were placed in a total immersion chamber, and an electrode was driven into the pyramidal cell layer until an extracellular single unit response evoked by LOT stimulation was recorded. A seven-barrel glass ionophoretic electrode was then driven into the zone of termination of the LOT fibers onto the apical dendrites of the pyramidal cells (100-200 u superficial to the recording tip). The barrels were filled with 1 M L-Glu, 1 M L-Asp, 1 M N-methyl-D-aspartate (NMDA), 1 M DL-homocysteate (HC), 1 M L-cysteate (Cys), 10^{-2} M kainic acid (KA), or 0.5 M NaCl, each at pH 7-8.

Single units driven by LOT stimulation were also excited by ionophoretic application of all six amino acids. Bath perfusion of 10^{-3} M APB blocked the activity evoked by LOT stimulation but was ineffective in blocking the responses evoked by L-Glu, L-Asp, or L-Cys. However, responses to the three other excitatory amino acids—NMDA, HC, and KA (which do not naturally exist in brain)—were markedly reduced by bath perfusion of 10^{-3} M APB. We tentatively conclude that (1) neither Asp nor Glu is the endogenous LOT transmitter, and (2) Glu, Asp, and Cys act at different receptor sites than do NMDA, HC, and KA.

184.13 EFFECTS OF KAINIC ACID, N-METHYL-d-ASPARTATE AND GLUTAMIC ACID DIETHYL ESTER ON UPTAKE OF 2-DEOXYGLUCOSE IN THE RAT BRAIN. N.H. Diemer[1]*, K.E. Sørensen[2]* and I. Divac[3]. 1) Institute of Neuropathology, University of Copenhagen, 2) Institute of Anatomy B, University of Aarhus, 3) Institute of Neurophysiology, University of Copenhagen.

Intraperitoneal injections of kainic acid (KA, 12-13.3 mg/kg) produced a characteristic change in distribution of uptake of [^{14}C]2-deoxyglucose (2-DG). In other animals, silver impregnated (degenerated) neurons were found after intracranial injections of ninogram amounts of KA in those brain formations which had relatively high uptake of 2-DG. Neither N-methyl-d-aspartate (26.7 mg/kg) nor glutamic acid diethyl ester (1200 mg/kg), also injected intraperitoneally, had a visible effect on distribution of 2-DG uptake.

184.14 CYCLOPROPANE ANALOGS OF GAMMA-AMINOBUTYRIC ACID: EFFECT OF RESTRICTED CONFORMATION ON RECEPTOR BINDING. James C. Schaeffer, Layton L. McCoy* and Mark S. Wassermark*. Dept. of Chemistry, University of Missouri, Kansas City, Missouri 64110.

Gamma-aminobutyric acid (GABA) is an extremely flexible molecule that can exist in a variety of conformations. In order to investigate the possibility that different GABA binding sites interact preferentially with different conformational states, we have synthesized two cyclopropane analogs of GABA. Cis-2-(aminomethyl)-cyclopropane-1-carboxylic acid (CIS-cGABA) was prepared in six steps from methyl acrylate and ethyl chloroacetate while trans-2-(aminomethyl)cyclopropane-1-carboxylic acid (TRANS-cGABA) was prepared from the same starting materials in eight steps.

The GABA receptor assay of Enna, Collins and Snyder (Brain Research, 1977), using 1 mM unlabeled GABA as the displacing ligand, was employed to assess the effect of restricted conformation on sodium-independent receptor binding. CIS-cGABA, TRANS-cGABA, and GABA gave I_{50} values of 30μM, 0.38μM, and 0.073μM, respectively. These data, coupled with the previously demonstrated preference of the GABA receptor for trans-4-aminocrotonic acid over the corresponding cis isomer, strongly suggest that the trans staggered conformation of GABA is preferred by its postsynaptic receptor. Recently, Johnston, Allan, Kennedy and Twitchin (Alfred Benzon Symposium 12) have prepared independently the same cyclopropane GABA analogs and reported similar binding results. However, they found that TRANS-cGABA bound slightly better than GABA while we have found that GABA binds five-fold better than TRANS-cGABA. In contrast to the data of Johnston, et al., our results may indicate that the binding site is a narrow crevice that cannot well tolerate the extra methylene of the cyclopropane ring.

The ability of the cyclopropane analogs to inhibit sodium-dependent GABA reuptake was also investigated. Like Johnston, et al., we have found both isomers to be poor inhibitors of GABA reuptake.

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184.15 SEROTONIN AND ENKEPHALIN CO-EXIST IN NEURONS INVOLVED IN OPIATE AND STIMULATION-PRODUCED ANALGESIA IN THE CAT. A.I. Basbaum, E.J. Glazer, H. Steinbusch* and A. Verhofstad*. Department of Anatomy, University of California, San Francisco and Department of Anatomy and Embryology, Catholic University, Nijmegen, The Netherlands.

Profound analgesia can be produced by electrical stimulation of the serotonin (5HT)-containing nucleus raphe dorsalis (RD), raphe magnus (NRM) and paragigantocellularis (PGL) (located lateral to NRM). Recently we reported significant numbers of enkephalin (ENK)-immunoreactive neurons in RD and PGL of the cat. In this study we describe neurons in these nuclei which contain both 5HT and ENK. ENK and 5HT were sequentially localized on the same section by a combined immunoperoxidase-immunofluorescence technique using specific antisera generated against either leucine-enkephalin-BSA or serotonin-BSA conjugates. Cats were pretreated with colchicine (III ventricle) and an MAO-inhibitor and were perfused with buffered 4% paraformaldehyde/0.2% glutaraldehyde. The brainstems were embedded in paraffin, serially sections at 10 microns and sequentially processed for ENK immunoperoxidase (PAP) and 5HT immunofluorescence.

Both 5HT and ENK labeled neurons are found throughout the rostral caudal extent of the RD. Two 5HT cell types were observed. Small, round neurons appear in the midline; larger multipolar neurons extend laterally into the periaqueductal grey (PAG), beyond the cytoarchitectural boundaries of the RD. ENK neurons of the RD are concentrated on the midline; others are found throughout the PAG. The specific 5HT immunofluorescence fills both the cytoplasm and nucleus of labelled neurons. In contrast the enkephalin peroxidase reaction product is limited to the cytoplasm. These different staining characteristics made it possible to distinguish a population of double-labeled neurons, showing both cytoplasmic peroxidase reactivity and nuclear fluorescence. A population of these double-labeled neurons was found within the midline RD intermingled with neurons containing only serotonin or enkephalin. The PGL also contains three populations of neurons: an enkephalin group, a serotonin group and a group containing both serotonin and enkephalin.

A comparison of the 5HT- and/or ENK containing cell groups of the rostral medulla with the known origin of descending pathways in the spinal dorsolateral funiculus reveals a variety of potential descending modulatory systems. These include 5HT-cells of NRM, ENK and 5HT cells of PGL and cells of the nucleus reticularis magnocellularis located dorsal to PGL which contain neither 5HT nor ENK. This study suggests that the analgesic action of RD and rostral medullary electrical stimulation reflects a simultaneous activation of 5HT and ENK neurons.
(Supported by NSF 7824762, PHS DA 01949 and NS 14627.)

184.16 RELEASE OF METHIONINE ENKEPHALIN FROM CAT MESENCEPHALON AND SPINAL CORD IN VIVO. Tony L. Yaksh, and Robert P. Elde. Dept. of Neurosurgical Res., Mayo Fdn., Rochester, MN 55901 and Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

Methionine enkephalin like radioimmunoreactivity (MELA) found within cells of the spinal cord and brain may reveal one of the transmitters for intrinsic opioid systems. To investigate the properties associated with the release of MELA from the mesencephalic aqueduct (MA) and spinal cord, cats were anesthetized with chloralose urethane, and prepared for perfusion by inserting a 22 ga inflow cannula into the caudal aspect of the third ventricle and an outflow catheter into the mouth of the aqueduct. To perfuse the spinal cord, an infusion catheter (PE-10) was inserted through the cisterna magna to the caudal edge of the lumbar enlargement. Outflow was collected by a second concentric catheter whose tip lay at the level of the thoracic cord. Artificial CSF contained albumin (120 μg/ml) and bacitracin (300 μg/ml). Samples were collected on ice and immediately lyophilized prior to radioimmunoassay. The mean resting levels of MELA was .8 ± 0.1 ng/ml and 1.3 ± 0.2 ng/ml for the MA and spinal cord superfusates, respectively. Bilateral stimulation of the sciatic and infraorbital nerves at high but not low intensity produced over a 2 fold increase in the levels of MELA in both the MA and spinal perfusates. That the elevated levels of MELA were not due to alterations in blood pressure as evidenced by the failure of vasoxyl, i.v., to alter enkephalin levels in cord or MA perfusates. Cold block at C-3 had no effect on the elevated levels of MELA in spinal perfusates, but reduced the release otherwise evoked from the MA. Column chromatography of pooled samples of ventricular and spinal perfusates prior and during high intensity stimulation revealed that the MELA activity was isographic with authentic methionine enkephalin. This in conjunction with the relative specificity of the antisera (cross reacts with leu-enk, less than 0.6%) strongly suggests that the material observed in the perfusate was methionine enkephalin. Results suggest 1) that the MELA activity in brain was dependent upon the activation of rostrad transmission systems, while the activity of MELA systems in the spinal cord were directly activated by afferent input and did not depend upon a descending link for their activity and 2) that the MELA systems in the central gray are activated by sensory input of diverse somatotopic origin. The observation of the increase in levels of MELA in regions where enhanced exogenous levels of opiate activity produce significant elevations in the nociceptive threshold, strongly suggests that high intensity somatic stimulation results in the activation of an intrinsic modulatory circuit. (This work was supported by Mayo Fdn., NS 14629 and DA 02148).

184.17 OPIOID PEPTIDES INHIBIT DRINKING BEHAVIOR AND THE PRESSOR RESPONSE TO HYPERTONIC SODIUM CHLORIDE. Joan Y. Summy-Long and Lillian M. Rosella*, Dept. of Pharmacology, College of Medicine, PA State University, Hershey, PA 17033.

Enkephalins inhibit angiotensin II-stimulated drinking behavior and pressor response by a naloxone-sensitive mechanism (Pharmacologist 21:288, 1979). Studies were designed to determine if endogenous opioid peptides inhibit: 1) drinking induced by relative cellular dehydration or hypovolemia and 2) the pressor response to intracerebroventricularly (IVT) administered hypertonic sodium chloride (NaCl). Adult male Sprague Dawley rats (300-400 g) were anesthetized with sodium pentobarbital and a cannula positioned in the left lateral cerebroventricle. In drinking experiments animals were placed in individual metabolism cages with food and water available ad libitum. Five days after surgery rats received 20% polyethylene glycol (PG; M.W. 20,000) or 1 M NaCl (HS) subcutaneously (15 ml/kg) at 9 a.m. Food and water were withheld for 3 hr (HS) or 6 hr (PG) after injection. Artificial cerebrospinal fluid (CSF, 10 µl) with or without β-endorphin (β-ED; 1 or 5 µg) was administered IVT 5 min prior to water access. Drinking onset and volume consumed for 2 hr were measured for each rat. Differences were evaluated by analysis of variance and Newman Keuls t. β-ED delayed ($p<0.05$) drinking onset (min ± SEM) stimulated by PG (CSF 4 ± 2 < β-ED 1 µg 78 ± 12, β-ED 5 µg 94 ± 12) and HS (CSF 6 ± 2 < β-ED 1 µg 33 ± 5 < β-ED 5 µg 71 ± 4). After drinking began animals receiving β-ED and HS consumed a volume similar to controls (CSF-HS) within 45 min. In other experiments blood pressure was continuously monitored directly from a femoral artery in conscious rats 2 days after surgery. Repeated (hourly) injection of 10 µl 1 M NaCl IVT to the same animal resulted in similar pressor responses (HS ↑ 32 ± 3 mmHg; CSF-HS ↑ 29 ± 2 mmHg; $p>0.05$, paired t, n9). Leucine^5-enkephalin (LE; 100 µg/5 µl) administered 15 sec prior to the second HS, IVT injection significantly reduced the pressor response (HS ↑ 31 ± 3 mmHg > LE-HS ↑ 13 ± 3 mmHg; $p<0.01$, paired t, n7). β-ED (0.5 µg/5 µl) produced a similar inhibition (HS ↑ 30 ± 4 mmHg > β-ED-HS ↑ 16 ± 2 mmHg; $p<0.01$, paired t, n6). Naloxone prevented LE and β-ED inhibition of the pressor response to HS, IVT. Opioid peptides inhibit: 1) drinking behavior stimulated by both intracellular dehydration and hypovolemia and 2) the pressor response to 1 M NaCl, IVT.

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184.18

Withdrawn by Author

184.19 CHRONIC STRESS INDUCES INCREASED BRAIN IMMUNOREACTIVE β-ENDORPHIN CONTENT, WHICH IS PREVENTED BY HYPOPHYSECTOMY. M. West*, V. Havlicek, V. Sundmark*, Dept. of Physiology, University of Manitoba, Winnipeg, R3E OW3.

Several studies have noted that experimental models of acute stress promote analgesia in rats, which is partially naloxone-reversible. Goldstein (Proc. Can. Fed. Biol. Soc. 17, 1974) has suggested that mild sustained nociception would be an optimal stimulus for the central release of endorphin. We studied the effect of chronic stress on the brain content of immuno-reactive (ir) β-endorphin, and the modulation of such an effect by previous hypophysectomy (hypox). Experiments were performed in Sprague-Dawley male rats (200-250 gm.) separated into 4 groups; sham hypox (SH); sham hypox and stress (SS); hypox (H) (by the oropharyngeal route); and hypox and stress (HS). Chronic stress was administered by placing rats on a warm plate at 50°C. for 2 min. four times daily for 7 days. Rats were sacrificed 18 hours following the last stress, by microwave irradiation. Three brain regions normally high in β-endorphin content were studied; hypothalamus, thalamus, and amygdala. Ir β-endorphin was measured by a double-antibody radioimmunoassay. Gel filtration of brain extracts in our laboratory has shown that 98% of β-endorphin immunoreactivity co-elutes with synthetic β-endorphin. Only a minor peak co-elutes with β-LPH.

Following chronic stress, ir β-endorphin content was increased in the regions studied. Prior hypox eliminated the stress-induced increase; in fact hypox rats had lower than control content of ir β-endorphin, as previously reported from this laboratory.

	ir β-ENDORPHIN CONTENT (ng/gm tissue) Mean ± S.E.M.			
Brain Region	SH	SS	H	HS
Thalamus	91 ± 17	153 ± 14[a]	45 ± 5	51 ± 6[b]
Hypothalamus	652 ± 123	2645 ± 935[a]	217 ± 13	285 ± 56[b]
Amygdala	63 ± 15	178 ± 53[a]	24 ± 5	37 ± 12[b]

a - significant difference between groups SH and SS $p<.05$, non-paired t test

b - no significant difference between groups H and HS.

The data suggest that chronic stress induces the mobilization of β-endorphin from the pituitary to the brain and that hypox eliminates this physiological response. (Supported by the MRC of Canada).

184.20 DEVELOPMENT OF A COMBINED HPLC - FLUOROMETRIC QUANTITATIVE ASSAY FOR ENKEPHALINS. K. M. Wu*, J. W. Sloan* and W. R. Martin. Department of Pharmacology, University of Kentucky, Coll. of Med., Lexington, Kentucky 40536.

Several techniques using UV, electrochemical or fluorometric methods have been used for detecting enkephalins in HPLC effluent. Here we are reporting a sensitive method for identifying enkephalins by a precolumn labelling technique in a HPLC - fluorometry system. A Waters C_{18} HPLC column and a Shoeffel GM 970 fluorometer were used. The elution was accomplished with 0.05M pH 7.4 Tris buffer (A) and 100% methanol (B). Two gradients were employed at a flow rate of 2 ml/min. In the first, the starting concentration ratio of A to B was 65:35 and the final ratio was 0:100. The second differed from the first in that the starting Tris - methanol ratio was 40:60. Starting at the injection of the sample, linear gradient was performed for 60 minutes at an ambient temperature of 21 ± 1°C. Enkephalins were reacted with fluorescamine in 0.05M, pH 7.4 phosphate buffer and 20 µl of this product was injected into the column. The effluent was excited at 390 nm and fluorescence detected at 470 nm. The retention times with their standard errors for fluorescamine reacted leucine-enkephalin and methionine-enkephalin were respectively 16.01 ± 0.04 minutes and 13.89 ± 0.03 minutes using the first gradient and 12.15 ± 0.04 minutes and 10.13 ± 0.08 minutes using the second-gradient. D-ala methionine-enkephalinamide was distinguished from methionine- and leucine-enkephalins using the 60:40 gradient in that it had a longer retention time than both. The least detectable amount was at least 2.5 ng. Standard calibration curves were linear. This combination of HPLC and precolumn derivatization procedure using fluorometry is a simple, economic and sensitive method for identifying and measuring opioid pentapeptides.

Supported by a grant from the University of Kentucky Tobacco and Health Research Institute.

184.21 LEVELS OF A CEREBROSPINAL FLUID ENDORPHIN ARE ELEVATED IN PLACEBO RESPONDING CHRONIC PAIN PATIENTS

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Our working hypothesis was that chronic pain patients should have lower than usual concentrations of at least one CSF endorphin during pain and prior to pain relief by a non-drug method and this endorphin should become elevated over the patient's usual baseline level.

We obtained CSF from 2 groups of patients; chronic lower back pain patients (n=20) and pain free elective surgery patients (n=14). All the chronic pain patients underwent a diagnostic spinal examination (DSE) at the University of Tennessee Pain Clinic. The DSE is briefly: lumbar puncture, CSF withdrawal, warmed normal saline (NS) injected followed by graduated doses of drug. The DSE is terminated if complete pain relief is achieved after any step.

Radioreceptor assay of this lumbar CSF following gel filtration chromatography indicated multiple opioid-like components. The major endorphin activities did not correspond to β-endorphin, met or leu-enkephalin. The levels of one component, Peak B, was correlated with the pain status of the patients. The CSF endorphin reported in 1975 (Terenius and Wahlström) and named Fraction I is chromatographically similar to Peak B.

It was found that those chronic pain patients who experienced an auto-analgesia during a pre-drug step of the DSE were members of a sub-population of the chronic pain patients. This sub-group was defined in terms of CSF Peak B concentrations. The CSF Peak B levels of the non auto-analgesia chronic pain patients (n=11) was significantly lower than those of both the pain free group and auto-analgesia chronic pain group ($p < .01$ and $p < .025$ respectively by the Mann Whitney U-test).

This auto-analgesia was usually reported to the doctor just after intrathecal injection of NS. This auto-analgesia may be referred to as a "placebo" pain relief response.

We now believe that a precise knowledge of the time course relationship between both the "placebo" stimulus and pain relief is crucial to the interpretation of Peak B levels and their role in the process of auto-analgesia (placebo response). We believe that the variability of the Peak B levels in the CSF of the placebo responding chronic pain patients could result subsequently to an uncontrolled environment cue. Present studies in progress involve multiple CSF sampling and more detailed pain assessment using a modified DSE. This approach may help elucidate the factors responsible for Peak B level variations in chronic pain patients.

184.22 AVERMECTIN B_{1a} INCREASES THE NUMBER OF GABA RECEPTORS IN THOROUGHLY-WASHED RAT BRAIN MEMBRANES. S. S. Pong* and C. C. Wang. Merck Institute for Therapeutic Research, Rahway, NJ 07065.

Avermectin B_{1a} (AVM), an antiparasitic macrocyclic lactone, reduces lobster muscle membrane resistance by opening the chloride channels (Fritz et al., PNAS, 34: 351, 1979) and blocks transmission from interneurons to motor neurons in the ventral cord of the parasitic nematode Ascaris (Kass et al., PNAS in press). Both actions are reversible by picrotoxin. The drug also stimulates GABA release from mammalian brain synaptosomes (Pong et al., J. Neurochem. 34: 351, 1980) and enhances ^{3}H-diazepam binding to the brain membranes (Williams and Yarborough, Eur. J. Pharmac., 56: 273, 1979) as well as the solubilized receptor complex in the membranes (Pong and Wang, unpublished); the enhanced binding is partially blocked by bicuculline, and unaffected by picrotoxin. When rat brain membranes were thoroughly washed with phosphate buffer through 5 to 7 cycles of freezing, thawing and centrifugation for assays of ^{3}H-GABA binding, AVM (10 μM) was found to increase significantly specific GABA binding by 40-80% without much effect on the non-specific GABA binding. The stoichiometry of drug effect appears to be complicated; AVM at 0.1 to 1.0 μM showed slight inhibition on GABA binding, but became stimulatory at concentrations above 1 μM with a linear dose dependence. Scatchard analysis indicates that AVM (10 μM) does not alter the apparent dissociation constant (23-26 nM) of GABA binding but increases the number of GABA binding-sites from 3.3 to 5.2 pmoles per mg protein. Bicuculline (10^{-4}M) completely blocked the original and the AVM-stimulated GABA binding, whereas picrotoxin (10^{-4}M) antagonized very specifically the AVM-stimulated GABA binding with an IC_{50} value of 4 μM. The AVM-stimulated GABA binding is also chloride-ion dependent; substitution of Tris-Cl with Tris-citrate or Tris-acetate in the reaction mixture resulted in total loss of the stimulatory effect of AVM. The optimal concentration of Cl^- was about 60 meq. for the drug action. These results suggest that the AVM action is very closely associated with the chloride ion channel or that AVM may have exposed a new population of GABA receptors which are sensitive to picrotoxin and dependent on Cl^- for ligand binding.

184.23 IMMUNOCHEMICAL COMPARISONS OF L-GLUTAMIC ACID DECARBOXYLASE FROM DIFFERENT SPECIES. Y.Y.T. Su*, J.-Y. Wu and D.M.K. Lam. Cullen Eye Institute and Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

L-Glutamic acid decarboxylase (GAD), the enzyme involved in the synthesis of GABA, has been purified from catfish brain and antisera against this enzyme were produced from rabbits. The species specificities of GAD were examined immunochemically using antisera against catfish GAD. The antibodies were found to cross-react with GAD from brains of goldfish, chick, frog and turtle in immunodiffusion tests. Enzymes from rabbit, rat, bovine, drosophila and crayfish did not show precipitin bands by these tests. Enzymes from crayfish, frog, chick, drosophila and goldfish were inhibited to different degrees by anti-GAD but bovine, rabbit or rat enzymes were not inhibited. The results from microcomplement fixation tests were consistent with those from enzyme inhibition and immunodiffusion tests. These immunochemical comparisons indicate that GAD from lower vertebrates and some invertebrates can be studied immunologically using antibodies against catfish GAD. Antibodies against mouse brain GAD, which have also been produced, cross-react only with the enzymes from mammals, chick and frog. Thus with the combination of the antibodies against mouse brain GAD and catfish brain GAD, it is now possible to study immunologically the GABA-ergic systems of almost all the vertebrate species and also some invertebrate species. Localization of GAD containing neurons in the chick retina using these antibodies were compared.

This work was supported by research grants from the U.S. National Institutes of Health.

185.1 RECEPTOR CHANGES FOLLOWING SHOCK AVOIDANCE. D.R. Cherek, J.D. Lane, M.E. Freeman* and J.E. Smith. Psychiatry Research Unit, Department of Psychiatry, LSU Medical Center, School of Medicine, Shreveport, LA 71130.

Shock avoidance has been used to study the effects of stress upon brain neuronal systems. This research project was initiated to determine if different lengths of exposure to shock avoidance and shock presentation resulted in changes in brain acetylcholine, benzodiazapine or dopamine receptors. Pairs of Fisher (F-344) littermate rats were exposed to one or four 8 hour sessions of electric footshock presentation. One of each littermate pair was placed in a chamber containing a response lever. Responding on this lever was maintained by a signaled Sidman avoidance schedule with an R-S and S-S interval of 40 seconds. The last 20 seconds of this interval was signaled by a flashing light and clicker. Each lever press during the first 20 seconds postponed shock presentation for 40 seconds and terminated the flashing light and clicker. The other member of the littermate pair was placed in an identical chamber with no lever present and was also presented with the flashing light and clicker. If the avoiding animal failed to emit a lever press for 40 seconds, both rats of the pair received an electric footshock (1 mA 500 msec). Four pairs were exposed to one 8 hour session and four pairs to four consecutive 8 hour daily sessions. Following the termination of the first or fourth session, both animals of the pair were sacrificed by decapitation and the heads stored at -70°C. Brains were removed at -20°C, and dissected into cortical regions, striatum, hippocampus and diencephalon. Tissue from four animals from each treatment condition was pooled and total particulate membranes prepared. The resulting membrane suspension was incubated at the appropriate time and temperature in the appropriate buffer with either 0.6 nM [^{3}H] QNB in the presence or absence of 10 µM atropine or 30 pM or 0.03 mM or .35 mM 350 pM [^{3}H] spiperone in the presence or absence of 1 µM (+)-butaclamol or 3 nM [^{3}H] diazepam in the presence or absence of 3 µM clonazepam. Although there were many differences when comparing avoider and yoked-shock animals, the most striking results were observed in the cortical regions. When comparing the yoked-shock animals with their avoiders after 32 hours, muscarinic acetylcholine receptors were increased, while benzodiazepine receptors were decreased. High affinity dopamine receptors did not appear to vary while low affinity dopamine receptors increased in the frontal cortex (mesolimbic system) and decreased in other regions. These changes probably result from emotional and conditioning components of this paradigm. (This project was supported in part by USPHS Grants DA-01999-04 and MH-31835.)

185.2 RECEPTOR CHANGES FOLLOWING CONDITIONED EMOTIONAL RESPONSE. J.D. Lane, M.P. Sands*, J.E. Smith and D.R. Cherek. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Conditioned emotional response (CER) is thought to be an animal model for anxiety. CER has frequently been used to evaluate behavioral-drug effects with anxiolytics clearly attenuating the emotional response. To evaluate the neurochemical changes associated with this paradigm, groups of three littermate Fisher F-344 rats were food deprived and shaped to lever press in a standard operant chamber. Lever pressing was maintained by a variable interval (VI) 1 min schedule of food presentation. Following stabilization of responding, one hour classical conditioning sessions were initiated. Classical conditioning consisted of presenting conditioned stimulus (tone) of varying lengths (2 min-10 min) and unconditioned stimulus (foot shock, 1 mA, 500 msec) approximately 8 times during each session. UCS was presented at the end of CS. One rat of each group received this classical conditioning for 10 sessions. To isolate the conditioning component from the shock history and animal activity, the other two littermates received either CS (tone) or USC (shock) only. On test day, the CS was presented to the triads for 15 minutes while lever pressing was maintained by the food presentation schedule, afterwhich the animals were decapitated and stored at -70°C until analysis. The pre-tone and post-tone responding on the VI1 schedule were compared. The CER animal responded very little (<<1 res/min) after tone presentation, while both controls continued at pre-stimulus rates (circa 15 res/min). Brains were dissected into discrete cortical and subcortical areas and total particulate membrane fractions were prepared. Membrane suspensions were evaluated for binding of tritiated QNB, spiperone, muscimol and diazepam in the presence or absence of atropine, (+)-butaclamol, GABA or clonazepam, respectively. When comparing the CER animals with their shock-only controls, frontal and associative cortical areas had decreased benzodiazepine and increased muscarinic acetylcholine receptors. These receptor data are correlated with multiple neurotransmitter utilization (turnover) in the same regions. This data is consistent with CER being a model for studying emotional behavior in the absence of an aversive stimulus. (supported by USPHS Grant MH-31835)

185.3 INTERACTION OF NEUROTRANSMITTER SYSTEMS IN THE MEDIATION OF HYPOTHERMIA IN THE MOUSE. C. L. Melchior and B. Tabakoff, Dept. of Physiology & Biophysics, Univ. of Ill. Med. Ctr. and West Side V. A. Med. Ctr., Chicago, IL 60612.

Hypothermia occurs in the mouse in response to agonists of the central noradrenergic, dopaminergic, serotonergic, and cholinergic systems. In order to determine how these neurotransmitter systems interact, we examined the effects of agonists of different systems in mice in which selective amine depletions were made with neurotoxins.

C57Bl mice were intraventricularly injected with 10 µl of artificial cerebrospinal fluid (CSF) or CSF containing 50 µg of 6-hydroxydopamine (6-OHDA) to deplete catecholamines or 75 µg of 5,7-dihydroxytryptamine (5,7-DHT) to deplete serotonin. All of the mice given 5,7-DHT and half of those given 6-OHDA were injected with 25 mg/kg of desmethylimipramine i.p. 45 mins. prior to the central injections to protect against noradrenergic depletion. The animals were tested for their temperature response to agonists of various systems 1-3 weeks after the neurotoxin treatment. Drugs were presented in a randomized block design with at least 48 hrs. between the i.p. injections. Body temperature was measured with a rectal probe before and every 15 mins. for 2 hrs. after injection. At the end of the experiment, the animals were sacrificed and the brains analyzed by a spectrophotofluorometric procedure for levels of norepinephrine, dopamine, and serotonin.

The α-adrenergic agonist clonidine produced a greater and longer lasting fall in body temperature in 5,7-DHT-treated mice than in CSF-treated animals. In contrast, the hypothermia induced by the serotonin precursor 5-hydroxytryptophan was blocked in the 5,7-DHT group but exacerbated in animals with norepinephrine depletion. These results suggest a reciprocal inhibitory action between noradrenergic and serotonergic neurons.

The hypothermic response to the muscarinic cholinergic agonist oxotremorine was attenuated by 6-OHDA treatment, indicating a noradrenergic link in cholinergic-induced hypothermia.

The fall in body temperature caused by the dopamine agonist piribedil was blocked in the 5,7-DHT group. This supports the idea that there is a serotonergic link in dopamine hypothermia. Although the mice given 6-OHDA did not show as great a fall in temperature as controls, they were far more activated. The activity of these animals may explain, at least in part, the diminished temperature response. In contrast, animals given DMI-6-OHDA to produce predominantly a depletion of dopamine manifested a greater sensitivity to the hypothermic actions of piribedil.

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185.4 BEHAVIORAL EFFECTS OF YOHIMBINE: ANTAGONISM BY ANXIOLYTICS. Ronald G. Browne, Dept. Pharmacol., Central Research, Pfizer Inc., Groton, CT 06340

Yohimbine, a naturally occurring alkaloid, is known to be a selective antagonist of presynaptic α- noradrenergic receptors. In man, this agent produces a number of effects including stimulation, hallucinations and anxiety. The clinical observation that yohimbine-induced "anxiety" could be reversed with diazepam suggested the present study where attempts were made to antagonize the discriminative stimulus properties of yohimbine with various anxiolytics.

Twenty-four male Sprague-Dawley rats weighing 250-300 g at the start of the experiment were trained to discriminate 3.2 mg/kg of yohimbine hydrochloride from saline in a 2 lever operant procedure. Fifteen minute sessions were conducted five days per week beginning 30 minutes after drug or saline administration. The rats were required to press the left lever under a FR-10 schedule of reinforcement on those days when yohimbine was administered, and to press the right lever during saline sessions. Incorrect responses were recorded but had no programmed consequence. Discrimination accuracy was established when the animals consistently emitted their first FR-10 responses on the appropriate lever. Following about 40 training sessions all animals were reliably discriminating yohimbine from saline as evidenced by having selected the correct lever in 9 out of 10 consecutive sessions.

Twice weekly test sessions were conducted in order to examine the similarity between yohimbine and various drugs, as well as to attempt antagonism of yohimbine's discriminative stimulus properties. The results of generalization testing indicate that piperoxane, another presynaptic α_2 antagonist with anxiogenic properties in man, produces yohimbine-like discriminative effects. In contrast to yohimbine and piperoxane, clonidine and prazosin were discriminated as saline, suggesting that yohimbine's discriminative properties are mediated through α_2 receptors.

When the rats were pretreated with diazepam, a dose related antagonism of yohimbine's stimulus properties was observed. Thus, all animals selected the saline appropriate lever following the simultaneous administration of 17.8 mg/kg of diazepam and 3.2 mg/kg of yohimbine. A partial antagonism of yohimbine cueing was also obtained with meprobamate, phenobarbital, chlordiazapoxide, and clonazapam. These results suggest that yohimbine discrimination in rats may be a useful model for detecting agents with anxiolytic activity.

185.5 DOSE-DEPENDENT TOLERANCE TO A BEHAVIORAL EFFECT OF NICOTINE. Glenn Daniel Todd* and John Dougherty. Depts. of Pharmacology and Psychiatry, University of Kentucky and Veterans Administration Medical Center, Lexington, Kentucky 40511.

We have previously reported that tolerance to the suppression of lever pressing by nicotine developed according to two time courses: rapidly (within hours) and slowly (over days) when 200 µg/kg (first 9 days) and then 350 µg/kg (next 10 days) of the base were injected i.p. twice daily at intervals of 1,2,4, or 8 hours on successive days (Soc. Neurosci. Abstr., 5:665, 1979). As an extension of that study, we now report the effects of chronic injections of higher doses (500 and 650 µg/kg/inj base) on the slowly developing tolerance to the effects of the first daily injection. Twenty male albino Wistar rats, divided into 4 groups of 4-6 rats each, were maintained at 80% of their free-feeding weight by limiting water access and were injected twice daily at the four dosing intervals listed above, with each injection followed by a 30-minute session of water-reinforced lever pressing according to an FR50 schedule.

We previously found that tolerance to the initial suppressant effect of the first daily injection of nicotine on responding developed slowly over successive days at doses of 200 and 350 µg/kg/inj (400 and 700 µg/kg/day). When the dose was increased from 350 to 500 µg/kg/inj (1000 µg/kg/day), the suppression of responding at the beginning of the session was lengthened, as was previously found when the dose was increased from 200 to 350 µg/kg/inj. However, unlike the findings at lower doses, tolerance at this increased dose was not evident after 12 days. Increasing the nicotine dose further to 650 µg/kg/inj (1300 µg/kg/day) produced an additional slight lengthening of the suppression duration. Tolerance at this dose was also not seen over four days of dosing. Dosing was stopped at this largest dose because of progressive weight loss and the increasing appearance of fasciculations and muscular rigidity.

These data indicate that the tolerance that slowly develops to a behaviorally suppressant effect of nicotine over a period of days is dose-dependent and does not occur with doses at or above 500 µg/kg/inj. These data are consistent with other research findings that show a lack of tolerance to the body weight lowering effects of the drug in rats. More significantly, our findings are compatible with the development of certain types of cigarette smoking patterns in which initially increasing smoking rates are followed by long periods of stable smoking frequency.

185.6 EFFECTS OF CLONIDINE ON ACTIVITY AND LEARNING IN 6-HYDROXYDOPAMINE AND NORMAL DEVELOPING RAT PUPS. B.A. Shaywitz, A.S. Wolfe*, D.J. Cohen*, J.G. Young*, G.E. Anderson* and M.H. Teicher. Lab. Develop. Neurobiol. and Develop. Neurochem., Yale Univ. Sch. Med. New Haven, CT 06510.

Intracisternal administration of 6-hydroxydopamine (6-OHDA) (preceded by desmethylimipramine) to neonatal rat pups results in selective reduction of brain dopamine (DA) to concentrations 10-25% of controls, hyperactive motor activity which abates with maturity and persistent cognitive deficits. Such behavior may result from a predominance of noradrenalin (NA) compared to DA and should be attenuated by reduction in NA activity. We have examined the effects of clonidine (CD), an agent which at low dosages will decelerate the utilization of NA and inhibit the firing rate of NA neurons in the locus coeruleus via inhibitory autoreceptors, an action which would be expected to reduce the hyperactivity and the cognitive deficits. Normal and 6-OHDA pups were treated with saline, or CD (50 µg/kg-low dose, 500 µg/kg-high dose) given 30 minutes prior to observation. Activity levels were significantly affected by age averaging 36.8%, 35.0% and 22.7% for 12, 19 and 26 days respectively; activity was also significantly influenced by dose averaging 28.4% at 0 dose, 39.4% for low and 26.7% activity at the high dose. Activities averaged 32.5%, 45.1% and 32.9% at 12 days, 17.7%, 53.8% and 33.6% at 19 days and 35%, 19.4% and 13.6% at 26 days for 0, low and high doses respectively. This indicated a significant age X dose interaction, with the low dose increasing activity at 12 and 19 days, while the high dose had no effect at 12 or 19 days, and both doses reduced activity at 26 days. Reduction of activity by 34% over the hour long observation period (habituation of activity) was observed for pups receiving 0 or the high dose, but habituation of activity was not demonstrated for those receiving the low dose. Avoidance learning in a shuttle box at 28 days was not affected by the low dose but increased by 82% indicating a significant impairment by the high dose. Interactions between CD and 6-OHDA were not observed for either activity or the avoidance task.

The impaired habituation of activity after low doses of CD indicates a role for NA mechanisms, with similarities to the dorsal bundle extinction effect proposed by Mason and Fibiger. Furthermore, our results underscore the important role of dopamine and noradrenalin in the ontogeny of motor activity and avoidance learning but suggest that the development of other neural systems (serotonin, GABA, substance P) may also influence the response to CD.

185.7 CHRONIC AMPHETAMINE INFLUENCES STIMULUS FILTERING. Venera Bruto, Hymie Anisman and Larry Kokkinidis. Carleton University and University of Saskatchewan.

When permitted to explore a multiarmed maze, mice tend to visit most frequently those arms least recently entered (spontaneous alternation). When treated with amphetamine (3.0-7.0 mg/kg) mice exhibit perseverative responding (i.e., successive visits between two arms). Following repeated drug treatment (5 - 10 mg/kg for 5 - 10 days) the perseverative pattern of responding ordinarily elicited by amphetamine is eliminated; however, alternation responding does not appear either. Rather, mice exhibit a random pattern of responding. Such behavior was evident even when mice were tested in the nondrug state. The effects of chronic amphetamine did not appear to be due to reduced effectiveness of the drug. Specifically, if mice were preexposed to the maze, thereby reducing the alternation tendency, the perseveration induced by amphetamine was maximized. Thus, doses that ordinarily reduce alternation to chance levels, induced marked perseveration follow apparatus preexposure. In contrast, preexposure did not influence behavior of mice that received chronic amphetamine. It is suggested that NE depletion provoked by chronic amphetamine treatment results in deficits of filtering information. As a result mice chronically treated with amphetamine do not adequately attend to apparatus cues and are unaffected by previous experience in the maze.

185.8 COMPARATIVE ASSESSMENT OF EQUILIBRIUM PERFORMANCE OF RHESUS AND CYNOMOLGUS MONKEYS: EFFECTS OF ATROPINE. C. T. Bennett, N. E. Lof*, D. N. Farrer*, and J. L. Mattsson*. USAF School of Aerospace Medicine, Brooks AFB, Texas 78235.

The primate equilibrium platform (PEP) consists of a small restraining chair that is pitched forward and backward from horizontal by a computer-driven, random signal (band-passed 0 to 0.4 Hz). Six M. mulatta (rhesus) and six M. fascicularis (cynomolgus) monkeys were trained by mild shock reinforcement to operate a control stick to maintain themselves in a relatively horizontal position. Cynomolgus monkeys took an average of approximately six weeks longer than rhesus monkeys to reach a similar level of performance. Previous clinical screening of the effects of atropine sulfate indicate rhesus to be approximately 5 to 10 times more sensitive, in terms of mydriasis and muscular weakness. The effects of four doses of atropine on performance in the PEP were tested. As expected, as atropine doses increased, the PEP performance decreased, with rhesus performance changing at lower doses than fascicularis.

185.9 SEIZURES INDUCED BY CHRONIC GABA ELEVATION WITH γ-ACETYLENIC GABA. S.E. Bachus, A.B. Young & E.S. Valenstein; Depts. Psych. & Neurol., Univ. Michigan, Ann Arbor, Michigan 48109.

The GABA transaminase inhibitor γ-Acetylenic GABA (GAG), reported to elevate brain GABA levels (Jung et al., Neurochem. 28: 717, 1977), has been proposed as an anticonvulsant (Schecter et al., GABA-Biochemistry & CNS Functions, ed. P. Mandel & F.V. De Feudis, Plenum, p. 43, 1979) and also tested clinically as a treatment for Huntington's Chorea (Böhlen et al., Brain Res. Bull.4:692, 1979) and tardive dyskinesia (Casey et al., Brain Res. Bull. 4:698, 1979). Two cautions have been noted: seizure-protection does not persist as long as does maximal GABA elevation (Wood et al., Neurochem. Res. 2:707, 1977); and single high doses of GAG can cause seizures (Schecter & Grove, Brain Res. Bull. 4:712, 1979). We now report results which indicate that chronic low doses of GAG can induce seizures during a period while brain GABA levels are declining to baseline values.

Male Sprague-Dawley and Long Evans rats with lateral hypothalamic electrodes (N=8) were administered daily i.p. injections of 50 mg/kg GAG. Grand mal tonic-clonic convulsions were observed during electrical self-stimulation and programmed stimulation: regularly at 23 hrs, occasionally at 8-12 hrs, but never at 4 hrs post-injection (when GABA elevation is maximal) or after saline injections. Seizures were first seen after the second or third daily GAG injection, and thereafter observed regularly for as long as 16 days. 8 naive rats without electrodes were then monitored during 8 days of GAG treatment. Spontaneous seizures were detected during the 20-24 hrs post-GAG period in 5 rats.

To test the hypotheses that these seizures might be due to below-baseline GABA during GAG withdrawal or to development of GABA receptor subsensitivity, naive rats were sacrificed at 4 hrs post-GAG (N=6), at 23 hrs post-GAG (N=6), and at 23 hrs post-saline (N=6) on the third day of treatment. GABA levels (Enna & Snyder, J. Neurochem. 26:221, 1976) and GABA synaptic receptors (Williams & Risley, J. Neurochem. 32:713, 1979) in frontal cortex were assayed. GABA levels were significantly elevated at 4 hrs post-GAG ($\bar{X}$=12.60 μmole/g) relative to saline ($\bar{X}$=5.55 μmole/g), and still slightly though no longer significantly elevated at 23 hrs post-GAG ($\bar{X}$=7.10 μmole/g). GABA receptor sensitivity, however, did not differ significantly at any time period. Localized brain changes in GABA might provide an explanation for the appearance of seizures after low doses of chronic GAG treatment. Meanwhile, the possibility of inducing spontaneous seizures with GAG treatment should be seriously considered before recommending the clinical use of this drug.

185.10 ACUTE AND CHRONIC NALTREXONE TREATMENT: DIFFERENTIAL EFFECT ON MORPHINE-INDUCED LOCOMOTOR ACTIVITY AND ANALGESIA. J.M. Ng Cheong Ton*, R.S. Blair*, L.J. Holmes* and Z. Amit. Dept. of Psychology, Concordia University, Montreal, Quebec, Canada.

Three studies were conducted to investigate the opiate receptor's role in morphine's biphasic effects on locomotor activity (LA) and morphine's analgesic properties.

In the first study, naltrexone (2 mg/kg) injected subcutaneously (S.C.) 2 hr prior was found to attenuate both the excitatory and the depressive actions of morphine (1, 4 or 8 mg/kg, S.C.) on LA in an open-field. These results suggest that the opiate receptor mediates the biphasic effects of morphine.

It has been shown that chronic treatment with opiate antagonists increase the number of opiate receptors. In the second study, we examined the effect of chronic naltrexone treatment on morphine's biphasic effect on LA. Animals were injected daily with naltrexone (10 mg/kg, S.C.) for 24 days. Three days after the termination of naltrexone treatment, animals were treated with morphine (1, 4 or 8 mg/kg, S.C.) and open-field LA was recorded. Naltrexone was found to shift the biphasic activity curve to the right. The shift appears to have resulted from an increase in the duration of depression for naltrexone treated animals.

In the third study, animals received naltrexone and morphine treatments identical to those in the second study. Following the morphine injection, animals were placed within a plexiglas cylinder on a hot-plate every 15 min for 90 min. Paw-lick and escape latencies were recorded. Naltrexone potentiated morphine's effect on paw-lick but not escape latencies.

The observation that chronic naltrexone treatment did not potentiate all effects of morphine suggests that only specific opiate receptor populations were affected.

185.11 FOOD INTAKE ALTERS BEHAVIORAL EFFECTS OF NALOXONE. Diane Snell and R. Adron Harris. Depts. Psychology and Pharmacology, Univ. Missouri and Truman V.A. Hospital, Columbia, MO 65212

Few studies have evaluated the behavioral effects of chronic administration of narcotic antagonists. The present investigation explores the effects of repeated administration of naloxone as a function of food intake. In food deprived rats, neither single nor repeated injections of naloxone or naltrexone produced any alteration of food-reinforced responding (Harris, JPET 213: in press, 1980). It was noted, however, that following one injection of 30 mg/kg of naloxone, which itself had little effect, free feeding animals performing on a shock avoidance task became so sensitive to subsequent injections that administration of as little as 0.1 mg/kg of naloxone lead to disruptions in performance. This lead us to postulate that repeated administration of naloxone might lead to sensitivity in free feeding animals but not in food deprived animals. In order to test this hypothesis, rats were trained to avoid shock under a continuous avoidance schedule. Naloxone was administered twice/week to free-feeding rats until they became sensitized to the drug. Sensitivity was measured by increase in shock rate. Animals were considered sensitized when their shock rates were at least 3 times higher than their control rates for 5 consecutive injection days. Following 6 injections of 0.3 mg/kg of naloxone none of the rats were sensitized. The dose was then increased to 3.0 mg/kg, which lead to sensitization in some of the rats, while others required 10 mg/kg to produce a disruption in performance. When the rats became sensitized, they were reduced to 80% of their free-feeding weights. Injections of naloxone were continued twice/week until the rats showed little or no disruption in performance in response to the same dose of naloxone which had produced sensitivity when the rats were free feeding. At this time the animals were given free access to food and sensitivity to the behavioral effects of naloxone was reinstated. In contrast, mice given 17 daily injections of 3 mg/kg of naloxone did not show alterations in hot plate latency or in the hyperalgesic activity of naloxone as measured by the hot plate test. The effects of chronic administration of higher doses of naloxone on the hot plate test as well as results obtained with other tests will be presented. In summary, chronic administration of naloxone sensitized free-feeding rats to behavioral effects of the drug, while no sensitivity was detected in rats which were food deprived. In addition, free feeding animals which had been sensitized were no longer affected by naloxone when their body weights were reduced. This points to a critical role of dietary factors in the regulation of sensitivity to narcotic antagonists. Supported in part by V.A. Research and PMAF.

185.12 LIMBIC NEUROTRANSMITTER CHANGES CORRELATED WITH INTRAVENOUS OPIATE SELF-ADMINISTRATION. J.E. Smith, C. Co*, M.E. Freeman*, M.P. Sands* and J.D. Lane. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

The self-administration of opiates has been shown to result from their rewarding properties. This study was initiated to determine if limbic neuronal systems are involved in opiate reward. The turnover rates for dopamine (DA), norepinephrine (NE), serotonin (5-HT), aspartate (Asp), glutamate (Glu), glycine (Gly) and gamma-aminobutyric (GABA) were measured in rats intravenously self-administering morphine (I) and in yoked-morphine infused (II) and yoked-vehicle infused (III) littermates. Eleven litters of three (conditions I-III) male F-344 rats were implanted with chronic jugular catheters and two littermates in each group made physically dependent upon morphine with hourly automatic infusions. One of the physically dependent rats was then allowed to self-administer morphine (10 mg/kg) by lever pressing. The other two littermates in each group received simultaneous yoked infusions. After stable baselines of self-administration were obtained an average interinjection interval was calculated for each self-administering animal. At 60 min (N=5 litters) or 90 min (N=6 litters) prior to a predicted self-infusion, 0.5 mCi of [^{3}H]-tryptophan, 1.0 mCi of [^{3}H]-tyrosine, and 0.2 mCi of [^{14}C]-D-glucose were injected through the jugular catheter and the litter sacrificed by immersion in liquid nitrogen at the predicted self-administration time. The brains were removed at -20°C and the frontal-pyriform cortex (FPC), nucleus accumbens (NA), septum (S), hippocampus (HI), amygdala (A) and hypothalamus (HY) dissected from serial coronal sections at -20°C. Content and turnover rates were determined by previously described procedures (Smith et al, Prog. Neuro-Psychopharm., 2, 359-367, 1978; Freeman et al, Anal. Biochem. in press). Passive morphine infusion (yoked-morphine vs yoked-vehicle) resulted in the following turnover changes: decreases in DA in the S, increases in NE in the HI, decreases in 5-HT in the FPC and S, increases in Asp in the FPC, S and HI and a decrease in the NA, increases in Glu in the FPC, S and HI and decreases in the NA and A, an increase in Gly in the S and increases in GABA in all areas except the S. The effects of morphine self-administration (comparison of the self-administration group with the yoked-morphine group) showed the following turnover changes: DA to be increased in the FPC and S, NE increased in S and HI, 5-HT increased in the FPC and S and decreased in the NA, Asp and Glu increased in all areas except the septum and GABA increased in the FPC, HI and A and decreased in the NA. Limbic neuronal systems appear to be involved in morphine reinforcement processes. (Supported in part by USPHS Grant #DA-01999-04 and MH-31835.)

185.13 PHARMACOLOGICAL DISSOCIATION OF SHAKING BEHAVIOR PRODUCED BY MET-ENKEPHALIN, TRH and SEROTONIN. Eugene G. Drust*, Robert S. Sloviter and John D. Connor. Department of Pharmacology, Pennsylvania State University, College of Medicine, Hershey, PA 17033.

A pharmacological comparison was made of shaking behavior produced by intracerebroventricular injection into rats of met-enkephalin (ME), thyrotropin-releasing hormone (TRH) and serotonin (5-HT). ME caused dose-dependent shakes in the dose range of 1-500 µg. Shakes occurred within 1-2 min after injection; virtually all shakes were observed in the first 5 min period after injection. TRH produced dose-dependent shakes in the dose range of 10 ng to 1 µg. TRH shakes began within 2-4 min after injection; the duration of the shaking response to TRH was dose-dependent (5-30 min). Shakes induced by intraventricular administration of 5-HT were studied in rats pretreated with the monoamine oxidase inhibitor, tranylcypromine (20 mg/kg, ip, 30 min before 5-HT) as described previously (Drust et al., Pharmacology 18: 299, 1979.

The role of 5-HT mechanisms in shaking behavior was examined by pretreating rats with p-chlorophenylalanine (pCPA), 5,7-dihydroxytryptamine (5,7-DHT) or methysergide (MS). None of these treatments significantly affected ME or TRH shakes, indicating that 5-HT mechanisms are probably not involved in these effects of ME or TRH. In contrast, 5-HT shakes were significantly potentiated by 5,7-DHT pretreatment and significantly decreased by pCPA or MS. The opiate receptor antagonist naloxone significantly inhibited ME shakes but had no effect on TRH or 5-HT shakes. The effects were also studied of pretreatment with the catecholamine neurotoxin, 6-hydroxydopamine (6-OHDA), or the tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine (α-MpT), on the shaking behavior produced by these substances. While 6-OHDA or α-MpT pretreatment had no significant effect on 5-HT shakes, TRH shakes were decreased by both 6-OHDA and α-MpT. ME shakes were not significantly affected by α-MpT pretreatment but were significantly potentiated after 6-OHDA. This suggests that central catecholamine mechanisms may be involved in shakes produced by ME and TRH; however, the possibility exists that 6-OHDA and α-MpT produce some of these effects by mechanisms unrelated to catecholamines.

These data indicate that shaking behavior produced by these substances is apparently elicited by 3 different mechanisms. In light of the proposed role of ME, TRH and 5-HT as neurotransmitters or neuromodulators and the presumed existence of separate receptors for these compounds, the shakes produced by these substances may be useful as quantitative models of central enkephalin, TRH and 5-HT activity, respectively.

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185.14 COMPARATIVE EFFECTS OF LITHIUM ON DRUG-INDUCED AND LESION-INDUCED DOPAMINERGIC SUPERSENSITIVITY. Eliot L. Gardner, Ira Hirschhorn, Thomas F. Seeger*, Michael Weiss* and Maynard H. Makman*. Depts. of Psychiatry, Neuroscience, Biochemistry and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, N.Y., 10461.

We have previously reported (Seeger and Gardner, Soc. Neurosci. Abstr. 5: 662, 1979) that chronic lithium (Li^+) administration attenuates the development of mesolimbic dopaminergic (DA) behavioral supersensitivity. A similar blocking effect of Li^+ on nigrostriatal DA supersensitivity has also been reported (Pert et al, Science 201:171, 1978). However, these studies dealt only with drug-induced DA supersensitivity, leaving open the possibility that the demonstrated effects are unique to the drug-induced condition. The present study was designed to test for an effect of Li^+ on the development of lesion-induced DA supersensitivity. Rats received unilateral 6-hydroxydopamine lesions of the substantia nigra to produce DA denervation supersensitivity of the ipsilateral striatum. Half of the animals were given Li^+ carbonate in their food (1600 mg/kg of powdered chow) for three weeks, starting one week before surgery. Control rats received powdered chow without Li^+. Animals were tested for rotational behavior following systemic administration of apomorphine (2 mg/kg) after three weeks of Li^+ treatment and, again, four weeks after termination of Li^+ treatment. The Li^+-treated animals showed behavioral supersensitivity after three weeks of Li^+ chow, but at only 65% of the magnitude shown four weeks after the termination of the Li^+ treatment, when they were completely (control level) supersensitive. Control animals were equally supersensitive at the end of three weeks of control chow as four weeks later. Partial attenuation of supersensitivity after three weeks was also indicated by biochemical studies of striatal DA-stimulated adenylate cyclase. Thus, while Li^+ significantly attenuated the development of lesion-induced DA supersensitivity, the effect was incomplete. We also observed only a partial attenuating effect of Li^+ on the development of chronic haloperidol-induced DA behavioral supersensitivity, as measured by DA-mediated intracranial self-stimulation. Thus, while chronic Li^+ attenuates both the magnitude and time-course of both lesion-induced and neuroleptic-induced DA supersensitivity, the effect is neither complete nor permanent. The impermanence of the effect has also been confirmed by biochemical studies, in which we find that chronic Li^+-treated animals become fully supersensitive, in terms of increased ^{3}H-ADTN binding, when taken off the daily Li^+ regimen.

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185.15 THE EFFECT OF LITHIUM ADMINISTRATION ON APOMORPHINE INDUCED ROTATION FOLLOWING UNILATERAL DESTRUCTION OF THE NIGRO-STRIATAL PATHWAY IN THE RAT. M. Gruenthal* (SPON:J.A. Simmons). Dept. of Psychol., Washington Univ. St. Louis, MO 63130.

Previous reports have indicated that combined lithium-haloperidol administration may prevent the development of haloperidol induced behavioral supersensitivity to apomorphine (Pert, A. et al., Science, 201:171, 1978). The present study was conducted to examine the effect of lithium administration on the development of apomorphine induced contralateral rotation following unilateral denervation of the striatum, a phenomenon which appears to be due to the development of postsynaptic receptor supersensitivity (Creese, I. et al., Science, 197:596, 1977).

Male albino rats (sixty days old) received unilateral intra-nigral injections of either 6-hydroxydopamine or of the vehicle alone. Beginning 24 hrs. after surgery subjects received daily injections of either lithium chloride (2meq/kg i.p.) or isotonic saline for 14 days. Seven days after the last injection all subjects received apomorphine (0.25mg/kg sc.) and 10 min. later were placed in automated rotometers for a period of 15 min.

Rats in the lesion-lithium group did not differ from subjects in the lesion-saline group with respect to apomorphine induced contralateral rotation. Preliminary observations, however, suggest that rats in a similar lesion-lithium group show decreased apomorphine induced contralateral rotation, relative to saline treated subjects with lesions, when apomorphine is administered 3 to 12 hrs. following the last lithium injection. These data suggest that lithium administration may transiently supress, but not permanently eliminate, development of the behavioral consequences of lesion induced supersensitivity.

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186.1 RESPIRATION IN ASTROCYTES FROM PRIMARY CULTURES AND ESTABLISHED CELL LINES DURING EXPOSURE TO ALTERED OSMOLARITY. James E. Olson and David Holtzman*, Dept. of Pediatrics & Neurology, Stanford University School of Medicine, Stanford, CA 94305.

During maturation isolated brain mitochondria become resistant to morphologic alteration and to respiratory inhibition in media of increased osmolarity (Holtzman et al., J. Neurochem 33:453-460, 1979). These properties, which may be confined to a sub-population of whole brain mitochondria, appear coincident with astrocyte maturation. To investigate the proposed localization of these hyper-osmolar resistant mitochondria in astrocytes, we have measured the rates of oxygen consumption in astrocytes from primary culture suspended in media of varying osmolarities. These results are compared with similar studies using cell lines of type II pneumocytes and 3T3 mouse-embryo fibroblasts.

Astrocytes from dissociated neonatal rat brain were grown for two weeks in an Eagle's minimal essential medium supplemented with vitamins, amino acids, and 20% calf serum. The confluent cultures were then grown for an additional five days with 0.25 mM dibutyryl cyclic AMP added to this medium. At the end of this time, cells were suspended in a respiration medium (pH 7.3) which included glucose 55 mM, KCl 2.7 mM, $CaCl_2$ 1.0 mM, $MgCl_2$ 0.50 mM, Na_2HPO_4 8.0 mM, KH_2PO_4 1.5 mM, and variable concentrations of NaCl ranging from 0-822 mM. Basal respiration, phosphorylation-independent respiration (5µM oligomycin added), and maximally uncoupled respiration (20-80µM dinitrophenol added) were measured polarographically using a Clark platinum cathode assembly.

Basal rates of respiration in medium of normal osmolarity (137 mM NaCl) were between 25-30 n at. equiv O/mg protein min for all cell types. The basal rates were reduced by about 50% in the 0 NaCl medium. Basal rates in the pneumocytes fell significantly in each of the hyper-osmolar solutions (NaCl of 274, 548, and 822 mM). Basal rates in astrocytes were constant from 137 to 822 mM NaCl. The respiratory rates in the presence of a maximally-inhibiting concentration of oligomycin were depressed in hypo-osmolar media compared to normal medium in pneumocytes more than in the astrocytes. In hyper-osmolar media up to 548 mM NaCl, oligomycin inhibited rates were increased 2-3 fold in astrocytes compared to a 50% increase in pneumocytes. In both astrocytes and pneumocytes peak uncoupled respiratory rates were found in media of normal osmolarity.

This work was supported by NIH grant NS 07012 and BRSG grant RR 5353.

186.2 AN IMMUNOCYTOCHEMICAL STUDY OF ASTROCYTES ASSOCIATED WITH THE RAT SUPRAOPTIC NUCLEUS. Adrienne K. Salm* and Glenn I. Hatton (SPON: R. A. Pax). Department of Psychology and Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Astrocytic neuroglia associated with the rat supraoptic nucleus was investigated using immunohistochemical techniques. Recent evidence (Cell Tiss. Res. 1977, 181, 59-72) for a functional role of neuroglia in hypothalamic magnocellular hormone release prompted further investigation of the astrocytes in this area.

The unlabeled antibody method of Sternberger (1974) was used to demonstrate immunoreactivity to glial fibrillar acid protein (GFAP) antiserum. (The antiserum was kindly provided by Dr. E. A. Zimmerman.) GFAP is only found in fibrous and protoplasmic astrocytes. Hypothalamic tissue from 12 normal adult male rats was studied in 60 µm, 10 µm, and 6 µm unembedded vibratome or paraffin embedded sections. Immunoreactivity was visualized with 3,3'-diaminobenzidine. This revealed a group of morphologically distinct astroglia, the cell bodies of which lie primarily between SON cell bodies and the pial surface of the brain. The cells are easily distinguishable from all labeled neuroglia encountered in other areas of the hypothalamus, including the internal median eminence, periventricular subependymal region and the medial paraventricular nuclear region. Unlike the latter groups, which display a stellate morphology, the SON astrocytes have extremely long and thin processes which radiate outward in a predominately dorsolateral direction. However, some of these processes are interposed between the SON and the optic tract. The cell bodies of these astrocytes form a tightly packed lamina along the pial surface, diminishing in number as they extend laterally toward the periamygdalar cortex. Astrocytic fibers exhibit their most dorsal extension in the area neighboring SON, where they project for distances up to three times the depth of the nucleus.

At its most caudal extent SON appears to be almost totally surrounded by astrocytic processes except where capillaries exit dorsally from the nucleus. These capillaries are also seen ensheathed by long, thin neuroglial processes. Glial processes have also been observed forming a reticulum around cell bodies of SON neurons.

This research supported by NIH grant NS 09140.

186.3 EFFECT OF DOPAMINE ON PROTEIN SYNTHESIS IN A RAT GLIOMA CELL LINE. L. Roel, V. May*, and K. Braas*, Dept. of Anatomy, Northwestern Univ., 303 E. Chicago Ave., Chicago, IL 60611.

Intraperitoneal (ip) administration of L-dihydroxyphenylalanine (L-DOPA) causes massive disaggregation of rat brain polysomes and inhibition of protein synthesis in vivo (Life Sci. 22: 1887, 1978). Polysome disaggregation occurs between 1 hr and 2 hr after L-DOPA administration, and is temporally correlated with the rise in brain dopamine (rather the rise in *L-DOPA, 3-O-*methyl DOPA, or the depletion of brain S-adenosylmethionine). In addition, pretreatment of animals with Ro4-4602 (800 mg/kg), an L-Aromatic Amino Acid Decarboxylase inhibitor, prevents the L-DOPA-induced brain polysome disaggregation. These findings suggest that the brain polysomal disaggregation following L-DOPA administration is mediated by dopamine.

In order to determine whether observed brain protein synthesis effects are occurring in neurons or glia, studies employing C6 cells (a rat glioma cell line) were conducted. In these studies, C6 cells were grown in Falcon petri dishes, in an atmosphere of 95% air, 5% CO_2 in Ham's F-10 medium supplemented with 10% fetal calf serum and 0.1 µg/ml gentamycin. Confluent cultures were incubated with dopamine hydrochloride (in a final concentration of 1×10^{-5} M), and at various times thereafter, 0.5 µCi ^{3}H-lysine was added to the culture medium. Thirty min later, incubation media were poured off, cells washed once with 5 ml ice cold saline, and cells harvested in 5 ml ice cold 5% trichloroacetic acid (TCA). Incorporation of ^{3}H-lysine into TCA precipitable protein was determined by standard procedures.

Dopamine in concentrations of 10^{-5} M depressed incorporation of ^{3}H-lysine into TCA precipitable protein 25-50%. The maximal effect occurred at 2 hr after addition of dopamine to the media. Protein synthesis rates returned to control values between 4 and 6 hrs after addition of dopamine to the medium. The effect of amphetamine on protein synthesis in C6 cells was also examined under conditions similar to those described above. Results indicate that protein synthesis is inhibited 30-40% 2 hrs after addition of 1×10^{-5} M amphetamine to the medium. Studies are being conducted to determine whether dopamine and amphetamine have similar effects on protein synthesis in a neuroblastoma cell line.

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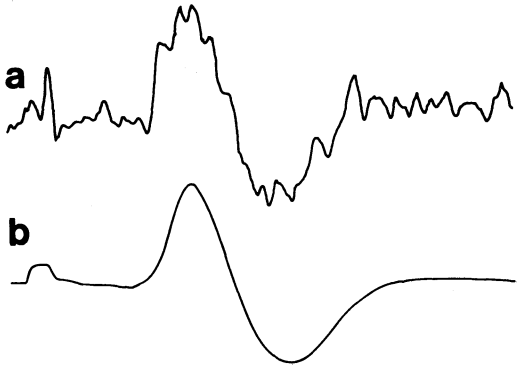
186.4 MAGNETIC MEASUREMENT OF INTRACELLULAR CURRENTS IN ISOLATED FROG SCIATIC NERVES. John P. Wikswo, Jr.*, John P. Barach* and John A. Freeman (SPON: Frank R.Freemon). Department of Physics and Astronomy and Department of Anatomy, Vanderbilt University, Nashville, Tenn. 37235.

We have developed a Supeconducting Quantum Interference Device (SQUID) magnetometer with a minature room-temperature toroidal pick-up coil that has sufficient sensitivity and frequency response to measure the magnetic field associated with the action potential of an isolated frog sciatic nerve (Wikswo, Barach and Freeman, Science 208:53, 1980). The nerve is immersed in Ringer's solution and is threaded through the ferrite core of the toroidal pick-up coil. The coil typically has a diameter of 2.6 mm and has 10 to 30 turns of No. 38 copper wire. The time-dependent magnetic field of the nerve induces currents in the pick-up coil that are detected by the SQUID. Recent improvements to the instrument provide a magnetically shielded environment for the SQUID in a liquid helium storage dewar so that the measurements of the weak magnetic field (100 picotesla) can be made in a typical laboratory. The (a) trace shown below is the output of the SQUID magnetometer electronics with a 1.0 Hz to 2 kHz bandwidth for a frog sciatic nerve threading the toroid. The (b) trace is an average of 1024 responses and clearly demonstrates that high quality magnetic measurements can be made on isolated nerves. Detailed theoretical calculations indicate that the intracellular currents provide the dominant contribution to the extracellular magnetic field, so that this technique provides a non-invasive measurement of the currents flowing inside a nerve.



186.5 Cl^- AND Na^+ EXCHANGE TRANSPORT PROPERTIES IN PRIMARY ASTROGLIAL CULTURES FROM RAT BRAIN. H.K. Kimelberg and S. Biddlecome*, Albany Medical College, Albany, N.Y. 12208.

We have previously reported (Neurosci. Abst. 5, 293, 1979) that Cl^- transport in primary monolayer astroglial cultures from rat brain (at least 80% pure by immunocytochemistry, Neurosci. Abst. 5, 759, 1979) shows kinetic properties consistent with much of the Cl^- being transported by a mediated process similar to the $Cl^- \leftrightarrow HCO_3^-$ exchange system described for red blood cells and other tissues. In further support of this concept we now find that the rate of efflux of $^{36}Cl^-$ from these cells is less in Cl^- and HCO_3^- free media and is increased when Cl^- or HCO_3^- is added back to the medium. Furthermore, addition of choline chloride to detached cells suspended in a Cl^--free, lightly buffered medium causes alkanization of the medium consistent with either intracellular HCO_3^- or OH^- exchanging with external Cl^-. As previously reported (Neurosci. Abst. 5, 293, 1979), the internal Cl^- concentration of around 30mM in these cells is some 3 times greater than expected from equilibration with an average measured membrane potential of -70mV. Since addition of furosemide, an inhibitor of Cl^- transport in other systems, decreases not only the initial rate of Cl^- uptake but the final steady state Cl^- levels by 3 to 5 fold this high $[Cl^-]_i$ could be a consequence of the Cl^- anion exchange system. Also, comparison of steady state Cl^- levels, measured with $^{36}Cl^-$, in a HCO_3^- buffered and HEPES buffered medium shows that $[Cl^-]_i$ levels were some 20% lower in a HCO_3^- buffered medium, suggesting that uptake of HCO_3^- competed with uptake of Cl^-.

These data indicate that the presence of an anion exchange system, perhaps operating in parallel with a much slower conductive system for Cl^-, results in a higher $[Cl^-]_i$ than expected from passive distribution. In other tissues, however, especially in secretory or absorptive epithelium, there is evidence that active transport of Cl^- involves co-transport with Na^+. In our astroglial cultures, however, the rate of uptake and final steady state levels of Cl^- measured with $^{36}Cl^-$ are unaffected by substitution of choline for Na^+ in the external medium. Also, it appears that Na^+ transport in astroglial cells includes a neutral $Na^+ \leftrightarrow H^+$ exchange system since addition of Na^+ to detached cells in a Na^+-free medium caused acidification of the medium. This acidification was partly inhibited by 10^{-4}M amiloride, as was uptake of $^{22}Na^+$ in cells pretreated with ouabain to raise $[Na^+]_i$. Thus, these studies suggest the presence of neutral homo- and hetero- ion exchange systems in astroglial cells which could have profound consequences on ion transport in the brain without markedly affecting the electric potentials developed across astroglial cell membranes. (Supported by NINCDS grant 13042).

186.6 ORTHOGONAL ARRAYS OF PARTICLES IN FREEZE-FRACTURED HYPOTHALAMO-NEUROHYPOPHYSIAL GLIA: DISTRIBUTION AND RELATIONSHIP TO INTERCELLULAR JUNCTIONS. James D. Hatton and Mark H. Ellisman. Dept. of Neurosciences, U. Calif. at San Diego, La Jolla, CA 92093

Orthogonal arrays of 65A particles have been demonstrated in the plasma membranes of several non-neural cell types. However, their relationship to other membrane specializations has not been carefully studied. Such a study might be especially useful in a system where non-neural cells react to physiological stimuli. Using freeze-fracture techniques, we have investigated membrane specializations of the glia of the hypothalamo-neurohypophysial system of the rat. In the paraventricular (PVN) and supraoptic (SON) nuclei, astrocytes in areas of high neuronal density (i.e., magnocellular regions) display orthogonal arrays of 65A particles solely near gap junctions, suggesting a function associated with the glial syncytium. Astrocytes in areas of lower neuronal density (i.e., parvocellular regions) contain additional arrays on membranes not displaying gap junctions. Arrays are especially numerous on astrocytic perivascular end-feet in both nuclei and on the laminations of the pial-glial limitans ventral to the SON. Ependymal cells near the PVN show arrays both on their lateral surfaces (displaying gap junctions) and their apical surfaces (facing the CSF). Tight junctions are not noted on astrocytes or ependymal cells of the dorsal third ventricle, but are noted on both the somas and myelin lamellae of oligodendroglia. Both of these latter membranes occasionally contain gap junctions as well; however, orthogonal arrays are never noted on oligodendroglia.

Pituicytes are the specialized glial cells of the pituitary which are similar in strucuture to the astrocytes of the CNS. The plasma membranes of pituicytes in the neurohypophysis display gap junctions, complex junctions, and tight junctions. Orthogonal arrays are noted near the first two of these, but not near the last. Arrays in the neural lobe appear in highest densities on membranes adjacent to subpial or perivascular spaces. Pituicyte membrane bearing orthogonal arrays appears infrequently near the neural stalk, increasing towards the distal end of the neural lobe. The distribution of orthogonal arrays in this system, as well as in other systems in which they have been noted, suggests a polarization of membrane activity, possibly providing for "trans-astrocytic" movement of substances.

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186.7 BIOCHEMICAL GENETICS OF GLYCOGEN METABOLISM IN CULTURED GLIAL CELL LINES. C. J. Cummins*, W. D. Lust, and J. V. Passonneau. Laboratory of Neurochemistry, NINCDS, NIH, Bethesda, MD, 20205.

The metabolism of glycogen may be predominantly glial in the mammalian brain. We have employed several glial cell lines to characterize the rate of glycogen formation and breakdown, the activities of the regulatory enzymes, and the role of cyclic nucleotides in glycogenolysis. Five glial preparations are considered: (1) primary cultures of neonatal rat astrocytes (RPA); (2) herpes virus transformed RPA cells, (HSV-TRPA);(3) SV-40 transformed adult mouse astrocytes;(4) & (5) glia derived from methylnitrosourea induced tumors in situ (B9 &B82).

The RPA and HSV-RPA cell lines show essentially the same features of glycogen metabolism: the rate of glycogen synthesis is rapid after feeding, accumulating up to 200 nmoles/mg protein at 2.5 hrs, and thereafter the level of glycogen decreases. The maximal level of glycogen is dependent on both the density of the cultures and the concentration of the extracellular glucose. Active ("a") and inactive ("b") forms of both glycogen synthase and phosphorylase exist, and the ratios of "a" to "b" forms are comparable to rat brain. Agents that increase the levels of cAMP promote the typical features of glycogenolytic cascade: quantitative conversion of phosphorylase "b" to "a", and the rapid degradation of glycogen.

B9, B82, and S22 all exhibit a deficient glycogen metabolism and appear not to accumulate significant glycogen. Phosphorylase activities for the B9 cell line are essentially similar to the HSV-RPA, but the active form of glycogen synthase is essentially absent, implying a defect in synthase phosphatase system. The active form of synthase is also low in in the B82 cell line, and the levels of active and inactive phosphorylase are essentially absent suggesting a pleiotropic effect on the metabolism of glycogen. For the S22 cell line, glycogen synthase activity is essentially similar to the HSV-RPA. However, the phosphorylase is found entirely in the active form. Thus, glycogen does not accumulate because the rate of breakdown exceeds the rate of formation, implying a defect in the phosphorylase phosphatase step.

186.8 INCORPORATION OF ^{3}H-LEUCINE BY A REACTIVE GLIAL CELL POPULATION IN THE LESIONED NEWT OPTIC NERVE. Linda L. Phillips and James E. Turner, Dept. of Anat., Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC 27103.

The glial cell population of the newt optic nerve has been shown to react to lesion within 30 minutes after injury (Soc. for Neurosci. Abst. IV:399, '78). This response involves a qualitative increase in filaments and cytoplasmic vesicles which has been associated with glial hypertrophy and lytic enzyme distribution (Soc. for Neurosci. Abs. V:431, '79). The purpose of this study was to determine if the apparent increase in such organelles is accompanied by an increase in amino acid incorporation into the glia. All newts were anesthetized, injected with 50μCi of ^{3}H-leucine (sp. act. 58.5 Ci/mmol.) in 200μl dH_2O and left optic nerves of the experimentals transected. Animals were sacrificed at 30 minutes post-lesion, along with sham-operated and unlesioned controls. Nerves were processed for liquid scintillation spectroscopy and light microscopic autoradiography. Results showed that lesioned and contralateral nerves from experimental animals had a 3.5 fold higher uptake of ^{3}H-leucine at 30 minutes post-lesion than the unlesioned controls (40.53 and 46.53 counts/min/μg protein in experimentals compared to 12.58 counts/min/μg protein in controls). Autoradiographic silver bromide grains were of higher density (41.0 ± 3.5 and 32.0 ± 2.6 grains/$\mu m^2 \times 10^3$) in the central neuroglial core of the lesioned and contralateral nerves than in the neurons of the nerve periphery (16.0 ± 2.7 and 18.0 ± 1.9 grains/$\mu m^2 \times 10^3$) at 30 minutes post-lesion. These central glial grain densities were significantly increased ($p<0.02$) over both sham-operated (11.0 ± 2.2 grains/$\mu m^2 \times 10^3$) and unlesioned (19.0 ± 3.5 grains/$\mu m^2 \times 10^3$) controls, while the peripheral neuronal densities were not different in the experimental and control groups. These results indicate that (1) lesion induces an increase over controls in both uptake and incorporation of an amino acid precursor to protein synthesis and (2) the contralateral optic nerve also reacts to lesion with an increased uptake and incorporation of amino acids during the early stages of Wallerian degeneration. These observations may be correlated with glial filamentous hypertrophy and lysosomal production in the newt optic nerve during the first 30 minutes post-lesion.

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186.9 BIOCHEMICAL PROPERTIES OF RAT GLIOMA TRANSPLANTED TO NUDE MOUSE. Y. Noda* and S. Hirano*. School of Medicine, University of Toho, Ohmori, Ohta-ku, Tokyo, Japan.

We studied the biochemical properties of neoplastic glial cells induced primarily in rat cerebral cortex by transplacental administration of ethylnitrosourea. Tumor cells were secondarily transplanted subcutaneously to the nude mouse. 1). The pattern of free amino acid content between the secondary glioma and rat brain cortex showed substantial differences. Glycine and alanine were higher, while glutamate and aspartate were lower in the glioma than in the rat cortex. In addition, the GABA content of the glioma was below our detection limits. 2). The incorporation of ^{14}C and ^{15}N from ^{14}C-U-glucose and ^{15}N-$(NH_4)_2SO_4$, respectively, into free amino acids were determined in secondary glioma and the host brain cortex of the nude mouse. The incorporation of ^{14}C to glutamate was the highest of all amino acids examined in both tissues. Aspartate and alanine were higher in the glioma than in the cortex. Notable incorporation of ^{15}N to glutamine was found in both tissues. A disproportionately high quantity of ^{15}N was incorporated into glutamine as compared to glutamate in the glioma versus the cortex. This indicates that glioma have a higher synthetic activity for glutamine than do neurons. 3). Slices of the secondary glioma tissue showed less oxygen consumption than slices of the cortex, without showing a potassium stimulating effect. 4). The utilization of glycolytic pathways was fundamentally different in the glioma and neuronal tissues. Glioma tissues showed higher activity in the pentose phosphate pathway than in the Embden-Meyerhof pathway.

187.1 CYTOCHEMICAL IDENTIFICATION OF NEURONAL AND NONNEURONAL CELLS IN MIXED AND PURIFIED CHICK EMBRYO RETINAL CULTURES. R. Adler[+], P. J. Magistretti[++*], A. G. Hyndman[+], and W. J. Shoemaker[++]. [+]Dept. of Biology, Univ. of Calif., San Diego and [++]The Salk Institute, La Jolla, CA 92093.

Different retina (NR) monolayer cultures can be generated by appropriate combinations of substrata and media. Cell suspensions from 8-day chick embryo retina undergo considerable clumping before attaching to substrata of low adhesiveness. These "clumped" cultures develop process-bearing (PB) cells with either Horse (HS) or Fetal Calf (FCS) serum, whereas only the latter supports development of "flat" cells, which migrate out of the clumps and generate a confluent carpet. Highly adhesive substrata, on the other hand, allow clump-free and flat cell-free cultures, rich in PB cells. To further extend this characterization as to the neuronal or nonneuronal identity of the cells, these cultures have been analyzed using i) ^{3}H-thymidine (^{3}HT) incorporation, as an indicator of mitotic activity, and ii) tetanus toxin (TT) binding, generally considered a neuronal "marker". Four culture conditions at day 1,3 and 6 in vitro were studied: i) highly adhesive substratum such as a) polyornithine or b) A30-0 collagen (Adler et al, Dev. Biol. 69: 424-435, 1979) in presence of optic lobe extracts, ii) low adhesive substrata, in presence of a) FCS or b) HS. Each dish was incubated with 1 µCi/ml of ^{3}HT. ^{3}HT was washed out 24 hours later and the cultures stained for TT immunofluorescence and processed for autoradiography. Main findings are: i) Neuronal identity of PB cells in clump-free and flat cell-free cultures is indicated by the fact that 100% of them are ^{3}HT(-), and 95% or more are TT(+). A significant number of small, process-free cells appears ^{3}HT(+) after 1 day in vitro, but their number decreases markedly when the labelling is applied at later in vitro stages; ii) After 1 day in vitro, cell clumps present on low adhesive substrata show TT(+) cells as well as ^{3}HT(+) cells both with FCS and HS. In FCS cultures, flat cells emerging from clumps between days 3 and 6 appear always TT(-), and are ^{3}HT(+) in the vast majority of the cases. The presence of ^{3}HT(+) cells within clumps in HS cultures at all the stages examined suggests that flat cell promotion by FCS does not result exclusively from its mitogenic properties. TT(+) PB cells are found within as well as outside cell clumps in both FCS and HS cultures. Preliminary experiments indicate that similar results are obtained when equivalent cultures are prepared using chick embryo optic lobe cell suspensions. Supported by USPHS grant EY-02854 from the National Eye Institute (to R.A.), and by grants from the March of Dimes Birth Defects Foundation (1-691) and NIAAA (AA-34504) (to W.J.S.). P.J.M. is a recipient of a Swiss National Sciences Foundation fellowship.

187.2 NEURONAL "BIRTH" AND DEVELOPMENT IN PURIFIED MONOLAYER CULTURES FROM CHICK EMBRYO RETINA. A. G. Hyndman and R. Adler. Dept. of Biology, Univ. of Calif., San Diego, La Jolla, CA 92093

It is now possible to obtain flat cell-free, purified neuronal cultures from 8-day chick embryo retina. They can be supported by media containing either fetal calf serum, horse serum or the serum-free supplement "N1" (Bottenstein et al., Exp. Cell Res. 125: 183-190, 1980) when cultured on highly adhesive collagen or polyornithine (PORN)--although the latter substratum must be pre-exposed to serum before cell seeding if serum-free medium is used. These different culture conditions were quantitatively compared using as parameters: i) total number of process-bearing neurons; ii) relative numbers of different neuronal classes (types A-F), using a classification based on neuronal polarities, and iii) neuritic development. Different tissue extracts added to the N1 medium, promoted both neuronal survival and neuritic development, without inducing detectable changes in the relative proportion of neuronal classes. When extracts from chick embryo optic lobe or telencephalon were added to the medium, however, a new cell type could be recognized. These "G" cells represent no more than 1% of the total number of neurons, and are characterized by a "super-long" neurite which is some 20-fold longer than neurites from other cell types.

Cultures from 6-day chick embryo retinas contain neurons and "neuroepithelial-like" cells. Autoradiographic analysis of ^{3}H-thymidine-labeled cultures provided evidence that some retinal neurons are "born" in vitro. Short thymidine pulses label almost exclusively the neuroepithelial-like cells, but with longer pulses increasing numbers of process-bearing, neuronal cells also appear labeled. Work is in progress to test the hypothesis, that some neuroepithelial cells undergo a last mitotic cycle, become postmitotic and differentiate as neurons in vitro.
Supported by USPHS grant EY-02854 from the National Eye Institute.

187.3 NEURONAL REPLICATION OF HERPES SIMPLEX VIRUS (HSV) IN VITRO IN THE ABSENCE OF VIRAL THYMIDINE KINASE. R.W. Price and R. Rubenstein*. Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Accumulating evidence supports the central role of neurons in the survival and spread of herpes simplex virus (HSV) in the human community. Experimental studies in animals have demonstrated that sensory and autonomic neurons are infected by HSV after inoculation of peripheral target tissues, that virus remains latent for prolonged periods in sensory and autonomic ganglia, and that virus in these ganglia can be reactivated. It has been speculated that the viral gene product, thymidine kinase (TK), may be important in latency and reactivation by facilitating viral DNA synthesis in non-dividing neurons in which only minimal (repair-related) DNA synthesis occurs.

In order to determine whether HSV TK is required for viral replication in neurons, we compared replication of a TK deficient viral mutant (TK^-) of HSV type 1 with the parent (TK^+) strain in two in vitro neuronal cell culture systems: 1. dissociated superior cervical ganglion (SCG) neurons, and 2. PC12 pheochromocytoma cells induced to neuron-like differentiation by NGF. In dissociated SCG neurons the kinetics of HSV replication, as judged by cytopathology, viral antigen expression and viral titers, were approximately equal for both the TK^- and TK^+ strains. Addition of the HSV inhibitor acyclovir, which requires the viral TK enzyme for its antiviral action, completely inhibited TK^+ virus replication but inhibited only slightly the TK^- virus. When methotrexate (MTX) was added to the culture medium, replication of TK^- virus was inhibited while TK^+ replication was unaffected. Results of studies of infection of PC12 cells were quite similar. In addition, TK activity was also measured in the PC12 cells during infection. No increase in either viral or cellular TK activity accompanied infection by TK^- virus, while viral TK activity was induced by TK^+ virus.

These results indicate that the metabolic machinery required for viral DNA replication is available or can be induced in isolated neurons, and that viral replication in these cells does not require virus-coded TK. The results of studies with MTX suggest that the de novo pathway involving thymidylate synthetase participates in DNA synthesis under these conditions, but further studies are needed to more precisely clarify both cellular and viral contributions.

187.4 RESPONSE OF SCHWANN CELLS IN VITRO TO ADDITION OF PURIFIED ZONAL FRACTIONS DERIVED FROM RAT CNS TISSUE. L.N. Minier and G.H. De Vries. Biochemistry Dept., Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.

Previous work from this laboratory and others has indicated that some factor(s) most likely associated with the mammalian axolemma has the ability to induce multiplication within a relatively quiescent population of Schwann cells maintained in vitro. Currently a method is available for the osmotic disruption and subsequent isolation of axolemmal and myelin membranes from brain using continuous sucrose gradients in a zonal centrifuge rotor. The resultant fractions from such a method were employed in efforts to determine the position and nature of a potential Schwann cell mitogen(s) derived from neuronal and/or nonneuronal membranes. White matter from rats of varying ages was homogenized and CNS myelinated axons were obtained utilizing flotation in a buffered salt-sucrose medium (Brain Res. 147:339, 1978). Purified myelinated axons were disrupted by hypotonic shock and were applied to a 20%-50% linear sucrose gradient in a Beckman Ti14 zonal rotor and were centrifuged to equilibrium. Aliquots of the resulting fractions were washed extensively in balanced salt solutions and then were added to cultures of rat Schwann cells for varying periods of time. Dispersed cultures of Schwann cells were obtained by the enzymatic and mechanical dissociation of dissected sciatic nerves from newborn rats. The growth of fibroblasts in these cultures had been arrested by the addition of the antimitotic agent cytosine arabinoside during the early stages of culture. After removal of the drug, tritiated thymidine was added as a single pulse for incorporation by mitotically active cells. The cells, on coverslips, were then processed for light microscope radioautography.

Initial evidence indicated that the addition of purified membranes from the zonal fractions caused a general increase of the mitotic index over control values when a twenty four hour labeling period was employed. Within a certain level of variation, the resting level of unstimulated cells was only half that of cells that had been stimulated by the presence of neuronal and nonneuronal membranes. The morphology of stimulated cells did not appear to be altered during the exposure to the mitotic signal in the culture medium. This is in contrast to the effects produced by the addition of cholera toxin to Schwann cell cultures; this known mitogen caused Schwann cells to assume a more flattened, polygonal shape from their normal bipolar, spindle morphology. (Supported by National Multiple Sclerosis Society Grant FG526A-1 and NIH Grant NS10821).

187.5 DEVELOPMENT OF BENZODIAZEPINE AND GABA RECEPTORS IN MOUSE CNS PRIMARY CELL CULTURE. D.B. Shibla, M.A. Gardell* and J.H. Neale. Dept. of Biology, Georgetown University, Washington, D.C. 20057.

CNS neurons obtained from fetal mice at 13.5-14.5 days of gestation, dissociated mechanically and grown in primary cell culture for three weeks become functionally mature. Receptors for benzodiazepines and GABA develop in both tissue cultured brain and spinal cord. The development of these receptors *in vitro* seems to parallel their development *in vivo*. The binding characteristics of ^{3}H-diazepam (^{3}H-DZP) in membrane homogenates prepared from cultured cells correspond closely with the binding characteristics of this ligand on membrane homogenates prepared from adult tissues. For ^{3}H-DZP binding in adult whole brain the K_D is measured at ~8.4 nM and the B_{max} at ~2.315 fmol/µg protein. The K_D for ^{3}H-DZP binding to cultured brain homogenates is ~8.7nM and the B_{max} is ~3.115 fmol/µg protein. Dissociated cell culture thus provides a model system for the study of receptor development and for a study of the effects of chronic drug treatment on receptor development.

We have evaluated the effects of chronic (1 µM) diazepam exposure to mouse whole brain grown in primary cell culture for three weeks and find no significant change in either the binding affinity of ^{3}H-DZP or the total number of ^{3}H-DZP receptors on these cells. We are currently investigating the effects of chronic diazepam treatment on GABA receptors as well as the effects of long-term exposure to GABA on the development of both benzodiazepine and GABA receptors.

187.6 K^+ - STIMULATED RELEASE OF ^{3}H-GABA FROM PRIMARY MONOLAYER CULTURES. B. R. Pearce*, D. N. Currie*, R. Beale*, G. R. Dutton, R. Hussey* and R. Pigott*. Department of Biology, The Open University, Milton Keynes, MK76AA, UK, and Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, IA 52242.

In order to investigate the release properties of putative neurotransmitter substances from primary monolayer cultures of perikarya isolated from brain regions of the rodent, we have designed and built a novel perfusion apparatus which allows the simultaneous study of efflux from two cultures each grown on 22 mm sq. glass coverslips [apparatus and methodology to be described in detail].

The data obtained demonstrate that in neuron-enriched cultures [85%; Brain Res. 183, 241 (1980)] of cerebellar perikarya which were prelabelled with ^{3}H-GABA, Ca^{+2} - dependent [5 mM] release with K^+ depolarization [50 mM] was observed. This phenomenon was abolished by *cis*-1-3-aminocyclohexanecarboxylicacid [ACHC, 1 mM], a specific inhibitor of neuronal GABA uptake, in the prelabelling medium. In addition, results obtained from different experiments could be pooled, meaned and standard errors calculated. For example, values of $P<0.001$ for the Ca^{+2} - dependent, K^+ - depolarized ^{3}H-GABA efflux [compared to background values] were obtained in three different experiments. Finally, this perfusion apparatus was designed to minimize the perturbing effects of media changes on both cell retention and cell surface disturbances known to cause non-specific release of putative neurotransmitter substances.

This work was supported by a grant from the British MRC [GRD]. B.R.P. was supported by an SRC scholarship. ACHC was a gift of Dr. N. Bowry, St. Thomas' Hospital Medical School, London.

188.1 AN ENERGY DETECTION MODEL FOR AUDITORY THRESHOLD TO PULSE STIMULI. Ronald L. Seaman. Biomedical Research Branch, Engineering Experiment Station, Georgia Institute of Technology, Atlanta, Georgia 30332

A model has been developed for detection of pulse stimuli (e.g., clicks) by the auditory system. The model is based upon energy detection in the frequency domain and is similar in some respects to models of energy detection in the time domain. Input is the frequency spectrum of the pulse stimulus given by its Fourier transform. Frequency-domain formulation has led to a straightforward analysis which shows the dependence of threshold intensity on pulse duration T_p.

The model comprises three separate elements. An ideal filter element with a passband from LF to UF represents the auditory system's frequency range. A detector element uses an absolute-value function as the nonlinear process of detection. An integrator element sums energies of the frequencies from LF to UF. The model's output is this summed energy which represents the response to a pulse input. The response is considered to be threshold when it is equal to a fixed, constant value.

In its simplest form, the model predicts that the relative threshold is a function of only $UFxT_p$, where UF is the upper frequency limit of response. When plotted against $UFxT_p$, there are two distinct regions of the threshold curve which become straight lines on a log-log plot. For small T_p, the slope is exactly -1; for large T_p, approximately -1/5. Transition between regions is gradual and occurs over roughly $0.3<UFxT_p<2$.

This derived threshold trend with $UFxT_p$ matches available experimental data. The shape of the threshold can be used to determine the auditory-system upper frequency limit by using pulse stimuli. This capability may be useful for experimental and clinical measurements.

188.2 ON THE ROLE OF AUDITORY NERVE INTER-FIBER DISCHARGE TIMING IN THE PERCEPTION OF PITCH. M. W. White*, G. E. Loeb, and M. M. Merzenich (SPON: K. Brown). Coleman Memorial Laboratory, UCSF, San Francisco, CA 94143 and Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Evidence for the combined role of temporal and spatial information in the encoding of complex spectral stimuli has been reported (Young, E.D., and Sachs, M.B., J. Acoust.Soc.Am. 66:1381, 1979). Various features of the temporal information might be utilized by the auditory nervous system. We here consider evidence that the auditory system is, in fact, sensitive to inter-fiber timing information and to which normally occurring aspects.

Electrical stimulation of the eighth nerve in cat causes strongly phase locked nerve fiber responses for stimuli up to 6 kHz. However, in frequency discrimination tests, implanted subjects were unable to utilize this phase locked information for frequencies above 500-1000 Hz (Eddington, D.K., Ann. Otol.Rhinol. Laryn. suppl. 53:1-39, 1978). Simmons (Arch. Otolaryng. 84:24-76, 1966) obtained similar results when stimulating a single group of eighth nerve fibers. In contrast, Simmons also discovered that his subject was highly sensitive to small changes in inter-pulse timing if each of the two pulses excited a different group of fibers. Inter-pulse timing changes of only 0.2-0.3 msec were detectable.

A neural network that performs a temporal cross-correlation between inputs from neighboring fibers would be highly sensitive to changes in inter-fiber discharge times. Because inter-fiber timing is a strong function of stimulus frequency and not amplitude (for phase locked frequencies, Greenwood, D.D., Psychophysics and Physiology of Hearing, Ed. E.F.Evans and J.P.Wilson, Academic Press, NY, 1977, pp. 43-54), such a neural network could be useful in pitch perception. In particular, it would be capable of relatively accurate and precise frequency discrimination over a wide dynamic range and in the presence of multiple frequency acoustic stimuli. We will discuss the relationship of this new model of pitch perception to other models and to existing psychophysical and neurophysiological data.

188.3 BRAINSTEM PROCESSING OF AUDITORY INFORMATION TO GENERATE A CENTRAL REPRESENTATION OF PITCH. G. E. Loeb, M. M. Merzenich, and M. W. White*. Coleman Memorial Laboratory, UCSF, San Francisco, CA 94143 and Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

A growing body of psychophysical evidence points to the existence of a central neural representation of the sound frequencies presented to a listener from which a decision about the overall pitch (and, perhaps, location) of the source is made on the basis of pattern recognition (Goldstein, J.L., J.Acoust.Soc.Am. 54:1496, 1973). However, the two existing theories of pitch perception (on the basis of cochlear place or neuronal phase-locked periodicity) cannot generate such a representation without supposing that complicated signal extraction processes such as Fourier analysis, template matching, or auto-correlation are occurring in the first few synaptic relays.

A new theory suggests that pitch could be extracted from the relative timing of activation of pairs of auditory nerve fibers sampling the basilar membrane motion at points some distance apart in the cochlea (White, Loeb, and Merzenich, this session). We here suggest a specific neuronal pathway which could perform the necessary calculation at a single synaptic level and which gives immediate rise to a three dimensional perceptual map function suitable for a pattern recognition determination of the pitches and spatial origins of multiple, simultaneous sound sources over a very wide dynamic range.

The critical requirement is for a convergent neuron capable of detecting the synchronicity of arrival of signals from two basilar membrane sites with a resolution of 10-20 μsec. Neurons capable of such fine temporal detection (cross-correlation) are already known to exist in the medial superior olive (MSO), where they subserve binaural sound localization by inter-aural delay detection. We suggest that the same neurons could actually perform a four-way synchronicity detection by convergence from pairs of anteroventral cochlear nucleus (AVCN) neurons phase locked to equally spaced points of basilar membrane motion in each cochlea. A given MSO cell would then be optimally stimulated by the existence of a certain sound frequency (giving rise to a unique spatio-temporal pattern of basilar membrane motion) at a certain position in space (causing a unique inter-aural delay). Physiological and psychophysical data supporting this notion and other testable predictions of this hypothesis will be discussed along with the implications for the design of an auditory prosthesis.

188.4 RESPONSES OF PRIMARY AUDITORY FIBERS TO COMBINED NOISE AND TONAL STIMULI. C. D. Geisler and D. G. Sinex.* Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706. Responses to tonal stimuli, with and without added noise of different bandwidths, were obtained from anesthetized cat auditory-nerve fibers using glass micropipettes. When low-pass noise with a cut-off frequency at least one octave below best or characteristic frequency (CF) was used, every neuron tested showed a suppression of the tonal response. This suppression did not cause a general reduction of neural responsiveness to all sounds, but rather took the general form of a frequency-specific reduction in the effective intensity of the tonal stimuli. That is, the principle effect of the noise was to shift the position of the rate-intensity curves to higher intensities without changing the shape or magnitude of the curves (cf. Javel, et al. J. Acoust. Soc. Amer., 63:1093, 1978). The suppression mechanism(s) involved thus adjust the sensitivity of these fibers to cover higher intensity ranges in the presence of noise. The frequency of the most severely affected tones was always at or near best frequency, in confirmation of previous work (Kiang and Moxon, J. Acoust. Soc. Amer. 55:120, 1974). The suppression gradually dropped off to zero as the frequency of the tones was decreased. The suppression is a direct but highly nonlinear function of the intensity and bandwidth of the noise. One of the most striking characteristics of the low-frequency noise was its great power: the activity of all neurons were affected by it, with suppressions of up to 40 dB being exhibited by some neurons.

The effects on tonal responses of wide-band noise were quite variable, sometimes causing suppression similar to that induced by the low-pass noise and sometimes causing only "strong-signal capture" effects (Rhode, et al. J. Neurophysiol., 41:692, 1978). By contrast to low-pass noise effects, low-frequency tones often caused suppression of the responses to the wide-band noise alone, particularly in neurons with low rates of spontaneous activity.

A model of noise-induced suppression has been developed. Each sound produces both an excitatory effect that is sharply tuned at best frequency and a suppressive effect that has its lowest threshold at best frequency but is more broadly tuned. The magnitude of the suppression is frequency-dependent, with the greatest effect seen at best frequency. Stimuli near best frequency mainly stimulate excitatory mechanisms, while tones with frequencies either above or below that also cause appreciable and sometimes dominant suppression. The balance between the excitatory and suppressive mechanisms determines the neural response.

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188.5 NEUROANATOMICAL MAPPING OF EIGHTH-NERVE FIBER PROJECTIONS FOLLOWING EXPERIMENTALLY-INDUCED COCHLEAR PATHOLOGY. G.K. Martin*, R.P. Wise* and B.L. Lonsbury-Martin (SPON: J.M. Miller). Dept. Otolaryngology, Univ. Washington Sch. Med., Seattle, WA 98195.

In the auditory system, afferent projections from the cochlea have been demonstrated by intracochlear injections of the tritiated amino acids leucine and proline which are taken up by the primary spiral ganglion cells in an unknown manner and label axonal terminals in the cochlear nucleus (CN). The results of these investigations have confirmed and extended the findings of many previous anatomical studies using classical degeneration techniques to describe innervation patterns to the CN. The purpose of the present study was to examine the feasibility of employing intracochlear injections of tritiated leucine to trace the remaining functional cochlear nerve-fiber projections to the cells of the CN following ototraumatic insults to the cochlea. To the extent that uptake of these tracers can be related to cochlear histopathology, these methods provide a powerful means to assess cochlear function in pathological ears. In order to assess the general applicability of autoradiographic tracers in revealing cochlear damage, we studied healthy guinea pigs that received end-organ damage resulting from one of the following experimental manipulations: exposure to intense sound, ototoxic poisoning, or mechanically-induced injury. Following one of these insults, subjects were allowed varying survival periods prior to the intrascalar injection of tritiated leucine. In all cases, the left cochlea of each experimental animal was injected with 50 µCi of [^{3}H] leucine dissolved in 5 µl physiological saline via the round window. Following a 24-hr survival period, animals were deeply anesthetized, perfused with 10% neutral buffered formalin and the brain and cochleas removed. A block of brainstem containing the CN complex was placed in a 30% sucrose solution for complete fixation and then frozen sectioned in the transverse plane. All tissues were processed for standard autoradiography and exposed for 4 wks. Sections were developed, stained, and examined microscopically for the presence of silver grains overlying the CN. All cochlear tissues were processed for midmodiolar sectioning or examined with a plastic embedding technique. All animals showed substantial damage as revealed by absence of projections to CN corresponding to regions of cochlear insult. In the most severely damaged ears that received neomycin poisoning, projections to CN were virtually absent although significant numbers of spiral ganglion cells remained. These preliminary results suggest that unmyelinated dendrites may play a significant role in the uptake of tritiated leucine following intracochlear injection.
(Supported by: Deafness Research Foundation and ONR N00014-75-C-0463).

188.6 MET-ENKEPHALIN-LIKE IMMUNOREACTIVITY IN EFFERENT NERVE FIBERS IN THE GUINEA PIG COCHLEA. J. Fex and R. A. Altschuler, Lab. Neuro-otolaryngology, NINCDS, NIH, Bethesda, MD 20205.

The distribution of Met-enkephalin-like immunoreactivity in the guinea pig cochlea was determined using the indirect immunofluorescence technique with antisera against Met-enkephalin. Two kinds of preparations were used, I and II. In both I and II, NIH strain guinea pigs were deeply anesthetized and perfused transcardially with 4% paraformaldehyde solution. The cochleae were removed and perfused with the fixation fluid. The bony cochlear spiral was dissected free from surrounding bone and vascular stria. I: The indirect immunofluorescence technique was carried out directly on the cochlear spiral. One to 2 mm segments of the organ of Corti were removed, forming surface preparations, and mounted for observation and photography under epifluorescent and ordinary illumination. II: The cochlear spiral was mounted in OCT and 10 micron cryostat sections were cut to give cross sections of the organ of Corti. The sections were incubated for immunofluorescence, observed and photographed.

Met-enkephalin-like immunoreactivity was found in unmyelinated fibers in the fascicles of the intraganglionic bundle immediately peripheral to the spiral ganglion. In the organ of Corti, the immunoreactivity was found in fibers of the inner spiral bundle and tunnel spiral bundle, in low tunnel crossing fibers and as patches at the bases of inner and outer hair cells.

We conclude that the cochlea of the guinea pig contains Met-enkephalin-like immunoreactivity in efferent nerve fibers. We have no evidence that hair cells or auditory nerve fibers contain any Met-enkephalin-like substance.

188.7 CENTRAL TERMINATIONS OF FUNCTIONALLY IDENTIFIED AUDITORY AND VESTIBULAR AFFERENTS. Hironori Koyama*, E. L. Leverenz*, R.A. Baird, and E.R. Lewis. Electronics Research Laboratory, University of California, Berkeley CA 94720.

After functional identification, individual afferent fibers from the auditory and otoconial organs of the bullfrog inner ear were injected iontophoretically with the fluorescent dye Lucifer Yellow and traced to their central terminations. The results to date are consistent with those of previous projection studies of auditory and vestibular populations in the frog (Gregory, K.M., Brain Behav. Evol. *5*: 70-88, 1972; Matesz, C., Neuroscience *4*: 2061-2071, 1979; Nieuwenhuys, R. & Opdam, P., in Frog Neurobiology (Springer): 811-855, 1976; Fuzessery, Z.M. & Feng, A.S., Soc. Neurosci. Abstr. *5*: 141, 1979), but refine the resolution to that of individual, functionally identified afferents. Three functional classes have been identified: auditory (responding to airborne sound), vibratory (responding to substrate-borne vibration), and vestibular (responding to head position, linear acceleration). Auditory afferents (from the amphibian and basilar papillae) and vibratory afferents (from the saccular macula and the striola of the lagenar macula) project to the dorsal medullary lobe. Vestibular afferents (from the utricle and the lagena) project to the cerebellum and to the medulla just ventral to the dorsal medullary lobe.

The central projections of the amphibian papilla occupy the lateral side of the dorsal medullary lobe in the immediate vicinity of the VIIIth nerve, and extend no more than 0.5 mm along the rostral-caudal axis. The projections of the basilar papilla units occupy the dorsal region of the dorsal medullar lobe, generally medial to the projections of the amphibian papilla, and extend approximately 1.5 mm caudally from the level of the VIIIth nerve. The projections of vibratory afferents occupy the lateral side of the dorsal medullary lobe, just ventral to the projections of the amphibian papilla, but extend well beyond them (approximately 2 mm) caudally from the level of the VIIIth nerve. Projections of the lagenar vestibular afferents occupy a region of the medulla immediately ventral to the projections of the vibratory fibers and immediately dorsal to those of the utricular afferents. Both lagenar and utricular vestibular projections extend approximately 4 mm caudally from the level of the VIIIth nerve and rostrally to the cerebellum. No afferent fibers of any type were found to project to the contralateral side.

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188.8 HORSERADISH PEROXIDASE DEMONSTRATION OF MOTONEURONS TO THE STAPEDIUS MUSCLE IN THE RHESUS MONKEY. Norman L. Strominger, Steven M. Silver*, Timothy C. Truscott and Jerome C. Goldstein*, Dept. of Anatomy and Division of Otolaryngology; Albany Medical College of Union University, Albany, New York 12208 and Samaritan Hospital Troy, New York.

The stapedius muscle was exposed in a series of rhesus monkeys using an endaural transmastoid approach with the aid of an operating microscope. The incus and head of the malleus were removed for better visualization of the contents of the middle ear. Horseradish peroxidase (20%-50%; Sigma Type VI) was injected into the stapedius through a micropipette. Dried flakes of the enzyme also were tattooed into the muscle belly in some cases. Animals were perfused transcardially 2-3 days postoperatively with 0.9% saline followed by 8% gluteraldehyde. Tetramethyl benzidine was used as the chromagen in the last several cases. Sections, cut in one of the three major planes were counterstained with neutral red or thionin.

After initial experiments demonstrated the completely ipsilateral disposition of stapedial motoneurons, subsequent cases were done bilaterally. A total of 20 muscles were injected. Labeled perikarya were located in the tegmentum immediately surrounding the facial nucleus. In some cases labeled cells occupied the facial nucleus itself; our data indicate that this was due to uptake of enzyme by fibers of the facial nerve. Labeled perikarya often were located in the tegmentum a distance superior to the facial nucleus along the course of the facial roots. Occasional cells were located at the interface between the rostral end of the facial nucleus and the lateral superior olivary nucleus.

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188.9 AUTORADIOGRAPHIC DEMONSTRATION OF EIGHTH NERVE PROJECTIONS IN RANA PIPIENS. Peter G. Aitken. Section of Neurobiology and Behavior, Division of Biological Sciences, Cornell University, Ithaca, N.Y. 14853

Approximately 10nL of tritiated proline solution (25uC/uL) were injected into the left eighth nerve ganglion of each of 5 Rana pipiens. Postinjection survival at 18°C was for 48 hrs (2 frogs), 72 hrs (1 frog), or 96 hrs (2 frogs). Paraffin-embedded brains were sectioned frontally (3 frogs) or horizontally (2 frogs) at 15u, mounted on slides, and coated with Kodak NTB-3 emulsion. Exposure at 4°C was for 45 days (1 frog) or 28 days (4 frogs). Slides were developed in Kodak D-19 and lightly stained with cresyl violet. The results described were essentially identical in all animals.

The labeled region, as determined by microscopic examination under both dark- and brightfield illumination, was limited to one contiguous area ipsilateral to the injected nerve, extending from slightly caudal to the entry of nerve X to the ventrolateral portion of the cerebellar granular layer; projections covered the caudal, ventral, and dorsal nuclei of nerve VIII described by other authors. At their caudal limit, projections formed two separate, thin bands bordering the fasciculus solitarius on its medial and dorsolateral edges. More rostrally, the bands enlarged, fused, and extended dorsally to become limited by the pial surface laterally and the ventricle medially. Ventrally, the projections were limited by the fasciculus solitarius, descending spinal tract of nerve V, and a line drawn from the fasciculus solitarius to the sulcus limitans. Rostral to the entry of nerve VIII, the ventral boundaries seemed to be the sensory nucleus and descending tract of nerve V; these boundaries were difficult to determine exactly because the limits of the labeled region were less sharp than was seen more caudally. Projections continued into the cerebellar peduncle, cerebellar nucleus, and the ventrolateral portion of the cerebellar granular layer, i.e., that portion bordering the cerebellar nucleus.

No projections were seen to the superior olive, contralateral cerebellum, cerebellar molecular layer, reticular formation, spinal nucleus of nerve V, or motor nuclei of any cranial nerves.

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188.10 EFFERENT INNERVATION OF THE ORGAN OF CORTI: ASSYMETRICAL TOPOGRAPHIC PROJECTIONS. John J. Guinan Jr. and W. Bruce Warr. Eaton-Peabody Lab., Mass. Eye and Ear Infirmary, Boston, MA 02114, and The Boys Town Institute for Communication Disorders in Children, Omaha, NE 68131

Warr and Guinan (1979, Brain Res. 173:152-155) have shown that olivocochlear cells in the lateral part of the superior olivary complex (LSOC) project bilaterally to the region under inner hair cells, whereas olivocochlear cells in the medial part of the superior olivary complex (MSOC) project bilaterally to the region under outer hair cells. We have used the same anterograde tracing methods (brainstem injections of 35-S-methionine followed by cochlear autoradiographs) to compare the projection patterns along the length of the organ of Corti of injections centered in different parts of the LSOC and MSOC.

LSOC injections show a clear ipsilateral topographic projection with more medial injections producing patterns of silver grains located more basally in the cochlea. A comparison of the tonotopic organizations of the cochlea and LSOC periolivary cells shows that this ipsilateral olivocochlear projection appears to connect regions of similar best frequencies. On the other hand, all of our LSOC injections produced silver grain patterns in the contralateral cochlea which were predominantly in the apex. Warr (1975, J. Comp. Neurol. 161:159-182) found that LSOC olivocochlear neurons which projected to the contralateral cochlea were located in the lateral part of the LSOC; some spread of injected 35-S-methionine to this lateral part of the LSOC occurred in all of our LSOC injections. Since both the apex of the cochlea and the lateral part of the LSOC are "low frequency" regions, the projections of LSOC olivocochlear neurons to the contralateral cochlea might also be between regions of similar best frequencies.

MSOC injections produced silver grain patterns which ranged from widespread distributions which were fairly symmetrical in both cochleas to distributions which were concentrated in a narrow region of one cochlea and were widespread in the other cochlea. We have not yet found a clear pattern of topographic projections from the MSOC to either cochlea.

188.11 INTRACELLULAR RECORDING AND STAINING IN CAT COCHLEAR NUCLEUS. W. S. Rhode*, P. Smith and D. Oertel. Dept. of Neurophysiology, University of Wisconsin Medical School, Madison, Wisconsin 53706.

Cells in cat cochlear nucleus (PVCN, DCN) were studied electrophysiologically and stained with horseradish peroxidase. Units were usually characterized extracellularly by their characteristic frequency (CF), threshold at CF, poststimulus time histogram (PST) for a 25 ms tone at CF, interval histogram, rate curve and response area when conditions permitted. A current pulse was used to enter the cell and intracellular responses were recorded to swept frequency tones. PST responses were used to classify cells according to the categories of Pfeiffer (Exp. Brain Res., 1:220, 1966).

An octopus cell (Osen, J. of Comp. Neurol., 136:453, 1969) exhibited a marked onset component followed by a slow sustained discharge. Small bipolar cells (8-9 μm) were sustained choppers and capable of discharge rates of 800-900 /s. Fusiform cells showed transient chopping or pausing responses. Giant cells (DCN) did not respond to auditory stimuli probably due to barbiturate anesthesia (E. F. Evans and P.G. Nelson, Exp. Brain Res., 17:402, 1973).

Many units exhibited chopper behavior. Sustained choppers were most often found in the PVCN and transient or weak choppers in the DCN. The latter exhibited lower maximum discharge rates and no tendency to phaselock to low frequency tones. Nearly every possible combination of PST types have been seen: onset-chopper, buildup-chopper, and pause-builder-chopper implying that categories based on PSTs are not always clear.

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188.12 OBSERVATIONS ON TONOTOPIC ORGANIZATION WITHIN THE RAT SUPERIOR OLIVARY COMPLEX AND COMPARISONS TO HIGHER AUDITORY STRUCTURES USING 2-DEOXY-D[1-^{3}H] GLUCOSE. J. Coleman, W.J. Clerici, A. Ryan*, A.J. Beitz, J. Buggy, and M.N. Huhns*. Dept. of Psychology, and Depts. of Physiology and Anatomy, School of Medicine, and College of Engineering, Univ. of South Carolina, Columbia, SC 29208.

Tonotopic organization is believed to occur at numerous locations within the central auditory pathways. This may be construed as a multiple representation of the basilar membrane in which divergence or convergence of information can be emphasized in particular brain areas. In the present study, the labeled 2-deoxyglucose method was used to investigate selected auditory regions where binaural processing is believed to occur. Awake restrained male albino rats catheterized 24 hr previous were monaurally or binaurally presented with constant pure tone stimuli at 1, 2, or 8 kHz at various intensity levels. Animals were given intravenous injections of 2-deoxy-D[1-^{3}H] glucose, sacrificed 45 min later, and the brain removed in unperfused or perfused cases. Frontal or parasagittal sections 15-30 μm thickness were exposed to X-ray film and later stained with thionin. Differential optical densities observed could be validated and quantified using a computer image processing system.

Major subdivisions of the superior olive complex, inferior colliculus, medial geniculate body and auditory cortex showed preferential activity to pure tone stimulation. Little or no distinctive activity was observed in controls with ears plugged. A prominent focus of activity occurred in the caudal part of the lateral superior olive (LSO) adjacent to the facial nerve. At 1 kHz, this activity was concentrated in the ventrolateral tip of LSO; 8 kHz stimulation produced maximal activity at the dorsal hilus of LSO. There was a greater response to binaural than monaural stimulation at the same intensity levels. The medial nucleus of the trapezoid body showed a band of activity at the dorsal or dorsolateral margin to 1 kHz stimulation and deeper bands to more intermediate frequencies. Diagonal banding observed in the superior paraolivary nucleus appeared particularly prominent at 8 kHz. The darkest bands in the auditory system appeared in the inferior colliculus. Multiple bands were observed in the medial geniculate body including the ventral and dorsal nuclei. Active bands observed in cortex including the primary area varied in width and depth. Results indicate selective banding patterns to pure tones at major levels of the auditory system and suggest potential for parallel and convergent processing. (Supported by NIH AG-1571).

188.13 AN EVALUATION OF THE ORIGINS IN THE BRAIN STEM OF THE SHORT LATENCY AVERAGED AUDITORY EVOKED RESPONSES IN THE ANAESTHETIZED DOG. S. J. Whidden* and R. W. Redding*(SPON: B. L. Bird)
School of Veterinary Medicine, Auburn University,Auburn,AL.36830

The origins in the brain stem of the short latency averaged auditory evoked responses in the anaesthetized dog were evaluated by surgical ablation(under visual control) of parts of the brain stem. Five normal adult dog's short-latency(<8msec) auditory evoked responses (A.E.R.) to clicks (70 dB S.P.L.) were recorded(512 averaged) after aspiration of different brain stem structures. Ten clicks per second were presented monaurally to the right ear and recordings were amplified, filtered(.32 Hz-3.2kHz), and averaged (512). These auditory evoked responses were analyzed statistically and assimilated into different models for each population after a given surgical procedure.The surgical suboccipital approach to the brain stem was used to expose the caudal aspect of the cerebellum, the dorsal aspect of the medulla and the fourth ventricle. The head was flexed nearly at a right angle to the cervical vertebrae. Bilateral precollicular decerebration was carried out by aspiration of both medial geniculate bodies rostral to the superior colliculi. Midbrain and later rostral medulla were aspirated,as well as the cochlear nucleus. After each of these lesions, a subsequent successful recording was obtained in all five of the manipulative surgical groups.

The results indicate that medial geniculate aspiration produced a decrease in amplitude of Wave IV, while WaveV remained nearly constant, and Wave VI was not clearly visible. With caudal colliculus aspiration, Wave IV seemed to decrease in amplitude , while Wave V was not clearly visible . With most of the brain stem removed, Wave III was not discernible. With the complete aspiration of the brain stem only Wave I remained in a reduced state. Based on these results it is possible to propose general locations for the generation of these potentials in the dog.

188.14 THE DISTRIBUTION OF ACTIVITY IN THE AVIAN AUDITORY SYSTEM TO MONAURAL SOUND STIMULATION EXAMINED WITH [14C]2-DEOXY-D-GLUCOSE AUTORADIOGRAPHY. W.R. Lippe* and R.B. Masterton. Depts. of Otolaryngology and Neurosurgery, Univ. of Virginia Medical Center, Charlottesville, Virginia 22908.

The laterality of auditory input at each level of the avian auditory system was studied using [14C]2-deoxy-D-glucose (2-DG) autoradiography. One to three week old chicks sustained unilateral basilar papilla removals. Three to seven days later they received injections of [14C]2-DG (16 μCi/100gm., IV) and were immediately exposed to 90 dB (SPL) broadband white noise stimulation. After 45 minutes of sound exposure they were sacrificed and autoradiographs were prepared from cryostat-cut frozen sections according to the procedure of Sokoloff et al. (J. Neurochem., 1977, 28, 897). The autoradiographs were then examined to determine the relative amount of 2-DG uptake contralateral vs ipsilateral to the remaining ear throughout the auditory system.

The distribution of 2-DG indicates that each hindbrain auditory structure except n. laminaris receives entirely or predominantly monaural input. In n. angularis, n. magnocellularis and the superior olive, the uptake of 2-DG occurs ipsilateral to the normal ear whereas in the nuclei of the lateral lemniscus there is uptake contralateral to the normal ear and only slight uptake ipsilaterally. In contrast, 2-DG uptake in n. laminaris occurs bilaterally and correlates with the known spatial segregation of afferent input to this structure (Lippe, Steward and Rubel, Brain Res., 1980, in press).

In contrast to hindbrain auditory structures, 2-DG uptake in midbrain and forebrain structures indicates that these structures receive binaural input which is only slightly dominated by input from the contralateral ear. In MLd ('inferior colliculus'), n. ovoidalis ('medial geniculate') and Field L ('auditory cortex'), the general pattern of 2-DG uptake is similar: the uptake is bilateral with only a slightly greater amount contralateral to the normal ear. The districution of 2-DG within MLd also suggests the presence of subfields in this structure which differ in dominance of binaural vs monaural input. This suggestion is further supported by the finding that pure tone stimulation results in high levels of 2-DG uptake at several separate areas within MLd.

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188.15 INGENIOUS USE OF PHASE BY THE OWL AUDITORY SYSTEM. Andrew Moiseff and Masakazu Konishi. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

A class of cells in owl's midbrain auditory nucleus (MLD) is known to respond to sounds from restricted regions of space. These cells have small spatial receptive fields, and are arranged anatomically to form a "neural map" of auditory space. We have exploited the organization of these cells to examine the role of interaural-time, -phase, and intensity differences in the extraction of directional information from sound. In this study we combine free-field and dichotic sound stimuli.

Single units were recorded with glass insulated Pt-Ir microelectrodes. The receptive fields were mapped using a free-field loudspeaker. Threaded plugs containing miniature speakers were then inserted into threaded tubes previously implanted into the ear canals allowing the presentation of dichotic stimuli. The phase of tone (in the range of 6-8 kHz) or noise stimuli delivered to each ear was varied, keeping intensity and arrival time constant, and the number of spikes resulting from each stimulus counted.

The results indicated that the responses of all MLD cells with restricted receptive fields varied as a function of interaural-phase differences. For example, cells with frontal receptive fields preferred zero phase difference between the two ears, whereas, cells having lateral receptive fields preferred the phase of the signal delivered to the ear contralateral to the receptive field to lag behind the signal delivered to the ipsilateral ear. The magnitudes of the preferred phase differences, which were within the physiologically relevant range, were correlated with the azimuth of the receptive fields. These data indicate that the owl auditory system is capable of using interaural-phase differences to determine the direction of high-frequency sounds.

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188.16 THE EFFECTS OF INTERAURAL TIME DELAY ON HIGH FREQUENCY CELLS IN THE CAT INFERIOR COLLICULUS. S. Kuwada*, T.C.T. Yin, and Y. Sujaku*. Dept. of Neurophysiology, University of Wisconsin Medical School, Madison, Wisconsin 53706.

A classical view of sound localization is that interaural time differences are the salient cue in the localization of low frequency (≲1500 Hz for humans) signals along the azimuth whereas interaural intensity differences are important in localizing high frequency stimuli. For sinusoidal tones the psychophysical, physiological and acoustical findings provide strong support for this duplex theory. However, results of recent psychoacoustical studies suggest that interaural time differences may also play a role in localizing high frequency signals. Human listeners in a dichotic situation can reliably lateralize complex high frequency signals (e.g. amplitude modulated) on the basis of interaural time differences alone.

We report here preliminary data on high frequency sensitive neurons in the central nucleus of the inferior colliculus (IC) which support the contemporary psychoacoustical findings. Platinum-and gold-plated microelectrodes were used to record extracellular single cell activity in barbiturate anesthetized cats. The stimulus to both ears was a train of high frequency sinusoidal tones which were gated on and off rapidly with a trapezoidal envelope. The carrier frequency was chosen to be within the response area of the neuron under study and the frequency (100-500 Hz) of the envelope was controlled by specifying the on-off time. The stimuli to both ears were identical except that the onset of the train to one ear was delayed in steps with respect to the other ear. The response of high frequency sensitive (>3000 Hz) IC neurons to such a stimulus was a cyclic function of interaural delay with a period corresponding to that of the envelope frequency. This response pattern closely resembles the commonly seen cyclic response of low frequency sensitive neurons to changes in interaural delay at the period of the stimulating frequency when pure tones are employed. Control experiments in which the carrier frequency was varied over a wide range demonstrated that these neurons were indeed sensitive to the high frequency component of the signal.

These data suggest that high frequency sensitive neurons are sensitive to changes in interaural time delay in a manner similar to their low frequency counterparts. These results are in accord with the recent psychoacoustical experiments demonstrating that time cues also play a role in the localization of high frequency signals.

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188.17 THE INFERIOR COLLICULUS OF MICE SUSCEPTIBLE TO AUDIOGENIC SEIZURES STUDIED WITH 2 DEOXYGLUCOSE AUTORADIOGRAPHY OR MULTIPLE UNIT RECORDINGS. J.F. Willott, N. Mogharreban*, and S.M. Lu*. Dept. of Psychol., Northern Illinois Univ., DeKalb, IL 60115.

Previous lesion-behavior studies have shown the inferior colliculus (IC) to be critical for the occurrence of audiogenic seizures (AGS) in mice. The present study therefore employed [^{14}C] 2-deoxyglucose (2DG) autoradiography or electrophysiological (multiple-unit) records to examine neural activity in the IC of two inbred mouse strains--the DBA/2 strain (susceptible to AGS) and the C57BL/6 strain (AGS resistant). For autoradiography, a 21-day-old mouse was injected with 5μCi 2DG and decapitated 20 min. later. The brain was removed, frozen, and sectioned in a cryostat. Subjects were DBA mice that had AGS induced (either wild running attacks only or full tonic convulsions) by exposure to intense broad band noise shortly before decapitation; C57 mice matched for noise exposure; mice of both strains exposed only to ambient sounds (in a glass chamber); and anesthetized mice of both strains, noise-exposed or exposed only to ambient sound.

In unanesthetized C57 mice, all IC nuclei were generally well labelled in both ambient sound- and noise-exposed animals. In DBA mice, the ventral portion of the central nucleus (ICC) showed limited uptake of 2DG irrespective of the occurrence or severity of AGS or acoustic conditions. Beneath the area of reduced uptake was a band of activity along the ventral IC border. Uptake of 2DG was also reduced in the external nucleus in DBA mice. Anesthetized mice showed reduced 2DG uptake, but noise exposure was associated with some increased labelling.

Multiple-unit records, obtained using high intensity stimulation in lightly anesthetized mice, found neurons showing abnormal afterdischarges (ADs) in the DBA IC (ADs have been correlated with susceptibility to AGS in previous studies). Most AD neurons were located near the area of transition to reduced 2DG uptake seen in the ICC of DBA mice. However, even here, the incidence of neurons displaying ADs was rather low.

These results indicate that (1) Although there are hyperactive (AD) neurons in the IC of DBA mice, there is not an abnormally high overall level of neural activity associated with AGS, as indicated by 2DG uptake. It appears that AD neurons comprise a relatively small subpopulation of IC neurons. (2) In DBA mice, the lack of difference in IC activity as a function of AGS suggests that paroxysmal activity associated with AGS arises in areas to which the IC projects, not within the IC itself. (3) The concentration of AD neurons in the ICC near the area of reduced 2DG uptake is consistent with previous studies showing a relationship between partially impaired sensitivity of the auditory system and susceptibility to AGS.

188.18 AUDITORY CENTERS IN THE ELASMOBRANCH BRAIN: DEOXYGLUCOSE LOCALIZATION AND EVOKED POTENTIAL RECORDING. Jeffrey T. Corwin and R. Glenn Northcutt. Dept. Neurosci., Sch. of Med., and Scripps Inst. Oceanogr., U.C.S.D., La Jolla, CA and Div. of Biol. Sci., Univ. of Michigan, Ann Arbor, MI.

An evolutionary branch point that appeared 400 million years ago is the most recent ancestral link between the elasmobranchs and all of the more modern jawed vertebrates. Yet, these groups still share many bodily traits. Some of the shared traits might be results of recent convergent evolution, but theory holds that the common characters that are widely distributed throughout these groups are most likely hereditarily linked through ancient ancestral forms. Therefore, by studying systems in elasmobranchs and comparing them to systems in other vertebrates we can infer the age of certain traits and some aspects of the primitive systems from which they arose.

In this inferential manner we have begun to approach the early evolution of the vertebrate auditory nervous system by investigating the brain of a modern elasmobranch, Platyrhinoidis triseriata. Candidate acoustic centers were identified through autoradiography of frozen sectioned brains from rays which had received ^{14}C-2-deoxy-D-glucose intravenously at 0.2 μCi/gm body wt. Following injections animals were exposed to 90 minutes of either intermittent airborne noise bursts or silence in a sound isolation chamber. Clear experimental increases in radioactivity were observed in the anterior and descending octaval nuclei of the medulla and a mesencephalic nucleus believed to be the torus semicircularis. Less distinct increases were apparent in the cerebellar auricles, the medial diencephalon, and the telencephalic dorsal pallium.

The electrical activity of these centers was then investigated through field potentials evoked by airborne clicks at +45 dB re 1 μbar. The prussian blue marking technique was used in the direct histological verification of recording sites. These experiments confirmed the acoustic activity of the medullary, cerebellar, and mesencephalic centers identified in autoradiography, but the forebrain centers have not yet been confirmed. In summary, this elasmobranch has specific auditory relay centers in at least the medulla and the midbrain. These appear to be partially separate from vestibular and lateral line centers and at least partially homologous to auditory relays in other vertebrates. While the auditory brainstem has been modified in many groups, these results suggest that the basic relay centers may already have existed 400 million years ago.

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188.19 EFFERENT PROJECTIONS OF THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS: AN AUTORADIOGRAPHIC STUDY. D.L. Griffith*, M.H. Cooper, and L.C. Massopust. Departments of Anatomy and Otolaryngology, St. Louis University School of Medicine, St. Louis, MO 63104.

The efferent projection system of the ventral system of the lateral lemniscus was investigated in the laboratory rat (Rattus norvegicus albinos) using an autoradiography technique. Tritiated leucine at a concentration of 50 μ Curies/μl was injected iontophoretically into the ventral nucleus of the lateral lemniscus (VNLL) of rats weighing between 290-325 grams with a micropipette which had an opening at its tip of approximately 70 μm. Following a survival period of five days the rats were intracardiacally perfused and the brains were processed for autoradiography according to the method of Cowan et al. (1972). The sections stained with cresyl violet acetate were studied under light field illumination for the topography of nuclear arrangement and under dark field illumination for the course and terminations of the efferent projection system of the VNLL.

Axons from the VNLL projected to a variety of structures both rostrally and caudally to the ejection site as well as to structures at the same level. Rostrally axons either ascended ipsilaterally in the lateral lemniscus or crossed the midline through the reticular formation to the contralateral lateral lemniscus. Terminal fields were present in the dorsal nucleus of the lateral lemniscus, inferior colliculus, and medial geniculate nucleus of both sides. The concentration of reduced silver grains was more prominent in the ipsilateral structures than in the contralateral structures. Within the dorsal nucleus of the lateral lemniscus the terminal fields were divided into two distinct zones, one dorsal and the other ventral. Caudal to the ejection site descending axons project to the cochlear nucleus of both sides. These axons reached the cochlear nucleus by traversing the reticular formation dorsally as well as ventrally. Labelled axons were also present in a separate group which was placed ventral to the reticular formation. At the level of the ejection site labelled axons crossed the midline through the reticular formation to the contralateral ventral nucleus of the lateral lemniscus.

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188.20 FOREBRAIN MECHANISMS IN SOUND LOCALIZATION: THE ROLE OF THE MEDIAL GENICULATE. Peter W. Judge* and Jack B. Kelly. Department of Psychology, Carleton University, Ottawa, Ontario, Canada, K1S 5B6.

Previous studies of sound localization by the rat have shown that large bilateral lesions of auditory cortex fail to produce the severe deficit in performance expected from studies of other species such as dog, cat or monkey (Kelly, J.B., Neuroscience Abstracts, 4:7, 1978). Even though auditory cortex is not essential for sound localization in the rat, it is possible that this function is mediated by other forebrain projections, for example, from medial geniculate to subcortical structures. In order to further clarify the role of the forebrain in sound localization, the effects of bilateral lesions of the medial geniculate were examined. Brain damaged and normal rats were trained to localize sounds in space in a two choice maze with variable speaker positions. Tests were carried out with speakers separated by 180 or 60 degrees and included single presentations of short duration noise pulses at the beginning of each trial. The stimuli contained either a broad band of frequencies (2kHz-32kHz) or narrow bands of high or low frequencies (around 32kHz or 2kHz). Histological analysis of brain damaged animals revealed massive lesions of the medial geniculate nucleus in every case. Damage to midbrain structures was minimal. The extent of damage to other forebrain structures such as hippocampus or other thalamic nuclei was variable from animal to animal. Pronounced deficits in performance were found especially under conditions of high frequency localization. Deficits were also noted under other stimulus conditions. However, with broad band and low frequency stimuli high levels of performance were found in spite of large lesions of the medial geniculate in a number of individual cases. These results indicate that while the medial geniculate is not essential for sound localization in the rat, deficits following forebrain lesions may be revealed under appropriate stimulus conditions.

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188.21 GIANT SPINE-POOR PYRAMIDAL CELLS IN AUDITORY CORTEX OF YOUNG AND AGED CATS. Anne S. Kaplan and Arnold B. Scheibel. Brain Research Inst. and Dept. of Anatomy, University of California, Los Angeles, CA 90024.

In studying the auditory cortex of aged (12-18 years) cats, we have discovered a population of unusually large and spine-poor pyramidal cells. These have been found in Golgi-stained, 120 μ thick coronal sections of the mid-ectosylvian gyrus, and in adjacent 10 μ thick Nissl-stained sections. The auditory giant pyramids (AGPs) are comparable in size (750-2600 μ^2 in cross-sectional area; 40-75 μ high by 20-25 μ wide) to the Betz and Meynert (1,2) cells of motor and visual cortices. Like these other giants, AGPs are found sparsely distributed in Layer V. In Golgi sections, each AGP has a wide skirt of basilar dendrites, and sends a thick (6-8 μ), mostly unbranched apical dendrite towards the pial surface. This shaft bifurcates in Layer III. Unlike Meynert cells, which are found in primary receptive cortex, AGPs are found most frequently in the auditory association areas AII and SF (suprasylvian sulcus).

Surprisingly, these AGPs are essentially spine-free. For example, they have fewer than 10 spines between the origin and bifurcation of the apical shaft, a distance which may exceed 400 μ. Cells of similar morphology, but with spiny apical shafts (25-250+ spines), invariably have smaller somata (400-850 μ^2).

In young adult cats (1.5-2 years), AGPs may also be found. There are fewer AGPs in young than in old cats, as confirmed by blind counts of giant somata in Nissl-stained sections. Also, the inverse relationship between size and spininess is less absolute in the young cat: occasional spiny giants (800-1000 μ^2) are found, and even the largest AGPs may be somewhat spinier than their aged counterparts. Most young cells having the dendritic morphology of the AGPs are densely spined, but have surprisingly small somata (200-550 μ^2).

The dendritic arrays of giant cells in both young and old cats are quite lush and extensive, and are free of the nodularities and distortions which typify dying neurons (3). Thus, the unique spine-free nature of the AGPs and their increase in number with age, does not seem to indicate an absence of viability.

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188.22 FREQUENCY ORGANIZATION OF AUDITORY CORTEX IN THE GREATER GALAGO. K.A. FitzPatrick, C. Welt, J.F. Brugge, T.A. O'Conner and B. Sprague*. Dept. of Neurophysiology, Waisman Center, Univ. of Wisconsin, Madison, Wisc. 53706.

Microelectrode techniques have been used to map the frequency organization of the auditory cortex of the greater galago (Galago crassicaudatus). Best frequencies of neurons and neuron clusters were recorded under sodium pentobarbital anesthesia. Their distributions were reconstructed in serial histological sections stained by the Nissl method. Auditory responsive cortex extends from the lateral surface of the superior temporal gyrus into the lateral fissure. It occupies much of the superior temporal plane in this prosimian. A primary field (AI) is present occupying a part of the dorsal and lateral surfaces of the superior temporal gyrus. Area AI contains a complete representation of the basilar membrane with low frequencies represented rostrolaterally and high frequencies caudomedially in the field. Another complete frequency representation is found posterior to AI in the posterolateral field (PL). Here low frequencies appear laterally whereas high frequencies are found medially contiguous with the representation of the highest best frequencies in AI. A rostral field (R) lies anterior to AI. We have mapped only a partial representation of the membrane in R. Its low frequency representation is contiguous with that in AI. Higher best frequencies (up to 12 kHz) are represented progressively more rostrally within R. The caudomedial field (CM) lies medial to AI and PL deep on the superior temporal plane. Its border is marked by a gradual or abrupt decline in best frequency from the highs which predominate at the medial sides of both AI and PL. Both high and low best frequencies are present in CM, but no obvious frequency organization is present. A few auditory responsive cells are encountered lateral to AI on the gyral surface. No particular frequency organization is evident. Fields AI, R, PL and CM appear to be homologous to areas completely or partially mapped in the rhesus and owl monkeys (Merzenich and Brugge, '73; Imig et al., '77).

188.23 TONOTOPIC ORGANIZATION AND DENDRITE ORIENTATION IN PRIMARY AUDITORY CORTEX OF THE RABBIT. E.M. Glaser and N.T. McMullen, Dept. of Physiol., Univ. of Maryland Sch. of Med., Baltimore, Md. 21201

We have been studying the frequency map in rabbit auditory cortex in order to determine whether its topography is related to the spatial orientation of the dendrites of its individual auditory neurons. Our experiments have been performed on urethanized Dutch Belted and New Zealand rabbits. The stimuli were slowly fluctuating tone bursts delivered at a 1/sec rate to the left, right, and both ears in a rotating sequence. Multiunit PSTs and average evoked potentials (AEPs) were observed with glass-coated microelectrodes that were advanced through the cortex along tangential tracks in the frontal plane. (See McMullen and Glaser, Soc. Neurosci. Abstr., 5,26, 1979 for details.) At 300 μm intervals within the responsive auditory area, we determined both best frequency and aural dominance by judging the vigor of the multiunit and AEP responses. In eight animals it was possible to make three or four parallel electrode penetrations 500 μm apart with each penetration passing through about 4 mm of 'mappable' cortex. Data were obtained from an average of about 50 sites per animal. We have found that: (a) The primary auditory area (as defined by its responsive and histological properties) is organized such that there is a generally monotonic decrease of best frequency from high (30-35 kHz) dorsally to low (ca. 400 Hz) ventrally. (b) The isofrequency contours are oriented in an anteroventral to posterodorsal direction. (c) The high (>15 kHz) frequency area is more extensive anteriorly while the area for frequencies below 3 kHz is more extensive posteriorly. (d) The largest part of the primary area is devoted to frequencies between 5 and 15 kHz. We have also obtained good evidence for a second auditory area dorsal and anterior to the primary one. Its best frequencies range from 1 to 10 kHz and are mapped with the low frequencies more dorsal than the high. With respect to aural dominance, we have found that 80% of the multiunit responses are contralaterally dominant and 20% ipsilaterally dominant. The responses exhibit binaural interactions that may be classified as summating (33%) or suppressive (67%). Alternating bands of response suppression and summation are sometimes seen. After electrophysiological mapping, the brains were processed according to the Golgi-Cox Nissl method. Using a computer microscope we have studied five pyramidal and five nonpyramidal neurons in layer IV of the primary auditory area of one such brain. A dendritic stick analysis was employed. The pyramidal cell basal dendrites show a clear preference for an anterior posterior orientation. There is also a good indication of a similar orientation of the dendrites of the nonpyramidal neurons. The results suggest that dendrites in layer IV are oriented similarly to the isofrequency contours.

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188.24 SOUND INDUCED HEAD ORIENTING IN UNRESTRAINED CATS, S.E. Fish. Dept. Neurobiology, North Eastern Ohio Univ. Col. of Med., Rootstown, OH 44272.

Cats will orient their heads toward a sound source of interest to them but this behavior is difficult to quantify in freely moving animals. A technique was developed to study the cat's orienting behavior and data were collected to document it.

A small lightweight projector was developed which could be fastened firmly to a cat's head. The position of a small spot of light emanating from the projector was photographed to determine the orientation of the subject's head in relation to a sound source (30hz square wave clicks, 75Db). Sound sources were baited with freshly killed (cervical dislocation) mice which the cats were allowed to capture and eat. Trials were run in the dark and the cats "hunted" (with great enthusiasm) for all of their food in this manner.

The two cats were able to orient their heads to within a few degrees both horizontally (mean error, 1.84° and 3.77°) and vertically (mean error, 3.25° and 5.25°) of a sound source. The horizontal figures are comparable with the cat's horizontal auditory localization ability. There was a significant tendency for a systematic error in vertical orienting that was not evident in the horizontal orienting. The cats tended to orient below targets positioned above them and above targets positioned below them. This systematic error was not correlated with stimulus duration and remains unexplained. After a momentary sound stimulus the cats were observed to maintain their accurate orientation to its position while approaching it.

These data document the prominent role for head orienting in auditory localization and predatory behaviors of the cat.

188.25 INFLUENCE OF SEMICIRCULAR CANAL AMPULLA, DUCT, AND UTRICULAR SHAPE ON ENDOLYMPH FLOW DYNAMICS C.M. Oman and E.N. Marcus*
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Theoretical descriptions of endolymph flow and cupula motion have traditionally been made employing a model (van Egmond, et al, J. Physiol. 110: 1 - 17, 1949) in which the entire membranous canal is represented as a hollow, thin torus of constant, circular cross section. The resulting 2nd order differential equation relating endolymph angular displacement about the center of the canal to head angular acceleration has two coefficients, π/Δ and θ/π. However, the cross section of an actual canal varies significantly in the ampulla and utricle. The effect of this variation has been considered only for simple variations in canal geometry (Van Buskirk, W.C., Ann. Biomed. Eng. 5: 1-11, 1977; Oman, C.M., Vestib. Function and Morphology, T. Gualtierotti, ed. Springer, N.Y., Ch.14, 1980). We have developed a more general theoretical model describing Newtonian endolymph flow where the canal lumen varies continuously in size and shape in a gradual fashion through the duct, ampulla, and utricle. Endolymph flow is more accurately described by a 2nd order differential equation with three coefficients, rather than by the traditional two coefficient model. The three coefficients (and hence the canal time constants and gain factor) analytically depend on the average of the inverse cross sectional area of the canal lumen (taken around the canal circumference) and also on the average inverse squared cross sectional area. To investigate the behavior of these functions for an actual canal, an optical technique was devised which was used to make the necessary dimensional measurements in the dissected, fixed canal of the skate, Raja erinacea. The numerical results obtained indicate that in most species, the inverse area functions (and hence canal response characteristics) are dominated by the influence of the narrow duct and ampulla cross sections, and by the fraction of the canal circumference which they occupy. The long time constant and the amount of cupula motion produced per unit head velocity (gain factor) should be heavily influenced by the relative length of the utricle and ampulla dilations. On the other hand, the short time constant of the model is nearly independent of the fraction of the canal torus occupied by the duct. One cannot legitimately "adjust" the two coefficients of the traditional model differential equation to allow for the presence of the ampulla and utricle, as has sometimes been claimed.

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188.26 TRANSFER CHARACTERISTICS OF ANTERIOR SEMICIRCULAR CANAL AFFERENTS IN THE ANESTHETIZED GERBIL. A.A. Perachio and M.J. Correia.
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Experiments were performed on 21 gerbils anesthetized with urethane and maintained on ketamine. Recordings were made from either the right or left vestibular nerve using stereotaxically guided glass micropipettes driven through the intact cerebellum. Using the rotational null point technique (Estes et al., J. Neurophysiol., 38:1232-1249; 1975), ampullary afferents from the anterior (n = 22) and horizontal (n = 22) semicircular canals were functionally identified. Responses of those afferents to single and complex sinusoidal oscillations were obtained over a stimulus bandwidth of 0.01 Hz to 5.0 Hz. Spontaneous discharge was also recorded. All tests were performed with the animal's head held so that the horizontal semicircular canals were in the plane of rotation, and coplanar with the earth horizontal plane. Unit responses were dichotomized based on the CV of the spontaneous discharge. Anterior ampullary units with CV $\leq$ 0.1 had a mean firing rate (± SEM) of 83.04 ± 8.1 Impulses/s ($I \cdot s^{-1}$) and a mean (± SEM) gain and phase re velocity of 0.077 ± 0.02 $I \cdot s^{-1}/deg \cdot s^{-1}$ and 42.94 ± 1.7 deg at 0.1 Hz; and 0.078 ± 0.02 $I \cdot s^{-1}/deg \cdot s^{-1}$ and 6.79 ± 1.0 deg at 1.0 Hz. Those units with CV > 0.1 had a mean firing rate (± SEM) of 48.29 ± 6.1 $I \cdot s^{-1}$, and a mean (± SEM) gain and phase of 0.154 ± 0.02 $I \cdot s^{-1}/deg \cdot s^{-1}$ and 55.14 ± 4.12 deg at 0.1 Hz and 0.166 ± 0.05 $I \cdot s^{-1}/deg \cdot s^{-1}$ and 29.06 ± 3.3 deg at 1.0 Hz. Comparable data for horizontal ampullary afferents include: (A) CV $\leq$ 0.1, mean (± SEM) firing rate 73.34 ± 2.87 $I \cdot s^{-1}$; mean (± SEM) gain and phase of 0.224 ± 0.06 $I \cdot s^{-1}/deg \cdot s^{-1}$ and 50.42 ± 5.07 deg at 0.1 Hz; and 0.312 ± 0.07 $I \cdot s^{-1}/deg \cdot s^{-1}$ and 14.48 ± 2.9 deg at 1.0 Hz, (B) CV > 0.1, mean (± SEM) firing rate 42.83 ± 6.73 $I \cdot s^{-1}$; mean (± SEM) gain and phase of 0.467 ± 0.08 $I \cdot s^{-1}/deg \cdot s^{-1}$ and 59.78 ± 2.8 deg at 0.1 Hz; and 0.914 ± 0.15 $I \cdot s^{-1}/deg \cdot s^{-1}$ and 29.45 ± 1.8 deg at 1.0 Hz.

The above findings on the response characteristics of horizontal canal afferents confirm those of Schneider and Anderson (Brain Res., 112:61-76; 1976). The new data on anterior canal afferents indicate that, for rotation in a plane nearly orthogonal to the vertical semicircular canals, the gain slope remains constant over the range 0.1 to 1.0 Hz while the phase angle of the response is comparable to that of horizontal canal afferents.

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188.27 ADAPTATION OF AN OTOLITH-SPINAL REFLEX DURING PROLONGED EXPOSURE TO ALTERED GRAVITY. D.G.D. Watt and H.A. Zucker*. Aviation Med. Research Unit, McGill Univ., Montreal, Canada H3G 1Y6.

Previous studies have demonstrated that gastrocnemius-soleus emg activity occurring 50 to 150 msec after the onset of a sudden fall is predominantly otolith-spinal in origin. Further experiments have shown that the size of this response may be reduced significantly by rotating the gravity vector 90° relative to the body, or by eliminating it entirely, as in parabolic flight in an aircraft. These experiments seek to identify any adaptive changes which might occur during prolonged 90° rotation of the gravity vector.

Three subjects were exposed to sudden, unexpected vertical falls of 15 cm while surface emg activity was recorded from their left calf muscles. Each was then suspended horizontally in the supine position and tested in the same way every 60 to 90 min for 8 hrs, substituting for gravity with bungee cords running from the waist to the wall. It should be noted that subjects were never allowed out of the supine position during this period, but were encouraged to remain active, and do such things as hop "up and down" on the wall. Vertical testing was then repeated immediately after returning to the upright position, and every 90 min thereafter for 3 hrs.

All subjects demonstrated the expected reduction of the otolith-spinal response on assuming the supine position (area of rectified and averaged response 15-30% of normal). All showed a steady, systematic increase of the response with time, however, with about 50% recovery by 8 hrs. The effect of returning to the vertical position was more complicated, consisting of an early suppression of the reflex, followed quickly by larger than normal responses and then a return to normal.

These results demonstrate neural adaptation to rotation of the gravity vector, comparable to changes seen in the vestibulo-ocular reflex during vision modification experiments involving reversing prisms or magnifying lenses. However, the present findings extend this phenomenon to the otolith organs and the peripheral musculature, and seem to indicate that adaptation can occur in the absence of any obvious sensory conflict. The latter finding is supported by the complete lack of motion sickness symptoms in these (and other) subjects while adaptation was occurring. This is very different from reversing prism experiments, in which nausea, etc. develops rapidly in most subjects.

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188.28 MORPHOPHYSIOLOGY OF SUPERIOR VESTIBULAR NUCLEUS NEURONS PROJECTING TO THE CEREBELLUM IN THE CAT. AN INTRACELLULAR HRP STUDY. A. Mitsakos*, H. Reisine, and S.M. Highstein. Dept. Neurosci., Albert Einstein College of Medicine, Bronx, N.Y. 10461

The superior vestibular nucleus (SVN) was explored with glass microelectrodes loaded with HRP in anesthesized, paralyzed cats. The IIIrd nucleus and both vestibular nerves (VN) were electrically stimulated. SVN neurons projecting to the cerebellum (VCb) were identified morphologically by their axonal trajectories. All VCb neurons receive monosynaptic EPSPs and di- or polysynaptic IPSPs following ipsi- and contralateral VN stimulation respectively. We imply that VCb cells are functionally Type I.

VCb neuronal somas are oval to round (long dia. 28-55μ) and lie centrally or lateral and dorsal in SVN. Axon hillocks of VCb neurons are located on the medial, dorsal or lateral soma.

VCb axons (d=2.5-4.5μ) traverse SVN in a dorsal or lateral direction before exiting SVN laterally. Two axonal trajectories are followed. Some VCb axons travel laterally across the restiform body towards the ipsilateral flocculus. Others travel dorsal, then medial curving around the brachium conjunctivum toward the ipsilateral fastigial nucleus. Other VCb axons course toward the fastigial nucleus while a thin collateral (d=1.5μ) travels toward the ipsilateral flocculus.

VCb neurons emit 3 to 7 primary dendrites with 2 to 3 disproportionally thicker dendrites, more heavily branched. Secondary and tertiary dendrites are both tortuous and straight, exhibit a few spines, short appendages with bulbous heads, and distal beaded branchlets. The dendritic aborization of VCb neurons is polarized along a rostro-caudal axis, covering a small field in SVN. This orientation is less pronounced than that of the typical radiate dendritic tree of SVN vestibulo-ocular neurons.

This study reveals the characteristic dendroarchitectonic appearance of VCb neurons and their axonal trajectories following two distinct courses.

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188.29 RESPONSE CHARACTERISTICS OF VESTIBULO-CORTICAL NEURONS TO ROTATIONS OF THE ALERT CAT IN THE DARK AND LIGHT. A.K. Sestokas* (SPON: M. Terman). Dept. of Psychology, Northeastern University, Boston,Ma.,02115.

While much is known about the processing of vestibular afferent signals in the brainstem, relatively little is known about the representation of this information by primary vestibulo-cortical neurons. The present investigations were undertaken to study the response characteristics of these cortical neurons to sinusoidal rotations in order to facilitate comparison with response properties of brainstem vestibular neurons. Recently, it has been reported that at rotational frequencies above 0.1 Hz single cell activity in primary vestibular cortex of the cat and monkey modulates in phase with head angular velocity, essentially imitating the activity of vestibular cells in the brainstem (Becker,Deeke and Mergner; Buettner and Büttner; Pflügers Archiv,373 Supplement,R87,1978). Furthermore, in monkey most primary vestibulo-cortical neurons also respond to optokinetic stimulation. The nature of visual input to cat vestibular cortex (anterior suprasylvian gyrus, ASSG) was investigated in this study by comparing responses of ASSG neurons during rotations of the cat in the dark with those in the light. Extracellular activity was recorded from restrained, alert cats during sinusoidal rotation at four frequencies between 0.18 and 0.75 Hz. These recordings confirm previous reports of Type I (activation by ipsilateral rotation, inhibition by contralateral) and Type II (activation by contralateral rotation, inhibition by ipsilateral) responses whose peaks were in phase with maximum head velocity. However, unlike the brainstem, cortical response patterns were often markedly distorted reproductions of the sinusoidal stimulation. Firing rates tended to increase much more rapidly during activation than they decreased during inhibition. Moreover, identical rotations in the light produced higher discharge rates and often accentuated the non-linearities causing a phase advance relative to activity in the dark. The influence of visual inputs in the ASSG was most evident in those cells which showed very weak responses to rotations in the dark. These neurons exhibited large amplitude, tightly coupled responses to head angular velocity only during rotations in the light. This is unlike activity described for the vestibular nuclei or vestibular cells in the thalamus and suggests that these cortical cells may receive a visual input other than that known to impinge on vestibular nuclei.

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189.1 EFFECT OF CHRONIC SALT TREATMENT ON THE ChAT ACTIVITY IN HYPOTHALAMIC MAGNOCELLULAR NUCLEI OF RATS. Dieter K. Meyer* and Cinda J. Helke. (SPON: Juan M. Saavedra). Laboratory of Clin., Science, NIMH, Bethesda, Md. 20205.

Choline acetyltransferase (ChAT) activity (a specific marker for cholinergic neurons) has been found in the supraoptic (SON) and paraventricular (PVN) nuclei which are responsible for the synthesis and release of vasopressin(AVP). The bulk of the ChAT activity in the SON appears to be in neurons which are intrinsic to the nucleus. Pharmacological studies have demonstrated that stimulation of hypothalamic cholinergic receptors alters the release of AVP (Endocrinology 101: 411, 1977). More recent in vitro studies suggest that cholinergic neurons mediate the release of AVP due to osmotic stimuli (Endocrinology 105: 367, 1979). Therefore, the purpose of this study was to investigate the ChAT activity (as a marker of the activity of cholinergic neurons) in magnocellular nuclei under conditions of chronic osmotic stimuli and to correlate the data with pituitary AVP levels.

Male Sprague Dawley rats were sacrificed (3,7 or 14 d) following chronic substitution of either 1% or 2% NaCl solution for drinking water. The SON, PVN, arcuate nucleus and dorsal hippocampus were microdissected and assayed for ChAT activity. Controls consisted of age matched rats supplied with normal drinking water.

Replacement of drinking water with 1% saline for 3, 7 or 14 d had no effect on ChAT activity in the hypothalamic nuclei investigated while 2% saline treatment reduced the ChAT activity in the SON at 7 and 14 d (to 84 and 67% of control) and in the PVN and arcuate nucleus at 14 d (to 59 and 58% of control, respectively). At no time was a decrease in ChAT activity observed in the dorsal hippocampus indicating the specificity of the effect. No change in ChAT was observed in any of these areas in salt substituted adrenalectomized rats (5 d). Thus, these effects do not appear to be due to nonspecific stress.

The AVP content of the posterior pituitary was decreased by 7 and 14 d of 1% saline treatment (70 and 60% of control). Two percent saline decreased the AVP content to 10% of control at 3 d of treatment, this decline was sustained at 7 and 14 d.

These results support the hypothesis that cholinergic neurons are involved in the regulation of AVP release due to osmotic stimuli.

189.2 ANTICONVULSANT ACTIVITY OF β-ENDORPHIN IN THE SEIZURE SENSITIVE MONGOLIAN GERBIL. J.G. BAJOREK*, D.H. CATLIN*, and P. LOMAX. Department of Pharmacology, School of Medicine and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

The opioid peptides, enkephalins and endorphins, were originally reported to induce electrographic seizures in rats. However, in the Mongolian gerbil, a natural genetic model of epilepsy, met-enkephalin (100-200 µg) injected intraventricularly (i.c.v.) did not change the incidence of seizures, nor did it modify the severity or duration of the attacks, or produce extensive epileptogenic electrographic discharges, although it did produce single paroxysmal spikes at a rate of 0.16 Hz. Intraventricular injection of β-endorphin (1-10 µg) reduced the incidence (51%) and severity (55%) of the natural seizures, as defined by characteristic behavioral and electrographic criteria. Naloxone (1 $mg.kg^{-1}$ i.p. 15 min previously) blocked these effects of β-endorphin indicating the involvement of opiate receptors. In addition to the anticonvulsant effect, β-endorphin induced consecutive periods of synchronization of the EEG to 6 Hz; concurrently the animals would flatten themselves down in the cage and clonic movements of the extremities occurred (LPS seizures). These LPS seizures comprised 9.4%-19.4% of the time in the period during which the animals were observed. Significant reductions in grooming (81%) and exploratory rearings (65%) are also evident. These effects were all dose dependent and were blocked by naloxone (1 $mg.kg^{-1}$ i.p.). Naloxone by itself has been shown to increase the incidence of the natural seizures in these animals. From these several observations it might be concluded that endogenous opioid peptides may modulate the seizures in the gerbil. There was evidence of an increase in intermittent abnormal spiking in the cortical EEG, which was increased, not decreased, by naloxone. It is of interest to try to disassociate the anticonvulsant and the LPS effects of β-endorphin and to localize the site at which the LPS effect originates. The lack of cortical paroxysms would tend to indicate a subcortical origin. Supported by ONR Contract N00014-75-C-0506.

189.3 HORMONAL MODULATION OF CENTRAL AND PERIPHERAL COMPONENTS OF "FAST" AND "SLOW" SKELETAL NERVE-MUSCLE SYSTEMS IN XENOPUS LAEVIS. G. Schneider*, N. Rubinstein*, F.P. Zemlan* and S.D. Erulkar Depts. Pharmacology and Anatomy, University of Pennsylvania Medical School, Philadelphia, PA 19104

Administration of exogenous androgens to male Xenopus laevis produces stereotyped sexual behavior including the "clasping" of the female during amplexus. The muscles of the front legs primarily involved in this action are the m. sternoradialis and the m. flexor carpi radialis. Using antibody staining techniques, we have shown that these muscles contain both slow tonic and fast twitch fibers. There is a sexual dimorphism; muscles from males contain higher numbers of slow fibers than those from females. The nerve in the m. sternoradialis has a multimodal distribution with respect to both fiber diameter and conduction velocity. In order to test neuronal effects of the androgens, the isolated spinal cord of Xenopus was used and the properties of neurons involved in clasp behavior studied in different hormonal states using intracellular recording techniques. Recordings were obtained from the nerve to m. sternoradialis in both castrated and sexually active (induced by testosterone or anterior pituitary administration) males. Dorsal root stimulation elicited a long latency diffuse response from neurons in cords from both castrated and induced males; the response was of shorter latency and more sustained in the latter. The most striking changes occurred in response to paired stimuli to the dorsal root. Testosterone administration to the bath caused a shortening of the latency, a reduction of the recovery period, an increased facilitation and a broadening of the interstimulus interval over which facilitation took place. Furthermore the pattern of activation was changed, the response to the second stimulus becoming more synchronized and periodic. Intracellular recordings from identified neurons in cords from induced animals revealed the existence of neurons that responded predominantly with repetitive discharge and after-depolarizing potentials, although our sample is small. The apparent effect of these changes brought about by the androgens is to allow more neurons to fire at faster repetition rates. Therefore hormones appear to modulate directly patterns of neural discharge in the cord consistent with the observed behavioral response. Moreover it is an interesting possibility that the differing patterns of neural input may influence the muscle fibers to change the character of myosin from slow to fast or vice versa. However a direct effect by the hormones on the muscle itself has not been excluded. (Supported by NS 12211).

189.4 EFFECT OF DELTA-9-TETRAHYDROCANNABINOL ON HIPPOCAMPAL EXCITABILITY IN ADULT MALE RATS. Michael R. Foy, Timothy J. Teyler and Richard M. Vardaris. Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272 and Kent State University, Kent, Ohio 44242.

Rodent hippocampal physiology has been shown to be altered following administration of behaviorally psychoactive doses of delta-9-tetrahydrocannabinol (delta-9-THC), one of the major psychoactive constituents of marihuana (Vardaris, Teyler and Weisz, Brain Res. Bull., 1977, 2, 181-187). Previous work has also demonstrated the modulating effects of gonadal steroids on in vitro hippocampal slices of normal adult rats (Vardaris and Teyler, Neurosci. Abs., 1978, 4, A1145) and castrated/ovarectomized rats (Teyler, Foy and Vardaris, Neurosci. Abs., 1979, 5, A1568). Due to the chemical similarity between delta-9-THC and the estrogenic steroids, further examination of the effect of delta-9-THC on hippocampal function may provide information concerning the physiological effect of this agent.

In vitro hippocampal slices were prepared according to standard methods (Teyler, Brain Res. Bull., 1980, in press) and tested in response to 1, 10 and 100 pM concentrations of delta-9-THC suspended in PVP applied in a random order. Electrophysiological measures were taken at 10 and 20 minutes following addition of drug to the bathing incubation media. CA1 excitability to monosynaptic stimulation of Schaffer collateral afferents displayed the greatest enhancement of extracellular population spike amplitude to 10 pM delta-9-THC. The 100 pM concentration resulted in a depression of population spike amplitude with respect to pre-drug controls, wheras the 1 pM concentration had little or no effect.

Within the examined dose range (1 pM, 10 pM and 100 pM), delta-9-THC initiated both excitatory and depressant postsynaptic responses. These results are consistent with the results of a similar study in the intact rat wherein hippocampal CA1 population responses to monosynaptic activation also displayed an inverted-U function over a 2-16 mg/kg dosage range (Weisz, Vardaris, Gunnell, and Teyler, unpublished). The time course of these actions was quite rapid, suggesting a membrane effect of this lipophilic drug. The initiation of both excitatory and depressant responses may be due to differential interractions with steroid receptors and/or neurotransmitters. These results indicate that delta-9-THC modulates the excitability of the rodent hippocampus and may do so by acting through mechanisms that can be studied in the in vitro preparation.

189.5 MELATONIN BINDING IN THE PINEAL GLAND: SPECIFICITY AND EFFECTS OF EXOGENOUS MELATONIN. W.R. Holloway*, L.J. Grota, & G.M. Brown. Dept. Psychiatry, Univ. Roch. Sc. Med., Roch., N.Y. 14642, and Dept. Neurosci., McMaster Univ., Hamilton, Ontario, Canada.

We have previously reported the presence of a melatonin (Mel) binding material in the rat pineal gland (PG)(Holloway et al., Neurosci. Absts.,1979,5,1515) using a semi-quantitative double antibody procedure (Holloway et al.,J. Histo. Cyto.,1980,28,255). Specific anti-Mel antibody (A-Mel) and FITC-labeled IgG were the first and second antisera, respectively. A CdS photometer measured fluorescence intensity (FI). With this method we found low levels of occupancy (30%) 1.5hr after lights-on with the maximum (100%) occurring 1.5hr after lights-off. In the following experiments we provide information oabout the specificity of this material and the influence of exogenous Mel on its binding ability.

Adult male rats on a 12:12 LD cycle were killed 1.5hr after lights-off, when the binding material is saturated with Mel. Cryostat sections of PG were incubated with A-Mel containing 0,1,10 or 1000 ng Mel/ml or with identical amounts of competing substances (N-acetyl-serotonin(NAS), serotonin(5-HT), and 5-Methoxy-tryptophol(5-M-ol)). 10 ng Mel/ml produced a significant fall in FI. NAS had a displacement curve identical to Mel. 5-HT and 5-M-ol did not displace Mel. These data indicate that indoles with an N-acetyl group on the terminus of the side chain will compete with Mel for binding sites.

Other studies have investigated the influence of Mel **in** vivo on the binding pattern of this material. Male rats were injected IP with Mel (MI) or vehicle (VI) 0.5 hr after lights-on (1mg/rat) and killed 1,6,or 11 hr later. MI animals had high plasma Mel levels at 1hr (16,500 pg/ml), returning to normal 11 hr later. Mel binding in the PG was markedly altered by the Mel treatment. VI rats had low FI at 1 hr, rising through the day to high levels 11 hr later. Mel injection prevented this rise. A second group of rats were injected with Mel (50ug), vehicle, or were not disturbed (NI) 1.5 hr before lights-out and killed 1, 3, or 9 hr later. MI rats had high plasma Mel 1 hr after injection (211 pg/ml), but at 3 and 9 hr they were identical with VI and NI rats. Mel treatment altered the pattern of Mel binding in the PG. Whereas NI rats had high FI at all sampling points, MI rats had significantly lower FI 1 and 3 hr after treatment, with a rise 9 hr later. VI rats exhibited a pattern of FI identical to the MI animals. The effect of repeated daily injections of Mel or vehicle on the binding properties of this material is under investigation.

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189.6 MELATONIN INHIBITION OF THE RESPONSE TO LHRH BY AN ENRICHED GONADOTROPH CELL PREPARATION. J. E. Martin, D. McKeel* and C. Sattler*. Departments of Pharmacology and Pathology, Washington University Medical School, St. Louis, MO 63110.

Melatonin, a secretory product of the pineal gland, can suppress the neonatal rat pituitary LH and FSH responses to LHRH. This action of the indole has been shown both in intact animals and in pituitary cultures, thus clearly demonstrating the pituitary gland as a target tissue of the pineal hormone. The present study has examined the effect of melatonin on an enriched gonadotroph cell preparation to determine whether the indole interacts directly with the gonadotroph or requires other pituitary cells to mediate inhibition of gonadotropin release. Monodispersed anterior pituitary cells were prepared from 15-day-old female rats by a modification of the procedure of Hopkins and Farquhar (J. Cell Biol. 59:276, 1973). This procedure, in contrast to certain other methods tested in our laboratory for monodispersion, produced cells maximally responsive to both LHRH and melatonin and comparable to those dissociated with collagenase in our earlier studies. The dispersed cells were fractionated by velocity sedimentation on a 1-3% BSA gradient. LH, FSH, TSH, PRL and GH concentrations in cell lysates of each fraction were measured by double antibody radioimmunoassay. The percentage of LH-, TSH- and PRL-containing cells was determined by immunohistochemistry. In 3 separate gradient fractionations, both procedures revealed a highly reproducible elution profile with a peak of gonadotroph cells enriched 5-10-fold over the unfractionated mixture. The gonadotroph peak was clearly separated from lactotrophs and somatotrophs but did contain some thyrotroph cells. Enriched gonadotrophs were cultured overnight in serum-containing medium, washed free of serum, and then incubated for 5h with increasing concentrations of LHRH either alone or in the presence of melatonin. Controls were incubated in medium only. LHRH-induced LH release by the enriched gonadotrophs was indistinguishable from that observed with unfractionated cells with regard to both dose-response relationships and percent of total LH released. Melatonin (1 μM) significantly suppressed the LH response to 0.1, 1, and 10 nM LHRH by 100%, 62%, and 43%, respectively. This inhibition was equivalent to results obtained with the unfractionated cells. These findings indicate that melatonin acts directly on the gonadotroph to inhibit responsiveness to LHRH. The availability of partially purified target cells of the pineal hormone will facilitate further studies of its mechanism of action. (Supported by NIH Grant HD11854).

189.7 EVIDENCE THAT INHIBIN CAN SUPPRESS FOLLICLE STIMULATING HORMONE (FSH) RELEASE AT THE HYPOTHALAMIC LEVEL. M.D. Lumpkin*, A. Negro-Vilar, P. Franchimont* and S.M. McCann (SPON: P. Shore). Dept. of Physiol., U. Tx. Hlth. Sci. Ctr., Dallas, Texas 75235.

Crude or semipurified inhibin extracts from the gonads of male and female mammals have been shown to suppress preferentially the secretion of FSH both in vitro and in vivo by a pituitary site of action. We report here that inhibin preparations purified from ram rete testis fluid (RTF) preferentially inhibit FSH secretion in the adult male rat by a hypothalamic mechanism. In three experiments, Sprague-Dawley male rats (300-400 g) were implanted with third ventricular (3V) cannulae. After one week of recovery, silastic jugular cannulae were implanted to facilitate blood sampling in unanesthetized animals. Also at this time the animals were bilaterally orchidectomized (ORDX), to be used 24 h later. Inhibin preparations dissolved in saline or the vehicle were injected into the 3V in a volume of 4 μl over a 1 min period. In the first experiment, 30 μg of a highly purified inhibin extract (RTF 38) or saline were given at time 0 and blood collected at 1,2,4,6,8,10 and 24 h post-injection. FSH levels increased steadily throughout the 24 h period in controls. Inhibin-treated ORDX rats showed significantly depressed FSH levels at 4 h ($p<.005$), 6 h ($p<.001$), 8 h ($p<.0025$) and 10 h ($p<.05$). By 24 h, the inhibin-treated group had FSH values near control levels. Plasma LH levels were not significantly different at any point, although both groups experienced a slight decline for up to 8 h. In a second experiment, 3V injection of an inhibin preparation with much lower (25%) potency, given at the same dose as above, failed to suppress FSH or LH levels significantly. This experiment is viewed as a pertinent control which shows that the osmolarity of the injected extract is not a factor in inducing hormonal alterations. In a third experiment, an inhibin preparation (RTF_1A) of lesser potency was given at a 3.3 fold higher dose. FSH levels at 2 ($p<.025$), 4 ($p<.05$) and 6 h ($p<.005$) were significantly lower than in vehicle-injected ORDX rats. Slight but significant decrements were seen in LH levels 2 and 4 h after inhibin treatment. An LHRH challenge (25 ng/100 g BW) was given at 6 h post-injection. Both LH and FSH at 15 and 45 min reached similar peak levels in inhibin and saline groups, thus arguing against a pituitary site of action of the 3V-injected inhibin. Moreover, systemic injection of an inhibin preparation at a dose 2.5 fold higher than the dose given centrally, failed to modify either FSH or LH levels for up to 8 h or responses to LHRH at 15 and 45 min post-challenge. These results provide the first evidence that inhibin in the male can preferentially suppress FSH release by a hypothalamic site of action, in addition to its well known pituitary site of action. Supported by NIH HD-09988 and AM-10073.

189.8 IPSILATERAL HYPOTHALAMIC HEMI-ISLANDS BLOCK INCREASES IN SERUM FSH FOLLOWING HEMI-CASTRATION. D.M. Nance, M. Wilkinson and W.H. Moger*. Dept. of Anatomy and Physiol. & Biophys., Fac. Med., Dalhousie University, Halifax, Nova Scotia, B3H 4H7.

Hemiorchidectomy of prepubertal male rats produces a selective increase in serum FSH with little or no change in serum LH levels (Moger, Biol. Reprod. 17, 661, 1977). This dissociation in LH and FSH secretion suggest additional mechanisms may stimulate FSH release. Since hypothalamic hemi-islands have been utilized to provide evidence that compensatory adrenal growth is mediated neurally (Holzwarth & Dallman, Brain Res. 162, 33, 1979), a similar procedure was used to test for a possible neural mechanism mediating the increase in FSH following hemiorchidectomy.

In two separate studies hypothalamic hemi-islands or sham surgeries were produced in prepubertal male rats with an extrudable Halász type knife (1.5 mm radius). Animals were then sham castrated or else hemi-castrated on the contralateral or ipsilateral side with respect to brain surgery. Following either an 8 or 21 day interval between surgery and collection of serum, hemi-castrated animals given sham knife-cuts showed a significant increase in serum FSH, relative to sham animals with intact testes. Also, animals given hypothalamic hemi-islands and hemi-castrated on the contralateral side showed a marked increase in serum FSH and were comparable to hemi-castrated animals with sham brain surgeries. However, hypothalamic hemi-island rats which were hemi-castrated on the ipsilateral side failed to show an increase in serum FSH and were similar to sham operated controls. Preliminary in vitro determination of pituitary sensitivity to GNRH indicated no difference between the ipsilateral and contralateral hemi-castrated animals with hypothalamic hemi-islands.

Since serum LH levels were found to be comparable among all the groups, these data verify a clear dissociation between LH and FSH secretion. In addition, the observed differences between contralateral and ipsilateral hemi-castrated animals with identical brain surgeries can only be accounted for by postulating that FSH release is, in part, under direct neural control.

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189.9 ELECTROCHEMICAL STIMULATION OF THE PREOPTIC AREA AND LH RELEASE IN THE RAT: THE EFFECTS OF FERROUS AND FERRIC CATIONS.
A.J. Carrillo and K.L. Evans.* University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

It is well known that electrochemical "stimulation" (ECS) of the medial preoptic area (MPOA) in the rat results in the release of LH. It is also known that this neuroendocrine effect is associated with the deposition of iron cations from the electrode tip to the surrounding neural tissue (Everett and Radford, 1961). This study was designed to further investigate the effect of depositing cations into the MPOA on the release of LH. In all cases, female rats on the day of vaginal proestrus were anesthetized with nembutal (37 mg/kg) and brain surgery performed (1400-1600 h). In one group ECS (100 uA anodal DC x 60 sec) of the MPOA was carried out with a stainless steel electrode (N=10). A sham group (N=9) had 200uA anodal DC x 60 sec passed through a platinum electrode. Blood samples for LH determination (RIA-NIAMDD) were obtained from the jugular vein at 0, 1 and 2 h post ECS. ECS of the MPOA resulted in a significant ($p<0.01$) elevation of plasma LH (0 h 39±12 ng/ml; 1 h 821±110 ng/ml; 2 h 368±52ng/ml). Sham stimulation did not have any effect on plasma LH levels (0 h 38±5 ng/ml; 1 h 54±10 ng/ml; 2 h 59±19 ng/ml). Two other rats underwent ECS of the POA and the brains were rapidly removed, frozen and cut in a cryostat and the sections stained for the presence of ferrous (Turnbull's blue) and ferric (Prussian blue) ions. This staining revealed both cations to be present in the ECS site. Other groups of rats had 1 ul of the following solutions infused into the MPOA: $FeCl_2$ (31.1 mM; 47.0mM; and 62.2 mM); $FeCl_3$ (20.7 mM, and 41.4 mM); $MgCl_2$ (62.2 mM) and $CuCl_2$ (62.2 mM). The smallest concentrations of the iron solutions corresponded to the number of equivalents of iron that were calculated to have been deposited from the stainless steel electrode tip during ECS (6.2×10^{-8} equivalents). Blood samples were collected as before for LH determination. Only infusion of $FeCl_2$(41.1 mM and 62.2 mM) and $FeCl_3$(41.1mM) resulted in a significant ($p<0.05$) rise in plasma LH levels when compared to the sham group. However, this stimulatory effect on LH release was significantly ($p<0.01$) less than that detected after ECS. The other solutions had no significant effect on LH release when compared to sham controls. These data suggest that ECS deposits both ferrous and ferric ions and that only iron cations infused into the MPOA can stimulated LH release. However, the effect of ECS on LH secretion cannot be attributed solely to the release of iron ions.
(Supported by USPHS Grant NS 14581)

189.10 COMPRESSED SPECTRAL ARRAYS OF THE AMYGDALA, PREOPTIC AREA, AND ARCUATE NUCLEUS IN CYCLING AND OVARIECTOMIZED RATS.
J. F. Masken and R. J. Morgan. Department of Physiology and Biophysics, Colorado State University, Fort Collins, Colorado 80523.

Adult female Sprague-Dawley rats were implanted with chronic electrodes in the corticomedial amygdala (AMY), medial preoptic area (POA) and arcuate nucleus median eminence area (ARC) in order to study electrical activity in these areas during the estrous cycle and following ovariectomy. Electrical signals from the three areas were recorded simultaneously on analog tape for ten minutes of each half-hour from 8:00h through 16:00h on each day of the estrous cycle. The animals were then bilaterally ovariectomized, allowed to recover, and recorded in similar fashion for four more days. The analog signals were digitized and analyzed for spectral density (1-32 Hz). Very little activity above 12 Hz was observed during estrus and diestrus-1 in any of the three areas (AMY, POA, or ARC). Higher frequencies, e.g. 12, 16, 24, and 28 Hz began appearing during diestrus-2, becoming more evident during proestrus. When the rats were ovariectomized and recorded, the power spectra of the three areas, i.e. AMY, POA, and ARC, showed frequencies similar to those seen during proestrus in the intact animals.
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190.1 VISUAL SPATIAL FREQUENCY AND ATTENTION: EVOKED POTENTIAL EVIDENCE FOR INTERACTION. Richard Coppola, NIMH, Bethesda, MD 20205 and Istvan Czigler*, Institute for Psychology, Budapest, Hungary

Previous evoked potential (EP) studies involving patterned and homogeneous field visual stimulation have shown attention by light intensity interactions. Other studies designed to investigate the presence of specific channels for size (spatial frequency) and orientation within the visual system have also shown effects of selective attention. Because in these studies pattern responses were simultaneous with luminance changes we undertook to investigate spatial frequency and attention while eliminating luminance change as a variable.

Vertical gratings at six spatial frequencies from .25 to 8 cycles/degree with either a sine wave or a square wave luminance profile were generated on a CRT display at a contrast of 44%. The screen went from a blank homogeneous field to the grating within 2 msec. The luminance change from the blank screen to the pattern was less than .005 ft-lamberts and was undetectable by a trained observer. Grass gold disk electrodes were placed on the midline at Oz and Cz with two electrodes spaced equally between them. The visual stimuli were presented concurrently with a series of different intensity tone bursts. The subjects were given instructions intended to direct their attention to either the visual patterns or the tones. Averaged evoked potentials time locked to the appearance of the gratings were recorded from 16 subjects. Half of the subjects had EOG electrodes placed at the outer canthi of each eye in order to control for eye movements. The EPs were measured using mean amplitudes for time bands of 100-200, 200-300, and 300-400 msec.

Analysis of variance indicated a significant attention by spatial frequency interaction for both sine and square wave. The most prominent aspect of the effect was the presence of a late positive component (400 msec) during the pattern attention condition which was missing for the tone attention condition. This component decreases with increasing spatial frequency. It is largest at vertex and diminishes at the more posterior leads. This result might suggest a greater saliance of lower spatial frequencies for visual processing.

190.2 NORMAL AND SCHIZOPHRENIC P300 LATENCY AND AMPLITUDE TO BACKGROUND, NOVEL, AND TARGET VISUAL STIMULI. A. M. I. Wagman, K. Keller*, A. Summerfelt and H. A. Burton*. Neuroscience Program, Maryland Psychiatric Research Center, P.O. Box 3235, Baltimore, MD 21228.

Eleven normals and 11 outpatient schizophrenics were tested on a visual evoked potential procedure that involved the presentation of background, novel, and target stimuli designed to differentially affect the P300 component. Slides were flashed for 8 millisec in 5 blocks of 40 stimuli, with intertrial intervals ranging from one to three sec. Background stimuli (vertical black and white stripes) comprised 40% of the total number of stimuli, while targets (black and white checkerboards) and novels (colored abstract art) each comprised 20% of the total. The order of presentation of the stimulus types was randomized, and subjects were instructed to respond to a touch switch upon seeing a target. Six electrode placements were used (left and right frontal, central, and occipital). P300 component amplitude (from pre-stimulus baseline) and latency were measured for each type of stimulus at each electrode site. Analysis of variance revealed significant ($p<.01$) group differences for both P300 latency (longer for schizophrenics) and amplitude (lower for schizophrenics), stimulus type differences for both latency (longest for novels) and amplitude (smallest for backgrounds, intermediate for novels, and largest for targets), and placement differences for amplitude (largest centrally and smallest occipitally). Analysis of the first few trials of the novel stimulus type is in process as a replication of the Courschene et al. (1975) effect. Because of the high between-subject variance, none of the interaction terms reached significance. However, one trend was apparent: for normals, across the three stimulus types, P300 amplitude was higher on the left at the frontal location and higher on the right at central and occipital placements. For schizophrenics, P300 amplitude was higher on the right at frontal and central placements and higher on the left at occipital locations.

190.3 MIDDLE LATENCY AUDITORY EVOKED POTENTIALS IN PATIENTS WITH TEMPORAL LOBECTOMIES. E. J. Hammond*, J. Bruni*, and B. J. Wilder. Neurology Service, VA Medical Center, Gainesville, Fl 32602.

The diagnostic use of human evoked potentials depends on identification of their generator sites. We recorded middle latency auditory evoked potentials in four patients before and after right temporal lobectomy for intractable epilepsy. Bilateral recordings were made from scalp locations C_3 and C_4 referred to P_Z; simultaneous recordings were obtained from these locations referred to linked earlobes. Stimuli were binaural clicks. In all patients a prominent positive potential at 28-30 ms (corresponding to the component P_a) was recorded pre-operatively. In three patients all tissue 6 cm from the anterior tip of the temporal lobe was excised. In these patients the P_a component was present before and after the operation. Although the exact extent of the primary auditory cortex (Heschl's gyri) was not determined electrophysiologically at the time of the operation, Heschl's gyri are generally located within the area of excision in these patients. In the fourth patient, the lobectomy extended past the primary auditory cortex into auditory association cortex (10 cm from the anterior tip of the temporal lobe). In this patient the P_a component was not present over the operated side.

This experiment suggests that the auditory P_a (P30) potential is not generated in the primary auditory cortex, but in a more posterior area of the temporal lobe.

190.4 ANALYSIS OF HUMAN AUDITORY BRAINSTEM RESPONSES TO CLICK TRAIN STIMULI P. J. Ainslie*, and J. R. Boston. Auditory Systems Laboratory, Carnegie-Mellon University, Pittsburgh, PA 15213

The temporal characteristics of click stimuli used to evoke auditory brainstem responses (ABRs) are important in determining the response. Recent studies have suggested that the use of high stimulus rates and click pair stimuli may have diagnostic significance. The purpose of this investigation was to examine the effects of the temporal characteristics in normal human subjects. ABRs were recorded from the scalp for monaural click train stimuli. Trains of one to four clicks with interclick times of 2, 4, 7, 15.4, 30.8 and 61.6 msec were presented to four subjects at 67 dBSL. Trains were separated by at least 110 msec for interclick times of 2, 4, and 7 msec and by at least 180 msec for interclick times of 15.4, 30.8 and 61.6 msec. Responses to each click in the click train stimulus were estimated using a linear model to cancel overlapping responses to other clicks in the train. Specifically, the response to the nth click of the click train was derived by subtracting the response recorded for n-1 clicks from the response recorded for n clicks, with all responses recorded during the same experimental session. The technique is based on two assumptions: 1) there is small variation among responses within an experimental session for a given subject for a constant stimulus condition, and 2) the response to the second click of a click pair does not affect the response to the first click (no backward masking effects.) Changes in the ABR to successive clicks in the click train were examined by comparing the response to each click, estimated as described above, to the response to one click. Several quantitative methods of comparison were evaluated, including cross-correlation, peak amplitude and peak latency measures. In particular, a wave peak corresponding to Jewett wave V was examined for changes in peak latency and peak to following trough amplitude as a function of click position. Wave V latency in derived responses to each click of the click train following the first click increased with respect to the wave V latency for the ABR to the first click. This latency increase depended on the interclick time and the number of preceding clicks. Latency changes were complete in the response to the last click of the click train for all interclick times except 2 msec. Amplitude changes also depended on interclick time and the number of preceding clicks. The variability in the amplitude data obscured any trends. Some additional data were acquired using noise or tone bursts preceding a click to compare the effects on the ABR generators of these stimuli to those of a click.

190.5 SIGNAL DISPERSION WITHIN A HIPPOCAMPAL NEURAL NETWORK IN THE RABBIT AND RAT. J. M. Horowitz, J. L. Giacchino* and K. S. Kott*. Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616.

Previous studies have described averaged evoked potentials (AEPs) recorded from populations of hippocampal neurons in the cat after stimulation of the fornix. Following single shock stimulation an AEP has the form of a damped oscillatory waveform lasting several hundred milliseconds. In the cat these AEPs have been related to post-stimulus time (PST) histograms of single pyramidal cells and to a model including an ongoing level of excitatory input to the pyramidal cell population (Intern. J. Neurosci. 5:113-123, 1973; Comput. Biol. Med. 5:283-296, 1975). The intent of the present study was to determine if PST histograms for single cells in the rat and rabbit hippocampus could also be related to oscillatory AEPs.

Tungsten microelectrodes were used to record PST histograms from hippocampal cells following commissural or fornix stimulation. Recording techniques were similar to those described previously for the cat. Rabbits and rats were anesthetized with sodium pentobarbital. A single unit was isolated using a WPI window discriminator and PST histograms were constructed using a Tracor-Northern digital signal analyzer. Following fornix stimulation in the rabbit and rat PST histograms showed multiple peaks, with successive peaks having a smaller amplitude and broader width. Commissural stimulation in the rabbit evoked similar PST histograms.

These results rule out the hypothesis that in the rat and rabbit each peak in an AEP is due to excitation of separate subpopulations of excited neurons, as PST histograms show that for a single shock one cell was excited to fire 2 to 4 times. As in the cat, the AEPs and PST histograms can be interpreted in terms of a network of pyramidal cells and inhibitory interneurons within the hippocampus. The first peak of the AEP reflects, at least in part, the excitation of pyramidal cells, and successive peaks reflect rebound excitations of some of the same pyramidal cells. The decreasing height and greater width of later peaks in both AEPs and PST histograms is attributed to dispersion of the signal as it circulates around the loop of pyramidal cells and interneurons. Rebound excitation occurs each time in the cycle there is a period of decreased interneuron activity so that the ongoing background activity excites pyramidal cells. (When background activity is reduced, as in hippocampal slices, previous studies have shown this type of damped oscillatory activity is absent). The network was simulated using differential-difference equations.

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190.6 ENDOGENOUS POTENTIALS GENERATED IN THE HUMAN HIPPOCAMPAL FORMATION AND AMYGDALA BY RARE EVENTS. Eric Halgren, Nancy K. Squires*, Charles L. Wilson, John W. Rohrbaugh*, Thomas L. Babb, Paul H. Crandall*. Brain Res. Inst., Mental Retard. Res. Ctr., Depts. of Psych. and Surg./Neur. (J.W.R. at Dept. of Psychiat., U. Nebraska, Omaha NE).

Long-latency potentials can be recorded from the human scalp that depend upon the cognitive context in which a stimulus occurs but are independent of the sensory characteristics of the stimulus. Typically these potentials ("N2","P3" and "slow-wave") occur when the subject is actively attending to the stimuli and the evoking event is rare. Recordings were obtained from 6 adults with bilateral electrodes chronically implanted in order to locate epileptogenic foci for possible surgical removal. Implantation was undertaken only after failure of more conservative techniques and with informed consent. Large endogenous scalp potentials are evoked by tone pips of two different fixed pitches presented at random intervals. The subject counts silently the 'rare' tones (comprising 20% of the total) randomly interspersed amongst the more frequent tones. We find that this task evokes large potentials in the hippocampus, hippocampal gyrus, and amygdala. The potentials evoked by rare tones are much larger than those evoked by frequent, even though the tones are of identical intensity and duration. Similar potentials are evoked by rare visual stimuli randomly intermixed with the more frequent stimuli of equal luminance, contour and contrast. In the auditory task, the limbic potentials are greatly attenuated if the same stimuli are presented while the subject is reading a book and explicitly instructed to ignore the tones. Our data strongly indicate that these potentials are locally generated: they are large, often exceeding 100 microvolts in amplitude, and sometimes exceeding 150; they can be recorded between two electrodes situated within the same medial temporal lobe, with tip separation as small as 1.0 mm; and, phase-reversals are commonly observed between different limbic sites. Direct evidence that locally-generated synaptic flows are present during these potentials are concurrent changes in limbic unit-activity. These data may permit investigators of endogenous scalp potentials to infer the effects of their manipulation on specific neural events in specific brain regions, which in turn can be related to the large body of knowledge concerning the structure and function of these regions in animals and humans. Current studies are attempting to determine whether some component of the scalp-potentials is in fact a passively volume-conducted reflection of the much larger limbic-generated potentials.

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190.7 HIPPOCAMPAL PYRAMIDAL CELLS DEPOLARIZE ON THE NEGATIVE PHASE OF DENTATE THETA RHYTHM IN URETHANE ANESTHETIZED RATS. S.E. Fox. Dept. of Physiol., Downstate Med. Ctr., SUNY, Brooklyn, NY 11203.

Rats were anesthetized with urethane (1.5 g/kg). Concentric bipolar stimulating electrodes (400 μm o.d.) were stereotaxically placed in the ventral hippocampal commissure. Recording macroelectrodes (150 μm nichrome--referred to skull screw) were placed in the right hippocampal formation under electrophysiological control, to record slow waves and evoked field potentials (0.1 Hz to 10 KHz) from the molecular layer of the dentate and the basal side of CA1. Theta rhythm of about 4 Hz ($>$ 0.5 mV p-p in the dentate) occurs spontaneously in such animals and is phase-reversed between two electrodes so placed. Theta rhythm can also be evoked by mild tactile stimuli. The left hippocampus was exposed by suction and covered with mineral oil. Glass micropipets filled with 3M KCl (30-90 MΩ) were inserted through a wax seal. All other openings were sealed with dental cement. Intracellular recordings ($\geq$40 mV membrane potential) were made during theta rhythm from CA1 and CA3 pyramidal cells (identified by depth and characteristic responses to commissural stimuli). Extracellular theta rhythms and membrane potential (21 to 106 cycles per cell) were normalized for cycle duration, then averaged, and crosscorrelation functions were computed.

Seven of the eight cells recorded thus far (5 in CA1, 3 in CA3) have shown membrane potential oscillations phase-locked to extracellular theta rhythm averaging from 1 to 3 mV p-p. In all these cases the maximum depolarization occurred within 45° of the maximum negativity of the dentate theta rhythm. That is, intracellular theta rhythms in CA1 and CA3 were 170° ± 30° ($\bar{X}$ ± s.d.) out of phase with extracellular theta rhythm from dentate, making them approximately in phase with CA1 theta rhythm. There was suggestive evidence for measurable phase-locked changes in conductance during theta rhythm in two of the three cells tested. The other cell was a CA3 cell which also showed no intracellular theta rhythm ($<$ 0.5 mV p-p).

Fujita and Sato (J. Neurophysiol. 27: 1011, 1964) and Artemenko (Neirofiziologiya 4: 531, 1972) previously reported oscillations of pyramidal cell membrane potentials phase-locked to theta rhythm in curarized rabbits, but they disagreed on the phase relation. These results agree with those of Artemenko, and disagree with Fujita and Sato. The present work is part of an attempt to determine the synaptic mechanisms of theta rhythm generation, and to discover explanations for the complexities introduced by these and several other recent studies which appear to be contradictory.

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190.8 EFFECTS OF HEMICHOLINIUM-3 AND CHOLINE ON HIPPOCAMPAL ELECTRICAL ACTIVITY DURING IMMOBILITY VS. MOVEMENT. Daniel J. Green* and Terry E. Robinson. Psychology Department and Neuroscience Laboratory, 1103 E. Huron, University of Michigan, Ann Arbor, MI 48109.

Earlier studies have suggested that there may be two neurochemically distinct inputs to the hippocampus, each of which is capable of producing hippocampal rhythmical slow activity (RSA or theta; e.g. Vanderwolf, JCPP, 1975, 88, 300). The RSA which may occur during behavioral immobility is abolished by systemically administered atropine (i.e. it is atropine-sensitive), while the RSA which invariably accompanies behaviors such as walking, running, or swimming in rats is not abolished by systemic atropine (i.e. it is atropine-resistant). However, in these previous experiments large doses of atropine sulfate (20-50 mg/kg, i.p.) have been required to abolish atropine-sensitive RSA. This raises the question as to whether the effects of these doses of atropine are due to possible non-specific toxic effects of the drug, or to the specific blockade of cholinergic synapses. The approach we took to answer this question was to manipulate brain cholinergic activity in ways other than through the use of post-synaptic receptor antagonists, and to determine if such manipulations had the effects predicted by previous studies with atropine.

We found that the immobility-related RSA (IRSA) elicited by electrical stimulation of the reticular formation in urethanized rats (which is known to be atropine-sensitive) was severely attenuated by intraventricular injections of hemicholinium-3 (HC-3; 7.5μg in 1.5μl saline into each lateral ventricle). HC-3 depletes brain acetylcholine, presumably by inhibiting the uptake of choline into cholinergic terminals. A subsequent systemic injection of choline chloride restored electrical stimulation-elicited IRSA in the majority of rats. Systemically administered choline has been reported to elevate brain acetylcholine. Intraventricular injections of the vehicle had no effect on hippocampal electrical activity. In contrast, HC-3 had no deleterious effects on the movement-related RSA (MRSA) recorded from freely moving rats during behaviors such as walking or struggling. Therefore, atropine-sensitive IRSA is also HC-3 sensitive, and atropine-resistant MRSA is also HC-3 resistant. These results support the hypothesis that there are at least two pharmacologically-distinct neurochemical systems capable of producing hippocampal RSA. It is suggested that acetylcholine is necessary for the production of IRSA, but is not necessary for the production of MRSA. An as yet unidentified noncholinergic system appears sufficient to produce MRSA. It is interesting to speculate that the effects of choline on hippocampal electrical activity reported here may relate to recent reports that choline administered to humans enhances performance on some learning tasks.

190.9 BEHAVIOR MODULATION OF HIPPOCAMPAL RESPONSE IN CA1 REGION OF THE RAT: MODEL AND EXPERIMENTS. L. S. Leung. Inst. Medical Physics TNO, 45 Da Costakade, Utrecht, The Netherlands.

Average evoked potentials (AEPs) were recorded in the hippocampal CA1 region in rats following electrical stimulation of the stratum radiatum (Schaffer collaterals). In a sequence of behavioral states characterized by increasing peak frequency of the EEG (e.g. awake immobility, head movement only, walking), the AEPs showed (1) decreasing initial peak amplitude, (2) increasing frequency of oscillation with (3) decreasing damping (Neurosci. Abstr., 5:277, 1979).

To account for these results, a previous model (Biol. Cybernetics, 31:219, 1978) has been extended and simulated with an analog computer. The model consists of a population of interneurons (INT) inhibiting a population of pyramidal cells (PYR). PYR are assumed to excite INT after a static, saturation type nonlinearity which passes on the excitation but not the inhibition of PYR. Therefore PYR excitation results in INT excitation and ends in PYR inhibition, simulating the responses in the anesthetized rat. In the awake rat, noise input from the brainstem provides a linearizing effect such that inhibition of PYR is followed by the disexcitation of INT, and then disinhibition of PYR (equivalent to excitation), and so forth. The feedback loop is completed and oscillation occurs. Increasing input from the brainstem, assumed to correspond with the sequence of behavioral states from immobility to increasingly vigorous movements, or with tetanizing the brainstem with increasing stimulus intensity, provides further linearization and results in the gradual change of the three AEP characteristics mentioned above. The model further predicts: (1) A decrease of response oscillation frequency if the intensity for evoking the AEP is increased; this has been observed experimentally. (2) For the intrinsic hippocampal circuit, a frequency response that peaks at 20 - 50 c/s, corresponding to the frequency range of the hippocampal fast EEG. (3) With high (recurrent inhibitory) feedback gain, a low amplitude limit cycle of 50 - 60 c/s. The latter is similar to the high frequency afterdischarges following a tetanus of high frequency of the hippocampus.

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190.10 ABNORMALITIES IN EEG AND SPATIAL PERFORMANCE FOLLOWING INTRA-HIPPOCAMPAL INJECTIONS OF NEUROTOXINS. R. J. Sutherland, I. Q. Whishaw and B. Kolb, Department of Psychology, The University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

Kainic acid, colchicine, or ferric chloride were microinjected into the dorsal hippocampus in rats. We found that kainic acid preferentially destroyed the CA3 and CA4 cell layers, colchicine preferentially destroyed the granule cells in the fascia dentata, and ferric chloride preferentially destroyed the CA1 cell layer.

The effects on EEG of unilateral intra-hippocampal microinjection of these neurotoxins were assessed in freely-moving rats implanted with recording electrodes bilaterally in the dorsal hippocampus and frontal neocortex. Injections of kainic acid or ferric chloride into dorsal or ventral hippocampus generated electrographic and behavioural seizure activity during the first 24 hr. On subsequent days, hippocampal and neocortical EEG activity returned to pre-injection levels. Colchicine did not have an immediate effect on EEG, but beginning 2-3 days after injection there was a marked and long-lasting decline in the amplitude of hippocampal theta activity recorded ipsilaterally to the injection site.

In other rats, pre-treated with kainic acid, colchicine, or ferric chloride unilaterally in the dorsal hippocampus, EEG profiles through the hippocampus and overlying cortex were obtained in an acute recording session. Normal hippocampal theta activity was found in both dorsal and ventral generators after destruction of CA3 and CA4. Theta amplitude was reduced if significant damage was produced in CA1 or the dentate granule cells. Following ferric chloride injection, normal theta activity was found in both hippocampal generators, but to date the destruction of the CA1 cells has not been complete.

In rats injected bilaterally in the dorsal hippocampus with kainic acid, colchicine, or ferric chloride, a disruption of performance was demonstrated in the Morris open-field water maze. The deficits in this spatial task are correlated with the abnormalities in hippocampal EEG profiles.

190.11 ARE RHYTHMICAL SLOW WAVES IN THE EEG RELATED TO MEMORY CONSOLIDATION? A RE-EXAMINATION. K.-P. Ossenkopp and C. H. Vanderwolf. Dept. Psychology, Univ. Western Ontario, London, Canada, N6A 5C2.

We have reinvestigated the claim of Landfield et al. (Science, 1972, 175, 87-89) that the EEG theta rhythm is related to memory consolidation. Rats with bipolar electrodes in the neocortex and hippocampus were given one training trial with footshock (1 ma) in a step through passive avoidance task. Neocortical and hippocampal EEG as well as general activity were monitored for 30 min following the footshock. High levels of hippocampal theta and associated motility in this post-shock period predicted poor passive avoidance performance on retests over the next 3 days ($r = -.79$, $n = 10$, $p < .01$) whereas high levels of neocortical 6 - 9 Hz spindles and associated alert immobility predicted good passive avoidance performance on the retests ($r = +.80$, $n = 10$, $p < .01$). Experiments were also carried out in a two-way shuttle-box. In both learning tasks hippocampal theta activity correlated with immediately concurrent motor activity while 6 - 9 Hz neocortical spindles or low voltage fast activity occurred during alert immobility. Further, frequent 6 - 9 Hz spindles predicted good performance in the passive avoidance situation where the correct response is immobility, but did not predict good performance in the shuttle-box where the correct response is running. We conclude that hippocampal theta activity and 6 - 9 Hz neocortical spindles are related to motor activity and not to memory consolidation.

190.12 PREDICTION OF P300 AMPLITUDE AND LATENCY FROM A COGNITIVE TEST BATTERY. A. T. Summerfelt, K. Keller*, A. M. I. Wagman and F. Funderburk*, Neuroscience Program, Maryland Psychiatric Research Center, P.O. Box 3235, Baltimore, MD 21228.

Nine normal and 13 schizophrenic subjects were tested on a battery of cognitive tasks and on an evoked potential procedure designed to differentially affect the P300 component. An attempt was made to predict P300 amplitude and latency from the cognitive variables. The cognitive tests included the Purdue Pegboard, Wisconsin Card Sort, Halstead Categories, Full Range Picture Vocabulary, Tapping Test, Tactual Performance Test, and Memory for Designs. A one way ANOVA between groups revealed differences on several Wisconsin variables (sets, cards, % error, efficiency), (normals better than schizophrenic), and set 5 of the Halstead (HCT5) (schizophrenic better than normal). There was no difference in age or IQ between groups, while the education of schizophrenics was slightly higher than for normals (15 yrs. vs. 12.9). Results of the evoked potential procedure, which involved the random presentation of background, novel, and target stimuli, are discussed elsewhere (Wagman, Keller, Summerfelt, and Burton, 1980).

The correlation matrix of all variables suggested a selection of cognitive variables relatively uncorrelated with each other but which significantly loaded on P300 amplitude and latency. These included from the Wisconsin, the number of sets completed (Wsets), the number of cards to criterion, set interruptions, and percent error; HCT5; from the Tactual Performance Test, scores from the non-dominant hand (Tnon), from both hands (Tboth) and the memory test; and the Memory for Designs (MFD). An all possible subset regression with Mallow Cp criterion, using these variables to predict P300 amplitude and latency for six electrode locations (left and right frontal, central, and occipital) revealed (with a strict criterion of $F>4.35$ df 1/20 for significance) that only MFD was a predictor of P300 amplitude, and only for target stimuli. MFD, Tnon, and Tboth were predictors of P300 latency. Separate stepwise regressions averaging across electrode placements for either amplitude or latency with coding for group and group X variable interaction, showed that MFD accounted for 33% of the target amplitude variance, while no other cognitive variable was a significant predictor of amplitude. Several group X variable interactions were significant with respect to P300 latency. These included Wsets, MFD, Tboth, and Tnon. In each case, the interaction was carried by the normal's linear trend, while the schizophrenics showed no relationship. In all comparisons the linear trend indicated that poor cognitive performance was associated with either decreased P300 amplitude or increased P300 latency.

190.13 ELECTROCORTICAL FREQUENCY DIFFERENCES IN HYPERACTIVE, LEARNING DISABLED, MIXED TYPE AND NORMAL BOYS. Phillip Holcomb*, Roscoe A. Dykman*, Peggy T. Ackerman* and D. Michael Oglesby* (spon. E. Powell), Behavioral Sciences Laboratory, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205.

In a previous paper (Dykman et al., J. Ner. and Men. Dis.,167: 288, 1979) we have contrasted differences in the behavioral performance of four groups of boys (hyperactive, learning disabled, learning disabled and hyperactive, and normals) engaged in a visual search conditioning task. We now report Principle Component analyzed EEG results obtained in the same paradigm.

Forty boys (WISC IQs>90) between 7 and 10 years of age severed as subjects. Group membership criteria were: Conners' teacher rated hyperkinesis scores of ≥ 15 for hyperactive and mixed type boys (<15 for all others); and Wide Range Achievement Test and Gray Oral Reading standard scores of ≤ 90 for learning disabled and mixed type boys (>90 for all others). On each trial subjects were awarded one penny for pressing the correct (one of twelve) transparent display windows behind which appeared one of 12 ASCII symbols (e.g., +, -,*,#). Over the experimental session visual fields progressed (as the child solved each successive search problem) from two to twelve simultaneously displayed symbols. EEG data (C_1,C_2,P_3,P_4, linked ear lobes; down 3 db at 1 and 100 hertz), which were recorded for one second pre and one second post stimulus onset, were Fourier transformed and averaged (Varian 100/F computer) over trials. Twenty frequency values (1 to 20 hertz) were submitted to a principle components analysis and subsequent component scores were analyzed with repeated measures analyses of variance.

Four components accounted for 87% of the variance. Component 1 which revealed primary activity between 16 and 20 hertz (beta) and secondary activity between 8 and 10 hertz (alpha) produced significant ($p<.05$) effects for group (control>mixed>hyper> learning disabled), pre vs. post stimulus onset (pre>post) and a pre-post onset by electrode site interaction. While Components 2,3 and 4 were less revealing , Component 3 (primary activity between 7 and 10 hertz) did produce significant pre-post onset and electrode site by pre-post onset effects similar to one.

These results agree with those of Grunewald-Zuberbier et al. (EEG and Clin. Neurophy., 38:149, 1975), who reported decreased alpha blocking and lower beta levels for hyperactive than non-hyperactive boys. The current study further suggests that: one, it is a combination of both alpha and beta frequencies that are responsive to group differences; and two, that learning disabled children are similarly (to hyperactives) depressed on this EEG component.

190.14 EEG FACTORS DERIVED THROUGH SPECTRAL ANALYSIS. Duilio Giannitrapani. Veterans Administration Medical Center, Perry Point, MD 21902.

A systematic evaluation of the degree of independence of EEG components has been attempted by subjecting EEG spectral analysis data to factor analysis. For this purpose the EEG of 56 11 to 13 year-old normal subjects was obtained from 16 scalp positions during 8 different behavioral states, each of 8 sec duration. Fast Fourier analysis was performed to obtain power density estimates for each of 17 bands (0 to 34 Hz), each 2 Hz wide, and averaged for the 8 conditions. A total of 272 variables (16 scalp positions X 17 frequency bands) was used in the factor analysis.

After preliminary analysis, principal component analysis with Varimax rotation of 10 factors was employed. The following rotated factors were obtained: Factor 1, loaded primarily on 29 Hz bilaterally; Factor 2, loaded primarily on 5 and 7 Hz and less on 9 Hz bilaterally: Factor 3, loaded primarily on 13 Hz and less on 15 Hz bilaterally; Factor 4, loaded exclusively on 15 and 17 Hz bilaterally; Factor 5,loaded primarily on 23 to 27 Hz in Fronto-Temporal areas on the left side; Factor 6, loaded exclusively on 11 Hz activity bilaterally; and four other factors accounting for minor portions of the variance.

The structure of the factors indicates a relative frequency-specific rather than brain-area specific factor structure. Factor 1, accounting for the greatest variance, relates to 29 Hz activity earlier found in greater amplitude in younger people, in females and in a portion of a schizophrenic sample. Some of the factors relate to traditional clinical EEG parameters (Factor 6, dominant alpha; Factor 2, theta activity). Other factors could be interpreted as representing newly discovered relationships with higher cortical functions (Factor 3, verbal functions; Factor 4, numerical functions) since in a previous study with the same data 13 Hz power was demonstrated to relate to verbal intelligence and 15 to 17 Hz to numerical processing. Factor 5 has an as yet unidentified role. The desirability of studying narrower band widths to resolve the composition of factors within the 2 Hz band width of this study is discussed.

190.15 AN EEG METRIC OF POST-SYNAPTIC DOPAMINE RECEPTOR ACTIVATION IN MICE. R. L. Lloyd* and P. K. Randall* (SPON:D.F.Lindsley).

On-going electrical activity was recorded from cortex and striatum of C57BL/6J mice following injections of the dopamine (DA) agonist apomorphine, and antagonist, haloperidol. Behavioral akinesia following 2mg/kg haloperidol was associated with large increases in striatal amplitude recorded from bipolar electrodes (1mm tip seperation) in anterior caudate. Power spectral analysis revealed a mean increase in power (1-15Hz) of the striatal signal of 254% 1 hr. following injection. Apomorphine, however, tended to reduce power 45-70% within 30min (3mg/kg); a time course similar to that of behavioral effects of apomorphine (e.g. stereotypy). However, 90 min following 3.0mg/kg apomorphine (IP), these animals, which manifested stereotypy earlier, underwent a rebound effect which was behaviorally and electrophysiologically similar to the effects of haloperidol: there was a decrease in motility, and catalepsy could be evoked. Ninety min following injection, striatal electrical power increased 272% over pre-injection levels. This effect could be produced with very small (IP) doses of apomorphine (0.01-0.05mg/kg), including high amplitude striatal signals, decreased motility, and catalepsy. Within 20 min striatal electrical power increased 43%.

These drugs also affected the simultaneous cortical EEG, with a slowing in frequency following 2.0 mg/kg of haloperidol. One hour after injection, the power spectrum changes from a difuse profile in the range 2-9Hz to a tightly constricted profile with a predominant frequency between 1.6-3.2Hz. The power in the band 0-3.25 Hz increased at this time by an average of 151%. During catalepsy, however, the predominant frequency shifted to a slightly higher range (3.25-6.0Hz) which increased in power by 202%. Following catalepsy, the EEG returned to the lower range predominating before catalepsy. Similarly, 90 min following 3.0 mg/kg apomorphine, the power in the 0-3.25Hz range increased by 216%.

These results are consistent with the selective effect of low doses of DA agonists upon the more sensitive presynaptic autoreceptors which would result in reduced DA release. The resulting DA deficit in the neostriatum would produce behavioral and electrophysiological effects similar to postsynaptic receptor blockade with haloperidol and suggests that recording of striatal gross-potentials may provide a convenient metric for post-synaptic DA receptor activation.

190.16 DEVELOPMENTAL CHANGES IN EEG PATTERNS OF NARCOLEPTIC DOBERMAN PINSCHERS. Arthur Foutz, Theodore L. Baker and William D. Dement, Stanford Sleep Disorders Center, Stanford, CA. 94305.

Narcolepsy is a disease occurring in humans and in several equine and canine breeds. It is usually characterized as a disorder of rapid-eye-movement sleep (REM) because many of the pathological manifestations suggest abnormal intrusion of REM into the waking state. For example, previous polygraphic studies of adult narcoleptic dogs in our laboratory have indicated: (1) sudden attacks of cataplexy (a REM-like inhibition of skeletal muscles) during periods of intense emotionality (2) REM-like desynchronized EEG during cataplexy, sometimes coupled with rapid eye movements and muscle twitches (3) sleep-onset REM periods. Experimental breeding of narcoleptic Doberman pinshers from our canine narcolepsy colony has yielded 35 puppies (6 litters) all of which have exhibited symptoms of narcolepsy within 4 wks. to 6 mos. of birth. We studied one male and one female (littermates) at intervals from 13 wks. to 16 mos. and a young adult male from a previous breeding of the same parents. EEG, EOG and EMG were recorded with implanted electrodes, and EEG signals were digitized and submitted to spectral analysis with a PDP-11 computer. Dogs were recorded during normal sleep-waking cycles and during cataplectic attacks elicited by play or food. The unanticipated finding of this study was that narcoleptic puppies showed not only the typical REM-like EEG patterns during cataplexy, but also periods of high amplitude slow-wave EEG activity. During some attacks, the EEG tracing consisted almost entirely of slow-wave EEG activity, which was indistinguishable from EEG recorded during normal non-REM sleep (NREM) periods. In some cases, a catapletic attack would initiate sustained, complete NREM sleep which did not spontaneously terminate. At other times, catapletic attacks were brief and consisted of alternating periods of both types of EEG patterns. In contrast, the young adult Doberman and the puppies re-recorded at 13 months showed only the REM-like EEG pattern during cataplexy. Catapletic attacks recorded in the more mature dog were much briefer and ended spontaneously. These findings indicate that narcolepsy may be manifested as a more generalized disorder involving both REM and non-REM sleep in the immature dog. Current studies are focused upon understanding developmental processes whereby narcolepsy becomes primarily a REM disorder in the adult dog.

191.1 SYNAPTOGENESIS IN THE VISUAL CORTEX OF NORMAL AND 6-OHDA-TREATED RATS. M. E. Blue*, J. G. Parnavelas and A. R. Lieberman* (SPON: H. Feit). Dept Cell Biology, Univ. Texas Health Sci. Ctr., Dallas, TX 75235 and Dept. of Anatomy, Univ. Coll. London, London, U.K.

The formation of synapses and their morphological maturation were studied by electron microscopy in the visual cortex of albino rats of several postnatal ages. The analysis was conducted on photographic montages of strips of cortex 50µm wide and extending from the pia to the white matter. The morphological features of all synapses were characterized and their positions were plotted on a scale map of the cortical strip on which the extent of the layers was marked.

There were few synapses (on the average 30 per montage) present in the visual cortex of animals 12 hr. of age, with the majority being located in the deep parts of the cortex. Synapses contained a few vesicles and appeared immature in their morphology; Gray's type I contacts predominated. There was a 6-fold increase in the number of synapses between 12 hr. and day 6 of postnatal life. At day 6, the distribution of synaptic contacts remained uneven, with the highest densities present in areas corresponding to layers I, IV and V/VI. Synaptic morphology was more mature and Gray's type I contacts remained the prevalent variety. Synaptogenesis continued at a high rate during the second postnatal week, and at day 14 there were approximately 3 times as many synapses as there were at day 6. The total number of synaptic contacts increased markedly during the third week, and by day 21 the number and morphological features of synapses resembled those of adult rats.

In addition, the influence of norepinephrine on cortical synaptogenesis was examined in rats that received intraperitoneal injections of the neurotoxin 6-hydroxydopamine (6-OHDA) in early postnatal life. Animals received an injection of 50µg/g body weight on each of the first two days and an injection of 100µg/g body weight on the third postnatal day; vehicle injected littermates were used as controls. Photomontages of 50µm strips of visual cortex were prepared from the 6-OHDA-treated animals and from their control littermates. Preliminary results from two ages (days 4 and 6) during the first week of postnatal life showed that there was a significantly higher number of synapses present in the visual cortex of animals whose cortical noradrenergic afferents were previously lesioned compared to their control littermates. However, at day 14, the number of synapses present in the cortex of lesioned and control animals were approximately equal. Further experiments are in progress to accurately describe the process of synaptogenesis in the visual cortex devoid of noradrenergic afferents.

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191.2 SYNAPTOGENESIS IN CHICK SYMPATHETIC GANGLIA. Kathleen A. Hruschak*, Victor L. Friedrich, Jr. and Ezio Giacobini (SPON: S. Maxson). Dept. of Biobehavioral Sciences, The University of Connecticut, Storrs 06268.

Chicks at the four ages: 10 days in ovo, 1 and 30 days after hatching and adult (360+ days after hatching) were fixed by perfusion and the lumbar paravertebral ganglia, L1-L4, were prepared for electron microscopy.

Total ganglionic volume increases progressively from 0.01 mm^3 per ganglion at 10 days in ovo to 0.09 mm^3 at 1 day after hatching to 0.13 mm^3 at 30 days after hatching to 0.80 mm^3 in the adult. The neuropil/soma compartment increases 100 fold over this period from .0046 mm^3 at 10 days in ovo to 0.46 mm^3 in the adult. The rate of increase is the greatest in the interval before hatching and is substantially less, subsequently.

The number of synapses per unit area (synaptic density) of ganglionic tissue (in thin sections) was determined for each of the four ages. At 10 days in ovo, synapses are sparse (240 synapses/mm^2); their density is only ten per cent of the adult value. The synaptic density increases over 17 fold from 10 days in ovo to 1 day after hatching (4200 synapses/mm^2) demonstrating that synapses are formed rapidly during the last week of incubation (days 10 to 21 in ovo). The synaptic density decreases approximately 50% from 1 day after hatching to adulthood with the greatest decrease, 35% (2800 synapses/mm^2) occurring between 1 and 30 days after hatching. In the adult, the synaptic density is very low, 2200 synapses/mm^2. This decline in synaptic density probably reflects a dilution of the synapses into a progressively larger total ganglionic volume. In addition, the rate of synaptogenesis may decrease substantially shortly after hatching. This issue will be resolved when the determination of total numbers of synapses based on synaptic density, currently in progress, is completed.

Our results indicate that synaptogenesis appears to be most intense between 10 and 21 days in ovo, and may decrease substantially after hatching.

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191.3 CORRELATION OF ACETYLCHOLINE RECEPTORS WITH ENDPLATE ACETYLCHOLINESTERASE AND NERVE TERMINAL PROCESSES AT DEVELOPING FROG NEUROMUSCULAR JUNCTIONS. K. Morrison-Graham* (SPON: M. S. Letinsky). UCLA School of Med., Dept. of Physiol., Ahmanson Lab. of Neurobiology, and the Jerry Lewis Neuro-Muscular Research Center, Los Angeles, CA. 90024.

The distribution of acetylcholine receptors (AChR) relative to the nerve terminal arborization and endplate acetylcholinesterase (AChE) was examined in the cutaneous pectoris muscle of Rana catesbiana bullfrogs from late-staged tadpoles to 7 month old post-metamorphic frogs. The muscle was removed and the AChR stained with tetramethylrhodamine-labelled α-bungarotoxin. Photographs of junctional receptors were taken, and the nerve terminal and postsynaptic AChE stained with the NBT/AChE method (Letinsky & DeCino, J. of Neurocytology, 1980).

In general, within any given terminal arborization, the distribution of AChE and AChR correspond closely with the NBT-stained nerve terminal processes. However, several different staining patterns were also observed, including: 1) most frequently, an absence of receptor staining underneath a growing tip (an axonal process extending approx. 5 µm past the confines of the endplate AChE and resembling the growth cones seen in tissue culture); 2) faint receptor staining under the initial part of a long growing tip (10-20 µm) without detectable AChE; 3) postsynaptic gutters revealed by AChE staining which were devoid of the normal overlying nerve processes and without demonstrable AChR; and 4) differences in the AChR staining intensity within an individual endplate. In addition, along NBT-stained processes which were varicose in appearance, the AChR and AChE staining patterns were similar with both being present under the varicosities but absent under the fine interconnectives. These differences in AChR, AChE, and nerve terminal staining patterns suggest ongoing growth and remodeling at established neuromuscular junctions. These were present at all ages studied, however, they were more prominent in the first weeks following metamorphosis when there is a rapid elimination of multiple innervation.

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191.4 SYNAPTIC DENSITY IN THE TECTUM: EFFECTS OF NUMBER AND SIZE OF OPTIC AFFERENTS. Adrienne E. Lynch,* John J. Brunette,* and Stephen A. George. Biol. Dept. and Neuroscience Prog., Amherst College, Amherst, MA 01002.

The tissues of triploid amphibians contain larger but fewer cells than normal diploids, so that structures such as eye and brain are of comparable size in diploids and triploids. Thus, animals of different ploidy provide a suitable system for studying how the number and size of afferent fibers influence the density of synapses formed by these afferents.

We counted the number of myelinated optic nerve fibers in 40 nerves from diploid (2N) and triploid (3N) Xenopus at 5 stages from mid-larval (stage 53) to post-metamorphic (stage 66). We measured the cross sectional areas of a sample of 3368 fibers from 16 of these nerves at stages 56 and 66. Differences in the number of fibers in 2N's and 3N's were maximal at the earliest stages. For example, at stage 53 the 2N nerves averaged 369±31 myelinated axons (mean ± S.E.), compared to 227±39 for 3N's. In contrast, differences in fiber area were greatest at later stages. For example, at stage 66, the average area of 3N fibers was 18% greater than the 2N average.

To assess the role of axon size and number in synaptogenesis, we exchanged left eye rudiments between 2N and 3N embryos at stage 28, and prepared controls that received transplants of the same ploidy. Synapses in both tecta of 5 postmetamorphic animals from this series were counted in mosaics of electron micrographs covering the full depth of the tectal neuropil. Three measures of synaptic density were calculated: the average number of synapses per μm^3 (N_V); the total number of synapses under 1 μm^2 of tectal surface (N_a); and the number of synapses per tectal cell nucleus (N_n). All three measures of density were strongly influenced by the ploidy of the optic nerve input. For example, N_V for 2N innervation was $1.27 \pm .09$ synapses/μm^3 while N_V for 3N optic nerves was $0.89 \pm .06$ ($p<.02$). However, there were no substantial differences in any of these measures of density when the ploidy of the tectum was used as the dependent variable (e.g. $N_V = 1.09 \pm .18$ synapses/μm^3 in 2N tecta, compared to $1.01 \pm .09$ in 3N tecta, $p>.4$).

Though it is not surprising that a reduction in the number of inputs during development (e.g. 3N input to 2N tectum) would lead to a reduction in synaptic density, it is noteworthy that synaptic density can be increased above normal levels (e.g. for 2N input to 3N tectum) by differences in the size and number of inputs that do not involve gross changes in the amount of tissue in the input pathway.

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191.5 FREEZE FRACTURE OF DEVELOPING NEUROMUSCULAR JUNCTIONS IN THE TADPOLE. K. Lynch*, C.-P. Ko, D.W. Pumplin and C.D. Harris*. Uniformed Services Univ. and Sec. on Funct. Neuroanat., LNNS, NINCDS, NIH, Bethesda, MD, and Univ. of Md. Med Schl., Baltimore, MD.

The rectus abdominis muscle of the tadpole is a mixed muscle which persists through metamorphosis. Due to the ease with which its neuromuscular junctions (NMJs) can be freeze fractured, we feel that it holds great promise for the ultrastructural study of neuromuscular development.

In R. pipiens, the muscle appears at about stage 24 (about 10 days) of embryonic development. Over several days, nerves enter the ventral body wall and muscle fibers develop, aligned in 5 or 6 tiers. The nerve bundles run along the boundary lines between adjacent tiers, dorsal to the myotendinous junctions. NMJs were located by staining for acetylcholine receptors (AChRs) with rhodamine-labeled α-bungarotoxin and for cholinesterase activity. The NMJs first appear near the myotendinous junctions and along the course of a few nerves that branch from the main trunks.

During the 2 to 3 months after embryonic development, NMJs become less frequent at the myotendinous junctions and larger and more numerous in the central part of each muscle tier. Freeze fracture of tadpole muscles during this period shows innervation sites which usually contain several nerve endings. Large P-face particles (interpreted as AChRs) in the postsynaptic membrane are concentrated in raised plaques separated by particle-free zones. Junctional folds, where present at all, are very shallow. No Schwann cell processes are evident between nerve and muscle. The presynaptic P-face contains particles that we interpreted to be active zone particles. In some NMJs, these are scattered or in short, randomly oriented double rows. In others, longer double rows are aligned on regularly spaced transverse ridges. The presence of ridges does not correlate with differences in terminal shape or receptor distribution. Where two or more nerve endings run parallel at a single innervation site with such ridges, their ridges are in register and appear to lie over shallow junctional folds. Cross-fractures and thin sections show that the nerve endings contain abundant synaptic vesicles and all other typical NMJ organelles. Freeze-fractured NMJs resembling those on frog twitch fibers have been seen in mature frog rectus abdominus; no slow junctions have yet been identified.

Our findings show some of the structural changes that lead to mature innervation of an amphibian muscle. The main changes observed were a shift in the position of the NMJs within the muscle, a shift from multiple to single innervation, enlargement of the junctions, and the appearance of active zones and junctional folds.

191.6 DENERVATION-INDUCED CHANGES IN ACETYLCHOLINE RECEPTORS AT GOLDFISH RETINOTECTAL SYNAPSES COMPUTED FROM INTRACELLULAR RECORDINGS IN TISSUE SLICES. John A. Freeman, Dept. of Anatomy, Vanderbilt University, Nashville, TN 37232, U.S.A.

In order to quantitate changes in the localization and density of nicotinic acetylcholine receptors (nAChR's) occurring during optic nerve denervation and reinnervation, intracellular recordings were made from neurons in tectal slices obtained at various times after optic nerve crush. The dendritic surfaces of individual tectal neurons, identified under Nomarski optics, were mapped iontophoretically for their sensitivity to ACh (Freeman, J. A., Soc. for Neuroscience, 5: 2495, 1979). In normal slices, ACh sensitivity was localized to 3 discrete zones corresponding to the regions of optic nerve synapses. The density of nAChR was estimated to be 1,500-3,500 molecules/μm^2 synaptic membrane from

$$p = 4\ A\ R_t/(\pi^2\ b\ r\ N_a\ V_t)$$

where A is Avogadro's number, R_t =moles ^{125}I-αbungarotoxin bound/slice volume V_t, N_a=areal density of synapses ($9X10^6/cm^2$, Norden and Freeman, Soc. for Neuroscience, this meeting), r=radius of synaptic disk (0.2μm) and b=fraction of nicotinic synapses (0.5, Oswald, Schmidt, Norden and Freeman, Brain Res., 187: 13, 1980). The Stoke's radius of the solubilized nAChR has previously been determined to be 80 Å (Oswald and Freeman, J. Biol. Chem. 254: 3419, 1979). Taken together, these density and size measurements suggest that acetylcholine receptors are packed very tightly at retinotectal synapses, probably constituting the major protein component of the subsynaptic membrane, and that the nAChR might be important for the structural integrity of the synapse, as previously postulated (Freeman, Nature, 269: 118, 1977). Following denervation 50% of nAChR's are rapidly lost. During reinnervation localized high density receptor aggregates reappear at synaptogenic sites. The detailed temporal relationship between receptor aggregation, seen with DTAF-alpha bungarotoxin, and synaptogenesis is presently being studied. Preliminary results suggest that regenerating fibers induce receptor clustering.

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191.7 DEVELOPMENT OF ORGANOTYPIC CULTURES OF NEONATAL RAT HIPPOCAMPUS R. L. Beach, S. L. Bathgate*, A. Y. Kwan* & C. W. Cotman. Dept. Psychobiology, Univ. of Calif., Irvine, CA. 92717.

To facilitate progress on cellular mechanisms of the development of plasticity of hippocampal neurons we have developed and characterized an organotypic culture system of hippocampus. Transverse slices of neonatal hippocampii were maintained in culture for up to 3 months. Explants were grown on polylysine coated coverslips for subsequent staining. Cell types in these cultures were positively identified with a number of specific markers. Neurons and neurites were labelled via immunofluorescence and immunoperoxidase techniques utilizing tetanus toxin and antitoxin. Astrocytes were identified by their reaction with antibodies to glial fibrillary acidic protein. Antigalactocerebroside was used to demonstrate oligodendrocytes. Fibroblastic and endothelial cells were characterized by their distinctive staining patterns with antifibronectin and antimyosin. In cultures grown in 20% serum, neurites elongated and glia migrated directly onto the substrate rapidly. After several days the neurites were primarily on top of the glia. By six days a number of granule cells had migrated onto the glial mat amid a dense meshwork of neurites. These cells could be identified by their position relative to the pyramidal cell layer in the explant and their size and shape under phase contrast and after silver impregnation. The great majority of the cells were astrocytes although patches of fibroblasts or endothelial cells, and occasional oligodendrocytes were seen. In older cultures pyramidal neurons could be recognized in the outgrowth after silver impregnation.

Synapses were seen in the outgrowth by the fifth day in culture, and rapidly increased in number. The earliest synapses were symmetric with few vescicles, but within a few days many of the synapses had pronounced postsynaptic densities and accumulations of vesicles. Cultures were also grown in serum free media (Bottenstein & Sato, PNAS 76: 514, 1979). In serum free cultures non-neuronal cell proliferation and migration into the outgrowth was markedly suppressed. The extent and rate of neurite outgrowth during the first week appeared similar to that achieved in the presence of serum, but a denser neuritic network grew directly on the substrate without serum. In these cultures non-neuronal outgrowth was never very extensive and did not overtake the neurite outgrowth. These cultures will be of use in studying the factors regulating differentiation synaptogenesis and plasticity in hippocampal neurons. (Supported by NIMH grant no. MH 19691 and NIH grant no. NS 08597.)

192.1 SYNAPTIC PLASTICITIES AT AN IDENTIFIED SYNAPSE IN APLYSIA ABDOMINAL GANGLION CAN BE CHARACTERIZED USING THE SPONTANEOUS FIRING OF THE PRESYNAPTIC NEURON. P.B. Guthrie & S.H. Barondes. Dept. of Neurosciences and Psychiatry, UCSD, La Jolla, CA 92093, and Dept. of Psychiatry, VAMC, San Diego, CA 92161.

Neuron RC1, monitored by its postsynaptic potentials in R15 (synapse RC1-R15), has been shown to be spontaneously active in an isolated Aplysia central nervous system preparation (Woodson & Schlapfer, Br.Res. 173:225-242, 1979). Since the RC1-R15 EPSP has been shown to exhibit pronounced plasticities (history-dependent amplitude variations) upon stimulation of the right connective, we wished to determine whether the frequency of the spontaneous bursting activity of RC1 elicited significant amplitude variations of this type.

We initially tested the manner in which these plasticities might be expressed at RC1-R15 in the isolated abdominal ganglion by choosing a stimulation pattern resembling the spontaneous firing pattern of RC1. Trains of stimuli (20-100 stimuli) at a given frequency (1/sec-5/sec) were separated by intervals of variable duration. Data collection was begun after 10 minutes of stimulation. Plots of the amplitude of the first RC1-R15 EPSP following the interval versus the interval duration show three phases corresponding to 1) short term facilitation, 2) early (rising phase) post-tetanic potentiation (PTP) and 3) late (falling phase) PTP. The magnitude of the amplitude variations representing the different plasticities varied as a function of the train frequency.

The amplitude of the spontaneous RC1-R15 EPSP in the isolated central nervous system preparation, plotted as a function of the preceeding interval, showed an identical complex curve. This suggests that the instantaneous frequency of RC1 is a major factor in determining the EPSP amplitude. To test this, we recorded the temporal pattern of RC1 and used this pattern to stimulate RC1-R15 in the same preparation. A one-to-one comparison between the spontaneous RC1-R15 amplitudes initially observed with the pattern and the stimulated EPSP amplitudes induced by artificial stimulation of the right connective with this same pattern showed a high correlation over the entire frequency range of RC1. This result also provides very strong evidence for the presynaptic nature of these synaptic plasticities.

This method of analysis of the spontaneous RC1-EPSPs has also been used to verify quantitative differences in PTP amplitudes observed at several different RC1-synapses upon right connective stimulation.

Supported by the Veterans Administration Medical Research Program.

192.2 POSTSYNAPTIC MODULATION OF SYNAPTIC EFFICIENCY BY A FAST, TRANSIENT OUTWARD CURRENT. Lawrence Salkoff* (SPON: W.S. CAIN). Dept. of Biology, Yale Univ., Box 6666, New Haven, Ct. 06511.

Changes in synaptic efficiency are usually associated with presynaptic changes involving the release of neurotransmitter substances. However, an exception is reported here that shows plasticity in synaptic efficiency due to a postsynaptic change in membrane excitability. In the dorsal longitudinal flight muscles of Drosophila depolarizing electrical activity lowers the voltage and current thresholds for the spiking response (Salkoff & Wyman, submitted). Thus, EPSPs which are below spike threshold prior to muscle membrane electrical activity may be above threshold following membrane electrical activity. This is illustrated in the accompanying figure where nerve stimulation applied at 2 Hz evoked 8 EPSPs of equal amplitude, shown superimposed in the figure. The first seven EPSPs produced identical subthreshold responses. 200 ms prior to the 8th sweep a priming pulse was delivered to the muscle through an intracellular electrode (the priming pulse, not shown, was 50 ms in duration and of sufficient current to elicit several spikes). The final EPSP, although no larger than the previous 7, then elicited the spike shown in the figure. The mechanism responsible for the membrane excitability increase involves the depression of an inhibitory, fast, transient outward current, the Drosophila A-current, which is similar to the molluscan A-current first analyzed by Conner & Stevens (1971, J. Physiol. 213: 21-53). Voltage clamp analysis has shown that the Drosophila A-current is suppressed by a prior depolarizing voltage step more positive than -55 mv. Suppression of the current persists for up to 1.5s following a depolarizing voltage step.

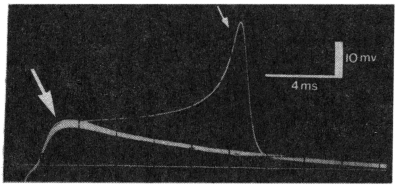

Figure Legend

large arrow: 8 consecutive EPSPs

small arrow: spike elicited by 8th EPSP following a priming pulse.

The effect of prior membrane activity on synaptic efficiency was also demonstrated entirely by nerve evoked responses. A single EPSP was below spike threshold, but a pair of EPSPs would summate to cause a muscle spike. This single spike sufficiently inactivated the A-current so that the next EPSP did elicit a spike. SUPPORTED BY USPHS NS07314 to R.J. WYMAN.

192.3 REPETITIVE NERVE FIRING IN DROSOPHILA MUTANTS: THE ROLE OF SPATIAL AND GENETIC INTERACTIONS. B. Ganetzky* and C. F. Wu. Dept. of Genetics, Univ. of Wisconsin, Madison, Wis. 53706 and Dept. of Zoology, Univ. of Iowa, Iowa City, Iowa 52242.

Shaker (Sh) mutants exhibit leg-shaking behavior under ether anesthesia and prolonged ejps at the larval neuromuscular junction (Jan et al., Proc. Roy. Soc. Lond. B 198:87, 1977). Abnormal repetitive firing of motor axons is also observed in Sh larvae. The behavioral and physiological phenotypes of Sh are suppressed by nap^{ts} (no action potential, temperature-sensitive), a mutation that decreases nerve excitability. A subcritical dose (15-25nM) of tetrodotoxin (TTX) coordinately reduces repetitive firing and ejp duration of Sh mutants in a manner similar to that of nap^{ts}. However, other evidence implicates the axon terminal in the generation of multiple nerve spikes: (1) A nerve severed near the terminal shows no repetitive firing. (2) After blocking the axonal Na spike with a high dose of TTX (>50nM) prolonged transmitter release can still be evoked by a strong electrotonic stimulus. (3) Repetitive firing is reversibly blocked when the presynaptic Ca current is blocked in 0[Ca^{2+}] saline or by 5mM Co^{2+}. (4) Simultaneous recording at two points along the nerve indicate that the extra spikes travel antidromically from the terminal. A positive feedback model involving the axon and the terminal is proposed. We suggest that the Sh terminal is abnormally excitable and capable of developing a presynaptic Ca action potential. The presynaptic Ca current serves as a source of depolarization that triggers additional axonal Na spikes, which in turn further depolarize the terminal or help maintain the depolarization. Interrupting the feedback cycle by reducing excitability at either the terminal or the axon inhibits the generation of repetitive nerve firing and prolonged ejps.

Repetitive nerve firing is also caused by a bang-sensitive (bas^{MW1}) mutation. The mechanism is not yet clear but it differs from Sh in that the terminal apparently plays a lesser role. The behavioral and physiological defects of bas^{MW1} are also suppressed by nap^{ts}.

192.4 LONG-LASTING HOMOSYNAPTIC AND HETEROSYNAPTIC POST-ACTIVATION POTENTIATION IN THE HIPPOCAMPUS. John M. Sarvey. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20014.

Following repetitive electrical stimulation of a monosynaptic input to pyramidal cells of either field CA1 or CA3 in hippocampus, a long-lasting potentiation of the excitatory response to single stimuli to that input has been found by several laboratories. Those groups recording from CA1 report that the potentiation is homosynaptic, i.e., restricted to the response to the repetitively stimulated input (Andersen et al. Nature 266:736, 1977; Lynch et al. Nature 266:737, 1977), while those recording from CA3 report that the potentiation is heterosynaptic, i.e., response to a second, unstimulated input is also potentiated (Yamamoto & Chujo. Exp. Neurol. 58:242, 1978; Misgeld et al. Exp. Brain Res. 37:217, 1979). It is difficult to decide whether the contrasting findings are due to differences in methodology or in the two hippocampal fields.

In 300-400 µm thick hippocampal slices from guinea pigs population spikes could be extracellularly recorded from stratum pyramidale of CA1 in response to "single" (0.37 cps) rectangular current pulses to either stratum radiatum (Schaffer, Sch) or stratum oriens (OR) in CA1. Repetitive (20 or 50 cps for 10 s) stimulation of either OR or Sch resulted in potentiation of the orthodromic population spike. This potentiation, which was followed for 30 min to 3 hr, was homosynaptic (restricted to the repetitively stimulated input).

In contrast, repetitive stimulation of the dentate hilus (mossy fiber, mf) of fimbria resulted in potentiation of the orthodromic response in stratum pyramidale of CA3 to single stimuli to either stimulation site. Similarly, heterosynaptic potentiation in CA3 resulted when the stimulation sites were mf and Sch. Failure of a conditioning shock to the heterosynaptic input (e.g., Sch) to facilitate the response to a test shock 20 ms later to a second input (e.g., mf) compared with consistent homosynaptic facilitation (e.g., mf-mf) insured that two separate inputs were indeed being stimulated.

The finding of homosynaptic potentiation in CA1 and heterosynaptic potentiation in CA3 in the same laboratory suggests that there are intrinsic differences in these two fields of the hippocampus.

192.5 QUANTAL ANALYSIS IN FASCIA DENTATA GRANULE CELLS: B. L. McNaughton and C. A. Barnes. Institute of Neurophysiology, University of Oslo, Karl Johans Gate 47, Oslo 1, Norway.

Synaptic response variability was analysed in granule cells of the fascia dentata in vitro in order to determine the quantal characteristics of the EPSPs from afferent fibres in the molecular layer. Hippocampal slices were incubated at 34 ± 2°C in a medium containing (in mM): NaCl 124; $CaCl_2$ 2; KCl 2; KH_2PO_4 1.25; $MgSO_4$ 2; $NaHCO_3$ 26; glucose 10. Standard intracellular recording techniques were used. The cells used in this study had the following mean characteristics: RMP 69 ± 2 mV; R_{in} 41 ± 3 MΩ; action potential 58 ± 1 mV; EPSP at discharge threshold 24 ± 2 mV.

In 40 cells, the stimulus intensity was adjusted to give a very small EPSP (.7 ± .08 mV) and 500 responses were collected at a frequency of 0.5 Hz. Fifteen data sets showing drift of resting potential of more than ± 3 mV or non-stationarity in response amplitude were rejected from the analysis. Three types of analysis were carried out: 1) a fourier transform method was devised to detect the most significant periodicity in the EPSP amplitude frequency histograms; 2) the mean quantal content ($\bar{m}$) was calculated from the logarithm of the total number of trials divided by the number of apparent response failures; 3) $\bar{m}$ was also calculated from the ratio of the mean response amplitude ($\bar{V}$) squared divided by the amplitude variance (after subtraction of an estimate of the noise variance). The quantal size was estimated by $\bar{V}/\bar{m}$. The three methods gave similar results (quantal sizes of .08 mV, .26 mV, and .14 mV respectively). Large reductions in $\bar{V}$ brought about by elevated Mg^{++} (12 mM) or reduced Ca^{++} (0.8 mM) resulted in a significant increase rather than a decrease in apparent quantal size (estimated by method 3). This indicates that the EPSP variability was not due to conduction failures. Detailed analysis of the minimally detectable EPSPs at stimulus intensities bordering threshold suggest that the mean quantal content of the EPSP due to a single afferent fibre is close to 1. Analysis of the role of non-linear summation of unitary EPSPs at multiple synaptic sites is still in progress. At present, however, the lower and upper bounds on the number of synapses required to discharge the average granule cell can be set at about 150 and about 1000 respectively.

192.6 INTRACELLULAR RECORDING OF SYNAPTIC INTERACTIONS BETWEEN PYRAMIDAL CELL AND INTERNEURON PAIRS IN HIPPOCAMPAL BRAIN SLICES. W. D. Knowles and P. A. Schwartzkroin, Dept. Neurological Surg., University of Washington, Seattle, WA 98195.

Simultaneous intracellular recordings were made in vitro from pairs of neurons in longitudinal slices of guinea pig hippocampus. Special attention was given to synaptic interactions between CA1 pyramidal cells and interneurons (identified per Schwartzkroin and Mathers, 1978).

Slices of adult guinea pig hippocampus were cut 500 μ thick in the dorsal-ventral longitudinal plane, and maintained in 35° C. Ringer's solution. Intracellular recordings were made using 50-100 megohm, 4M K^+ citrate + .01 M KCl filled electrodes. Possible synaptic interactions were tested for by injecting depolarizing intracellular current pulses (.5 to 1 nA x 100 ms) to evoke spike trains in one cell while noting postsynaptic potentials in the second cell.

Synaptic interactions between CA1 pyramidal cells were rare, occurring in 13% of 101 pairs. Only hyperpolarizing postsynaptic potentials were seen. These hyperpolarizations were usually low amplitude (<5mV), slow, and smooth, and lasted for the duration of the spike train in the presynaptic cell. Occasionally individual IPSPs corresponding to each presynaptic spike could be detected. We did not observe any signs of electrotonic coupling. The same cell was penetrated with both electrodes more frequently than penetration of synaptically coupled cell pairs. Such double penetrations must be kept in mind when studying interactions.

Of more than 360 cells successfully penetrated, less than 6% were identified as interneurons. Simultaneous recordings were made from 19 different interneurons and 43 different pyramidal cells. Induced spike trains in the interneuron caused IPSPs in the pyramidal cell in 30% of the 43 pairs. These IPSPs were similar to the IPSPs seen in pyramidal cell pairs. Stimulation of the pyramidal cell caused EPSPs or spikes in the interneuron in 28% of the pairs. In 53% of the pairs, no interactions were seen.

These results provide the first direct confirmation of the hypothesis (Andersen, Eccles, and Løyning, 1964) that IPSPs may result from recurrent inhibition mediated by inhibitory interneurons. The magnitude of inhibitory interactions in our study, however, was surprisingly small considering that large IPSPs were produced in pyramidal cells by extracellular stimulation of afferent pathways.

192.7 SYNAPTIC TRANSMISSION: DEPENDENCE ON CALCIUM LEVELS IN HIPPOCAMPAL SLICE. G. Somjen and Raymond Dingledine. Dept. Physiol., Duke Univ., Durham, N.C. and Dept. Pharmacol., Univ. N. Carolina, Chapel Hill, N.C.

Measurements in the brain and spinal cord of interstitial calcium activity ($[Ca]_o$) with calcium-sensitive microelectrodes have demonstrated that changes in $[Ca]_o$ accompany epileptiform seizures, spreading depression and, to a smaller extent, repetitive stimulation of synaptic afferent systems. We have asked whether such changes in $[Ca]_o$ could influence local transmitter release. The question here is the sensitivity of synaptic transmission to small departures of $[Ca]_o$ from its resting level of 1.2mM. Rat hippocampal slices (350 μ thick) were perfused at 33-36°C in vitro with a solution of the following composition (in mM): NaCl 130, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 24, $CaCl_2$ 1.2, $MgSO_4$ 1.2, glucose 10. A tungsten microcathode was inserted among the afferent fibers running in the stratum radiatum of the CA1 region, while a Ca-sensitive microelectrode was positioned nearby in the same layer and used to record both $[Ca]_o$ and extracellular focal potentials. Weak electrical stimuli evoked a typical two-component field potential, interpreted to reflect the presynaptic volley, and the excitatory postsynaptic current (the focal EPSP: Andersen et al., Brain Res., 144:11, 1978). By varying the stimulus intensity different numbers of presynaptic fibers were recruited, and input-output curves for the activated synapses could be constructed by plotting the size of the presynaptic fiber volley against the size of the focal EPSP. With moderate intensities of stimulation such input-output curves were linear, the slopes of which were taken as measures of the effectiveness of synaptic transfer.

Following a step change in [Ca] at the inflow valve, bathing fluid surrounding the slices equilibrated to the new level within 3-5 min, while the interior of the slices required 30-60 min for complete equilibration. During an imposed gradual decrease in $[Ca]_o$, from 1.2 to 0.5mM, the slope of the input-output transfer became progressively less steep. When $[Ca]_o$ was raised to 3mM the slope increased in a graded manner. A curvilinear relation was found between the normalized slope (the slope at 1.2mM $[Ca]_o$ being defined as 100%) and log $[Ca]_o$, the form of which suggests that synaptic transfer depends on the second power of $[Ca]_o$. For deviations from the "normal" 1.2mM level, a change of 0.1mM $[Ca]_o$ produced about 15% change in the slope of the input-output function. Under certain pathological conditions changes in $[Ca]_o$ are large enough for local synaptic transmission to be significantly affected.

Supported by NS11933 and DA02360.

192.8 TRANSMISSION AT THE Ia FIBER-MOTONEURON SYNAPSE IN THE CAT. T.C. Cope and L.M. Mendell. Duke Med. Ctr., Durham, N.C. 27710

We have previously demonstrated that EPSPs produced by the action of single group Ia fibers in α motoneurons fluctuate both in amplitude and latency from trial to trial. In the present work we have examined these fluctuations in greater detail. EPSPs were recorded intracellularly in motoneurons; in anesthetized preparations sweeps triggered by the group Ia impulse recorded in the dorsal rootlets were digitized and stored on tape at 20 μsec resolution. Latency of each EPSP was measured and EPSPs in each latency bin were summed on an averaging computer. We found a linear inverse relation between amplitude and latency. We also noted a systematic highly significant decrease in rise time (RT) for EPSPs as latency increases; the slope of this relationship averaged -1 μsec(RT)/μsec(latency). These results indicate that fluctuations in EPSP parameters are not due to activation of different portions of the soma-dendritic membrane by a distributed system of boutons given off by single Ia fibers since large, short latency EPSPs would be expected to exhibit the shortest rise times rather than the longest ones. These fluctuations are not correlated with changes in motoneuron membrane potential or motoneuron time constant, and so they probably represent variability in a presynaptic process. One possibility is invasion of different boutons in the ensemble from trial to trial, but the arrangement of boutons would have to be highly regular with respect to the Ia fiber axon and motoneuron soma at different synapses in order to give the uniform correlations which have been observed. Variability in the action potential from trial to trial due for example to fluctuations in terminal polarization (e.g. PAD) might be postulated to explain our results. However, any decrease in the amplitude and rate of rise of the terminal action potential causing a decrease in EPSP amplitude and an increase in latency would be expected to prolong EPSP rise time due to broadening of the action potential. The uniform correlations we have observed are best predicted by a model similar to that proposed for the neuromuscular junction (Barrett and Stevens, J. Physiol. 227:665, 1972). Arrival of the impulse at the terminal causes increased probability of release of transmitter. Release of a quantum at any time during the period of increased probability is independent of previous release. Therefore short latency EPSPs (i.e. first quantum released early) will tend to be the largest (i.e. most quanta released since period of release is longest) as well as having the slowest rise time (i.e. each increase of 1 μsec in period of release causes a 1 μsec increase in rise time). Our findings support the quantal hypothesis for synaptic transmission at this synapse. (Supported by NIH; NS08411; NS14899.)

192.9 PROPERTIES OF EPSP LATENCY DISTRIBUTIONS AT Ia FIBER-MOTONEURON SYNAPSES IN THE CAT. L.M. Mendell and T.C. Cope (Spon. S.G. Nelson). Duke Univ. Med. Ctr., Durham, N.C. 27710

Individual EPSPs produced in a motoneuron by the action of a single Ia fiber fluctuate in onset latency from trial to trial. Latency distributions at all synapses are unimodal but differ in variance (Collatos and Mendell, Neurosci. Abst. 5, 1979). We have analyzed 11 synapses whose EPSPs exhibited large amplitudes (178 μV<A<1090 μV) and brief rise times (140 μsec<RT<460 μsec); these differ not only in standard deviation ($\sigma=\sqrt{\text{variance}}$; 41 μsec<σ<116 μsec) but also in mean (μ; 281 μsec<μ<534 μsec) measured from arrival of the Ia impulse in the terminal region (i.e. from the terminal potential). A key finding is the linear correlation between μ and σ (r=0.81, n=11). The intercept (μ for σ=0) is 216 μsec. The slope is 2.66 yielding the relation $(216-\mu)/\sigma=-2.66$. Assuming normality (satisfied by most of these distributions), we conclude that the probability of an EPSP with latency < 216 μsec is small ($p<0.01$; i.e. $z=-2.66$); this latency is the same for each distribution. Our interpretation is that the expected minimum latency is similar at each of these synapses. The measured minimum latency fluctuated from synapse to synapse with a mean of 204±15 μsec (SEM); this was not correlated with μ. This variability arose from the difficulty in fixing the minimum latency accurately due to the relatively small number of EPSPs with short latency, as well as possible contributions from other active fibers synapsing on the motoneuron. Our estimate of minimum latency (216 μsec) is less than that obtained by Munson and Sypert (260 μsec - J. Physiol. 296:329, 1979) using averaged EPSPs whose onset always occur at a longer latency than the minimum measured from single trials. It follows that minimun synaptic delay (= synaptic latency − postsynaptic electrotonic delay) is about 45 μsec less than the 170 μsec estimated using averaged EPSPs (Munson and Sypert, ibid). We speculate that minimum synaptic delay is fixed at all synapses of this type, and that our finding of fixed minimum EPSP latency is a consequence of similar postsynaptic electrotonic delays for these very brief EPSPs. Synaptic efficacy is related to μ and σ since mean EPSP amplitude is inversely correlated with the square of the coefficient of variation ($C.V.=\sigma/\mu$) of the latency distribution ($p<0.05$). This may be explained by the finding (Kuno and Miyahara, J. Physiol. 204:405, 1969) that EPSP amplitude fluctuations are Poisson distributed (mean EPSP amplitude α quantal content α $1/C.V.^2$), and that EPSP amplitude and latency are linearly correlated at single synapses (Cope and Mendell, this volume). We propose that these synapses vary in kinetics of transmitter release, and that this is correlated with the average number of quanta released. (Supported by NIH-NS08411; NS14899.)

192.10 EFFECTS OF STIMULUS RATE ON POSTSYNAPTIC POPULATION POTENTIALS EVOKED BY IMPULSES IN SINGLE IA FIBERS. Hans-R. Lüscher, Paul Ruenzel* and Elwood Henneman. Dept. of Physiology, Harvard Medical School, Boston, MA 02115.

Evidence from studies on post-tetanic potentiation of EPSPs in motoneurons (Nature 282: 859, 1979) suggests that conduction in terminal arborizations of Ia fibers is influenced by the recent history of activation of the arborization. This has led us to investigate how the frequency of impulses in single Ia fibers influences the amplitude of the responses they evoke in motoneurons. To do so, we have measured changes in the amplitudes of postsynaptic population potentials (PSPPs), elicited by stimulating single Ia fibers electrically at different frequencies. The PSPPs were recorded from ventral roots perfused with isotonic sucrose, using a recently developed technique (J. Neurophysiol. 42: 1146, 1979). Input to the spinal cord was restricted to a single Ia fiber by dissecting the muscle nerve into branches and further into bundles for stimulation. All the dorsal roots on the left side were cut from L_5 to S_2, except for a filament containing the single Ia fiber. The PSPP measured at each frequency was an average of 256 sweeps. All but 1 of the 15 Ia fibers tested showed a relatively standard frequency response curve characterized by low frequency depression and high frequency facilitation. The lowest frequency used (1 Hz) was sufficient to produce a slight depression of PSPPs. With increasing frequency, a progressive depression of PSPP amplitude developed that was maximal at 2-5/sec. With further increases in frequency, PSPP amplitude increased to a maximum at 10-20/sec. It is likely that oscillations in the excitability of the membrane following action potentials modulate conduction through regions of low safety. Low frequency depression may be due to increases in branch point failure when successive action potentials coincide with phases of depressed membrane excitability following preceding impulses. High frequency facilitation may be due to less branch point failure when successive action potentials coincide with the hyperexcitable period. The observation that PSPP amplitude is a function of stimulation frequency suggests that the architecture of the terminal arborization together with the impulse pattern conducted through it play decisive roles in determining the total synaptic action of Ia impulses. Perhaps most important of all is the conclusion that the information from the periphery, carried to the terminal arborization and coded as a pulse train, can itself modulate the coupling between cells.

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192.11 SYNAPTIC EFFICACY DEPENDS ON MOTOR UNIT SIZE IN NORMAL FROG NEUROMUSCULAR JUNCTIONS. Alan D. Grinnell and Albert A. Herrera. Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA. 90024.

Neuromuscular junctions of the frog sartorius often exhibit low safety factors, some eliciting only subthreshold EPPs on single stimulation (Grinnell & Herrera, J. Physiol. in press). We have shown that when 1/2 of the muscle is removed, so that the motor axons reinnervate a reduced number of muscle fibers, they form stronger synapses, releasing an average 4.2X more transmitter per unit length of terminal (Herrera & Grinnell, Soc. for Neurosci. Abs., 1979). Reinnervated whole muscles do not show this enhancement. This suggests that a reduction in the number of terminals a motoneuron must maintain makes the remaining terminals stronger.

The enhanced transmitter release in muscles with reduced motor unit size might also suggest that, in normal muscles, junctions belonging to the largest motor units would show lowest safety factor, and vice versa. We have examined this hypothesis by measurement of tensions elicited by stimulation of single motor units, obtained by dissection of the sciatic nerve in nerve-muscle preparations from Rana pipiens. We find that the opposite obtains: the largest motor units have synapses of uniformly high safety factor, all suprathreshold to a single stimulus, with no change in twitch tension to changes in external Ca^{++} concentration between 3.0 and 1.0mM in the Ringer (normal = 1.8 mM). Progressively smaller motor units show smaller and smaller safety factors and an increasing proportion of subthreshold junctions. The twitch tensions of small motor units increase in size in 3 mM Ca^{++} Ringer, and drop sharply with reduction of Ca^{++} to 1.0 mM. The smallest motor units (approximately 5-10 mg twitch tension in the frogs we used) showed a tetanus/twitch ratio of 50-100, compared with a ratio of approximately 2 for motor units of more than 300-400 mg twitch tension. Visual inspection of contraction reinforces the conclusion that axons forming the smallest motor units innervate up to 50X as many fibers as they can drive with a single stimulus. This implies that there are many more subthreshold junctions in the sartorius than have been recognized to date. It seems probable that most of these subthreshold synapses constitute polyneuronal inputs to junctions innervated by other, suprathreshold, inputs. The subthreshold components may be so small that they are undetectable by the usual physiological techniques of assessing polyneuronal innervation. (Supported by USPHS grant NS06232, an MDA grant, and an NIH postdoctoral fellowship to A.H.)

192.12 PRESYNAPTIC EFFECTS OF γ-AMINOBUTYRIC ACID ON INTRASPINAL SINGLE CUTANEOUS AFFERENT C- AND Aδ-FIBERS. M. Randić, K. Semba and K. Murase*. Dept. Vet. Physiol., Iowa State Univ., Ames, IA 50011.

A high density of GAD-positive terminals is found in the superficial parts of the dorsal horn, the area where primary afferent fibers are known to terminate. Furthermore, recent observations on the distribution and synaptic relationships of the GAD-positive terminals within laminae II and III of the rat spinal cord (Barber et al., Brain Res., 141, 35-55, 1978) have provided additional support for the concept that GABA-containing terminals are involved in synaptic pathways which may influence excitability of primary afferents terminating in this region. While GABA is known to enhance excitability of the terminals of large A-fibers, its action on the excitability of C- and Aδ-fibers was not previously investigated.

In cats either spinalized or anesthetized with pentobarbital, small filaments of the sural nerve were prepared for recording, and thresholds for the antidromically-evoked nerve impulses of single afferent fibers were measured during intraspinal stimulation (Aδ-fibers: 1.6-8 μA; C-fibers: 4-15 μA) with a fine platinum microelectrode positioned within laminae I-III of the dorsal horn. GABA (0.5 M, pH 4.5), bicuculline hydrochloride (5 mM, pH 3-5), and piretanide (13.8 mM, pH 7.3) were iontophoretically ejected through glass micropipettes glued to a stimulating electrode (intertip distance <15 μm). Bicuculline was also administered intravenously (1 mg/kg).

The responses of C- and Aδ-fibers to GABA (50-100 nA) were not uniform: both threshold decrease and increase were observed. In the majority of C-fibers tested, GABA decreased threshold in a reversible and dose-dependent manner (range: 66-95% of control), although in some fibers, electrical excitability at the threshold value was decreased (105-139%). In Aδ-fibers, both elevation (n=7) and reduction (n=5) in threshold was produced by GABA. In some of C- and Aδ-fibers, bicuculline and piretanide reversibly suppressed the GABA-produced decrease in threshold.

The complex actions of iontophoretically applied GABA on the electrical excitability of intraspinal cutaneous afferent C- and Aδ-fibers observed in our experiments may reflect the complexity of the GABA synaptic relationships recently demonstrated in the superficial parts of the dorsal horn. Our results provide a direct pharmacological evidence that GABA is capable of modifying the electrical excitability of cutaneous afferent C- and Aδ-fibers.

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192.13 RECRUITMENT OF POSTGANGLIONIC NEURONS BY BURST PATTERNING OF PREGANGLIONIC INPUT IN THE CAT STELLATE GANGLION. R.I. Birks, W. Laskey* and C. Polosa*. Physiology Department, McGill Univ., Montreal, Canada H3G 1Y6.

Burst patterning of the preganglionic neural input to the perfused cat superior cervical ganglion (SCG) has been shown to increase acetylcholine (ACh) output up to 3 times that found in response to stimulation with equally spaced pulses at the same mean frequency as the patterns (Birks, R.I., J. Physiol. 295, 51-52P, 1979). It has now been found that patterns of pulses that increase ACh stores and ACh output in the SCG are equally effective in increasing the efficacy of synaptic transmission in the cat stellate ganglion in situ.

Stimulation of the preganglionic thoracic sympathetic trunk or portions of it (between rami T4 and T5) with 0.5s trains at 40/s every 10s increased the area of the postganglionic compound action potential (CAP) recorded from the inferior cardiac nerve. Stimulation with equally spaced pulses at 2/s did not increase the CAP. In five experiments using 40/s trains, the mean increase in CAP response to patterned stimulation was 258% the control CAP before patterning. This recruitment of the neuronal pool was as great as the maximum post-tetanic potentiation of the CAP response, indicating saturation of the pool. The increase in CAP was 200% in 6 trials using a train frequency of 20/s in the patterns, and 160% in 4 trials with a train frequency of 10/s. The pool was not saturated by the patterned stimulation in these tests at reduced train frequency. The greater proportion of the increase in CAP occurred within 1 min of the start of stimulation with a smaller increase occurring over 4-9 min of further stimulation.

The time-course and magnitude of the increase in postganglionic CAP are similar to the time-course and magnitude of the increase in ACh output in SCG found earlier. These similarities, and the fact that burst patterning at a mean frequency of only 2/s can recruit into the discharge zone the entire available neuronal pool, confirm and reinforce the hypothesis (Birks, R.I., J. Physiol. 280, 559-570, 1978) that burst patterning of preganglionic input is a major mechanism of modulation of the transmission process in sympathetic ganglia.

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192.14 THE EFFECTS OF CALCIUM AND MAGNESIUM ON QUANTAL CONTENT AND PROBABILITY OF RELEASE. George J. Blake* and Kenneth L. Dretchen. Dept. of Pharmacology, Schools of Medicine & Dentistry, Georgetown University, Washington, D.C. 20007.

The effects of limited Ca^{++} availability on quantal content (m) and probability of release (p) were studied on the rat hemidiaphragm "cut muscle" preparation. Miniature end plate potentials (MEPPS) and end plate potentials (EPPS) were recorded using standard techniques, and m was determined by the direct method (EPP/MEPP) at low frequency (0.1 Hz). The normal bathing medium contained 2 mM Ca^{++} and 1 mM Mg^{++}. Release was binomial, p ranged from 0.7 to 0.99 and was not significantly affected by stimulation rate. The highest values for m were obtained at low stimulus frequency (0.1 Hz); as stimulation rate increased m was depressed. High frequency (150 Hz, 1 sec.) stimulation produced a biphasic effect - an initial facilitation followed by depression of EPP amplitude to a "plateau" with m = 72% ± 4 of the first EPP. Decreasing the Ca^{++} concentration to 1 mM decreased m throughout the range of stimulus frequencies used, but p did not change. Elevation of Mg^{++} to 2 mM had no significant effect alone, but in combination with decreased Ca^{++} (to 1 mM) there was a decrease in m, n, and p, but release remained binomial. The extent of depression of these parameters was found to be dependent on the rate of stimulation. The effect of this treatment on m was the reverse of control: m measured at 0.1 Hz was depressed to 30% of control, and increased to 47% of control at 150 Hz. In addition, high frequency stimulation produced EPP's that were initially depressed and increased to 153% ± 15 of the first EPP. Although p measured at 0.1 and 2 Hz was significantly depressed to 55% of control, the difference was no longer significant when measured at 150 Hz. MEPP amplitude, frequency, rate of rise and duration were unaltered by these treatments. These results show that under conditions of depressed m, quantal release remains binomial. The depression in m, n and p produced by 2 mM Mg^{++}/1mM Ca^{++} treatment can be overcome by high frequency stimulation.

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192.15 FURTHER OBSERVATIONS ON CHANGES IN EXTRACELLULAR a_K AND a_{Ca} EVOKED IN RAT HIPPOCAMPUS BY REPETITIVE ACTIVATION. M.E. Morris, K. Krnjević, R.J. Reiffenstein* and N. Ropert*. Anaesthesia Research & Physiology Depts., McGill University, Montreal, Canada.

We have previously reported that in rats under urethane, repetitive stimulation of the fimbria or entorhinal cortex can lead to marked changes in hippocampal a_K and a_{Ca} (Krnjević et al., Can. J. Physiol. Pharmacol. 58: May 1980). At low frequencies of stimulation (< 3/s), only small increases in a_K are detectable, but there is no change in a_{Ca}. At higher frequencies, when firing in bursts is generated - and especially if electrographic seizures are manifested by desynchronized firing and after-discharges - the rise in a_K becomes very large (to 9-12 mM), and it is initially accompanied by a major fall in a_{Ca} (from 1.5 to 1.0-0.5 mM). For both ions, the maximal changes are recorded at the superficial pyramidal layer (where fimbrial stimulation elicits the largest positive field); but a sharp fall in a_{Ca} is evident only at this level, whereas a substantial rise in a_K is seen over a wide range of depth (> 1 mm).

More recent experiments have confirmed these findings during both urethane and ketamine anaesthesia; the principal difference seen with ketamine was that unusually low intensities of fimbrial stimulation were adequate to evoke population spikes, seizure discharges and corresponding large changes in ions. Moreover, systematic measurements in the CA1 and CA3 regions, combined with marking of critical points of recording by local release of small amounts of pontamine blue, showed no major difference in the pattern of depth distribution, frequency sensitivity, etc. of changes in a_K and a_{Ca}. In view of the probable existence of a cholinergic (septo-hippocampal) input, a possibly serious source of error in our measurement of a_K is that the "classical" ion-selective microelectrodes (prepared with the Corning liquid-ion-exchanger) are even more sensitive to ACh^+ than to K^+; therefore, similar recordings were made using valinomycin microelectrodes (Oehme and Simon, Analyt.Chim.Acta. 86:21,1976) which are much more sensitive to K^+ than to ACh^+, though having a slightly slower response time. The results were essentially indistinguishable from those obtained with "classical" a_K electrodes, and so confirm the identity of the signals previously ascribed to a_K. Finally, by varying the intensity of repetitive fimbrial or entorhinal stimulation, we have obtained further evidence of a close correlation between the appearance of population spikes and the sudden large increments in a_K or falls in a_{Ca}. This reinforces the conclusion that, in contrast to the situation in the spinal cord and the dorsal column nuclei, in the hippocampus changes in extracellular a_K and a_{Ca} are generated mainly by post-synaptic neuronal firing.

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192.16 PROPERTIES OF POTASSIUM CHANNELS IN SYNAPTOSOMES MEASURED WITH ^{86}Rb. M. P. Blaustein, R.K. Ickowicz* and H. Rasgado-Flores*. Dept. of Physiol., Univ. of Maryland, School of Med., Baltimore, MD. 21201

Potassium channels play an important role in the modulation of neurotransmitter release from presynaptic nerve terminals. To gain new insight into the physiology of K permeability in presynaptic terminals, we investigated some properties of ^{86}Rb efflux from presynaptic terminals (synaptosomes) from rat forebrain. Synaptosomes, isolated by the method of Krueger et al. (J. Membrane Biol. 50:287, 1979), were loaded with ^{86}Rb, a K analogue known to pass through voltage-sensitive K channels (Hille, J. Gen. Physiol. 61:669, 1973). When ^{86}Rb was added to standard physiological salt solution (with 5 mM K and 145 mM Na), the tracer accumulation was ouabain-sensitive (i.e. Na-K pump mediated). Tracer uptake rose to a steady level, equivalent to ~400 μmoles K/gm synaptosome protein, with a half-time of about 5 min at 30°C.

The ^{86}Rb-loaded synaptosome suspensions were diluted 40-fold into "efflux solutions" of desired composition; these solutions often had reduced Na concentrations, with some Na replaced isosmotically by K or choline. Following incubations of 5 to 60 sec. at 30°C, the suspensions were rapidly filtered on glass fiber filters (Whatman GF/C) and washed with three 4 ml aliquots of tracer-free ice-cold physiological saline containing 25 mM TEA (tetraethylammonium). The washed filters were analyzed for ^{86}Rb content by liquid scintillation spectroscopy; a reduction in ^{86}Rb counts on the filters, with increasing time of incubation or promoted by special incubation conditions, was taken as a measure of Rb efflux.

The rate coefficient for Rb efflux, from ^{86}Rb-loaded terminals, into control (5 mM K) solutions was 0.03-0.1 min^{-1}. The Rb efflux rate coefficient increased with increasing $[K]_o$, to ~0.4 min^{-1} at 90 mM $[K]_o$ and ~0.6 min^{-1} at 150 mM $[K]_o$. Rb efflux was not increased when external Na was replaced by choline at constant $[K]_o$. The fraction of the Rb efflux promoted by K-rich media was almost completely blocked by 25 mM TEA and by 1.25-2.5 mM 4-aminopyridine, two agents known to block K channels in excitable cells. Phencyclidine (100 μM), which is also known to block K channels (Albuquerque et al., Proc. Natl. Acad. Sci. USA, 77:1224, 1980), reduced the K-stimulated Rb efflux by about 50%. These data indicate that tracer flux methods can be used to study some of the physiological and pharmacological properties of K channels in mammalian presynaptic nerve terminals in vitro. [Supported by NIH and by a CONACYT fellowship to H.R.F.]

192.17

Withdrawn by Author

192.18 DEPLETION OF SUBSTANCE P IN A SYNAPTIC VESICLE FRACTION DERIVED FROM VERATRIDINE-DEPOLARIZED SYNAPTOSOMES. E. Floor and S.E. Leeman. Dept. Physiol., Harvard Medical School, Boston, MA 02115

Synaptic vesicles containing a peptide neurotransmitter, substance P (SP), were partially purified and evidence that at least some are involved in transmitter release was obtained. Synaptosomes prepared from rat brainstem by homogenization in 0.32M sucrose, differential centrifugation and banding on a linear Ficoll/sucrose density gradient were lysed by osmotic shock, centrifuged to remove debris, and the resulting crude vesicle supernate fractionated according to particle size on a glass bead column of average pore size 290nm. Acid extractable, radioimmunoassayable SP eluted in a major peak of material slightly larger than ^{14}C-labelled bacteriophage P22 (diameter about 60nm). This peak probably represents the large (100nm) SP-reactive synaptic vesicles seen by immunohistochemistry. SP-like immunoreactivity from whole rat brain was shown to be identical to synthetic SP by reverse-phase HPLC. In the peak fractions from the glass bead column SP was enriched 5 to 10-fold (per mg protein) over the initial homogenate. Vesicular SP in the crude vesicle supernate was not attacked by endogenous proteases at 0^{o} or 37^{o} although added, synthetic SP was degraded at both temperatures.

If the vesicle population identified above were active in transmitter release, strong depolarization of synaptosomes might be expected to reduce its content of SP. Veratridine (VA) has been shown to depolarize rat brain synaptosomes and to induce a tetrodotoxin(TTX)-sensitive, calcium-dependent release of transmitters. Synaptosomes were placed at $37^{o}C$ in a physiological buffer (Schenker et al., Nature 264: 790, 1976) and bubbled gently with O_2 for 4 min. Parallel batches were then oxygenated an additional 5 min under various conditions, lysed, and the crude vesicle supernate run on the glass bead column. Recoveries of SP in the vesicle peak after treatments with VA (75 µM) or TTX (1 µM) were as follows (percent of control ± SEM): VA, 73 ± 3% (n=5); TTX + VA, 109 ± 4% (n=4); and VA in calcium-free buffer, 96 ± 7% (n=3). The mean recoveries of protein under each of the three experimental conditions in synaptosomes after $37^{o}C$ incubation and in the crude vesicle supernate after lysis were within 4% of control values. Recoveries of vesicular SP were normalized with controls in each experiment on the basis of these protein recoveries. These results show that veratridine induces a substantial TTX-sensitive, calcium-dependent depletion of SP in the vesicle fraction and suggest that part of the readily releasable pool of SP in synaptosomes is vesicular.

192.19 ACETYLCHOLINE SYNTHESIS FROM [^{14}C]-GLUCOSE AND [^{3}H]-CHOLINE IN SYNAPTOSOMES FOLLOWING HIGH-AFFINITY CHOLINE CARRIER INHIBITION BY CHOLINE MUSTARD AZIRIDINIUM ION. B. Jane Rylett and E. Howard Colhoun*. Dept. Pharmacology, University of Western Ontario, London, Canada, N6A 5C1.

In our laboratory, the nitrogen mustard analogue of choline, choline mustard aziridinium ion (ChM Az), has been shown to be a potent and irreversible inhibitor of both high- and low-affinity choline transport into rat forebrain synaptosomes. Although not a selective blocker of the high-affinity choline uptake, this choline analogue shows a greater potency with respect to the sodium-dependent, high-affinity choline carriers in synaptosomes. In addition, synaptosomes incubated with ChM Az show a decrease in intrasynaptosomal choline acetyltransferase activity which paralleled the time-dependent alkylation of sodium-dependent, high-affinity choline carriers by the nitrogen mustard compound. In the present study, the ability of ChM Az-treated synaptosomes to synthesize acetylcholine (ACh) from either [6-^{14}C]-glucose or [^{3}H-N-Me]-choline was investigated to obtain more information about the sources of choline for the synthesis of ACh, and the contribution which choline supplied by the high-affinity choline carrier and choline present in the nerve terminal cytoplasm make in the biosynthesis of the neurotransmitter. Synaptosomes incubated with 1 µM [^{3}H]-choline at 37°C for 7 min showed 50% acetylation of the accumulated radioactive precursor, while those exposed to 8.2 µM (60.5 µCi/µmol) [^{14}C]-glucose at 37°C for 1 hr incorporated the [^{14}C] label into a quaternary compound with an R_f the same as ACh. Treatment of synaptosomes with 50 µM ChM Az resulted in 80% decrease in the total transport of [^{3}H]-choline into synaptosomes (high-affinity transport was completely blocked) and blocked [^{3}H]-ACh formation. The same concentration of ChM Az, following a 10 min exposure of synaptosomes to the nitrogen mustard analogue, gave a 30% decrease in the synthesis of [^{14}C]-ACh from [^{14}C]-acetylCoenzyme A stores formed in synaptosomes incubated with [^{14}C]-glucose. The results indicate that although choline transported on the high-affinity choline carrier contributed to the available choline pool for the synthesis of ACh, the transmitter may be formed also from endogenous choline in the nerve terminal. Inhibition of the high-affinity choline carrier along with inhibition of choline acetyltransferase decreased [^{14}C]-ACh synthesis which suggests either blockade of [^{14}C]-ACh formation from cytoplasmic choline at the level of choline acetyltransferase, or inhibition of unmeasureable high-affinity uptake of non-isotopic choline released from synaptosomes into the incubation medium by phospholipid degradation. Would similar results be obtained with a stimulated nerve-muscle preparation? Supported by National Research Council of Canada.

192.20 INVESTIGATION OF THE RELATIONSHIPS BETWEEN CYTOPLASMIC AND MEMBRANE ASSOCIATED ACETYLCHOLINE IN SYNAPTOSOMES. G.E. Duncan*, M.J. Clark* and P. Rowell. Dept. of Pharmacology and Toxicology, Univ. of Louisville Sch. of Med., Louisville, KY 40292.

We have investigated the time course of accumulation, the effects of varying external choline (Ch) concentration, and the effects of ions on the subsynaptosomal distribution of acetylcholine (ACh) in rat cerebral cortical synaptosomes. The ^{3}H-ACh synthesized from ^{3}H-Ch was found to be located in the cytoplasmic fraction and in membrane of hypotonically shocked synaptosomes ("H-band fraction," Whittaker, Prog. Brain Res. 31, 211, 1969) as determined by sucrose density centrifugation. No ^{3}H-ACh was associated with monodisperse synaptic vesicles. The time course of accumulation of ^{3}H-ACh associated with the synaptosomal membranes paralleled that of cytoplasmic ^{3}H-ACh as determined by hypotonic shock in distilled water. The membrane-bound ^{3}H-ACh also paralleled the cytoplasmic ^{3}H-ACh after incubating synaptosomes in varying ^{3}H-Ch concentrations. When synaptosomes were disrupted in hypotonic solutions containing 62mM Na^{+}, there was a 2-fold increase in the membrane-associated ^{3}H-ACh compared to synaptosomes disrupted in 62mM K^{+}. These hypotonic solutions were essentially as effective as distilled water in releasing the intrasynaptosomal contents. The "shocking" solution containing 62mM Na^{+} resulted in a 3-fold increase in membrane-associated ^{3}H-ACh compared to synaptosomes disrupted in distilled water.

This finding and other preliminary results indicate that disrupting nerve terminals in distilled water (a standard procedure used in subcellular distribution studies of ACh) may lead to a gross underestimation of the amount of ACh associated with nerve terminal membranes and an over estimation of the amount of ACh in the cytoplasm.

192.21 ACETYLCHOLINE AND CHOLINE LEVELS OF RAT TISSUE AND THE EFFECT OF β-BUNGAROTOXIN. M.W. Newton*, C.B. Gundersen and D.J. Jenden. Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024

The presynaptic neurotoxin, β-bungarotoxin (βtx), causes a threefold increase in acetylcholine (ACh) in the rat diaphragm (Gundersen et al., Brain Res. 182:486, 1980). We have studied the effect of βtx on ACh and Ch content of other tissues. For peripheral tissues βtx (5 µg i.p.) was injected in male rats (130-150 g). Rats were decapitated after 1.5 hr and each tissue excised. For brain regions, rats were cannulated and allowed to recover for 2 days. βtx (1 µg in 5 µl) or saline (5 µl) was injected intraventricularly. After 105 min rat brains were fixed by microwave irradiation (5 kW for 2.1 s). ACh and Ch were extracted using 15% 1 N aqueous formic acid in acetone and assayed using GCMS (Freeman et al., J. Neurochem. 24:729, 1975). Results were as follows:

	ACh ± S.E.		Ch ± S.E.	
	Control	Treated	Control	Treated
Units:nmol/gm wt wgt				
Str	64.7 ± 4.1	84.4 ± 6.2*	22.5 ± 2.0	46.0 ± 3.7**
Hip	23.4 ± 1.8	33.0 ± 2.4**	21.4 ± 3.4	42.6 ± 3.0**
Ctx	22.7 ± 3.2	24.1 ± 1.5	17.6 ± 1.0	39.0 ± 2.9**
Dia	0.91± 0.2	3.79± 0.2**	85.3 ± 4.7	71.8 ± 7.7
Hrt	16.3 ± 1.2	20.3 ± 1.4	61.2 ± 4.9	82.8 ± 6.9*
Ile	18.8 ± 1.2	20.0 ± 1.1	493.0 ± 43.2	1410.0 ±420.0*
Tem	1.08± 0.1	1.47± 0.1*	95.9 ± 12.7	69.8 ± 3.9
Units:pmol/tissue sample				
Scg	136.6 ± 8.8	140.9 ± 7.8	450.0 ± 41.7	551.0 ± 57.4
Adr	45.0 ± 2.6	84.1 ± 5.9**	3060.0 ±410.0	4750.0 ±380.0
Eye	608.0 ±56.0	761.0 ±68.0	4610.0 ±440.0	4150.0 ±340.0

* $p < 0.05$; ** $p < 0.01$

Str=striatum; Hip=hippocampus; Ctx=cortex; Dia=diaphragm; Hrt=heart atria; Ile=ileum; Tem=temporalis muscle; Scg=superior cervical ganglion; Adr=adrenal. N = between 4 and 13.

Results indicate the most marked increases occur in diaphragm ACh and brain Ch content. We conclude that the effect of βtx on ACh and Ch levels is consistent in direction but variable in degree among tissues. (Supported by USPHS grant MH-17691.)

192.22 DIVERSE EFFECTS OF β-BUNGAROTOXIN ON ACETYLCHOLINE METABOLISM IN MAMMALIAN PREPARATIONS IN VITRO. C.B. Gundersen and D.J. Jenden, Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024

The neurotoxic phospholipase A_2 β-bungarotoxin (β-Btx) causes up to a threefold increase of diaphragm acetylcholine (ACh) content (Gundersen et al., Brain Res. 182:486,1980). To characterize the mechanism of this effect we have used a GCMS assay for ACh and choline (Ch) to study the action of β-Btx on ACh turnover in P_2 fraction, brain minces and diaphragm of rat, and in longitudinal strips of guinea pig ileum. In P_2 fractions β-Btx causes a dose-dependent stimulation of the appearance of endogenous Ch in the medium. Using 0, 5 or 10 min preincubations followed by a 4 min incubation with $[^2H_4]$Ch (2 µM), β-Btx (1.25 µg/ml) causes a 40-60% increase of $[^2H_0]$Ch in the medium. We observed no inhibitory effect of β-Btx on the uptake and acetylation of $[^2H_4]$Ch or on the pellet content of $[^2H_0]$ACh and $[^2H_0]$Ch. These results contrast with those of Sen & Cooper (J. Neurochem. 30:1369,1979), who reported a significant inhibition by β-Btx of $[^3H]$Ch uptake in synaptosomes. Our data indicate that the apparent inhibition by β-Btx of Ch transport observed by Sen & Cooper may have resulted from a dilution of $[^3H]$Ch specific activity by the release of endogenous Ch. This Ch "releasing" effect of β-Btx is not seen in Ca^{++}-free solutions. Certain non-neurotoxic phospholipases A_2 and high K^+ media cause smaller increases of medium Ch (5-20% relative to control) while gramicidin D (2 µg/ml) and dinitrophenol (0.125 mM) have no significant effect. Increased "release" of Ch is not observed in diaphragm or ileum preparations treated with β-Btx (1.4 µg/ml). However, after prolonged incubation (2 hr) with toxin (1 µg/ml) a substantial rise of medium Ch is seen in experiments using brain minces. From these results we suggest that the effect of β-Btx to raise medium Ch may be an indirect action associated with the phospholipase activity of the toxin. While brief exposure to β-Btx (0.14 or 1.4 µg/ml for 30 min) raises the ACh content of diaphragm, sustained (3 hr) treatment first increases, then reduces the ACh content to a level only slightly above control. This decline of diaphragm ACh is accompanied by a significant increase of ACh efflux. β-Btx (1-2 µg/ml) does not significantly increase the ACh content of P_2 fractions, brain minces or ileum preparations. However, long term treatment of brain minces with β-Btx causes a massive outpouring of ACh and a decline of the ACh content to approximately 20% of control. The findings in these experiments are consistent with the postulate that the effects of β-Btx on ACh metabolism are highly dependent on the tissue used and the dose and time of exposure to toxin. (Supported by USPHS grant MH-17691.)

192.23 INDUCIBILITY OF MYOTONIA IN NEONATAL RATS. J. W. Gerst*, F. C. Garb* and R. A. Brumback* (SPON: W. Beatty). Zool. Dept., North Dakota St. Univ. and Neurol. Ser., V. A. Med. Ctr., Fargo, ND 58102.

Anthracene-9-carboxylic acid (9-AC, 9-Anthroic acid) is a potent inhibitor of membrane chloride conductance (G_{Cl}) and induces transient myotonic behavior in adult rat muscle. 9-AC (60 mg/kg, ip) was injected into 5-7, 10-12 and 28-30 day old Wistar/Furth rats. Electromyography (EMG) was performed on anesthetized rats 1-2 hours after 9-AC dosing. EMG of hind leg muscle was compared in uninjected, solvent injected and 9-AC injected neonates. The grading scheme was: 0 -- insertional irritability, individual motor unit activity clearly discernible (normal neonatal response); 1 -- insertional irritability with bursts of high frequency discharges obscuring individual motor unit activity; 2 -- high frequency discharges and short, waxing-and-waning (myotonic) discharges during needle insertion and/or needle movement; 3 -- sustained myotonic discharges during needle insertion and/or needle movement; 4 -- persistent myotonic discharges, either spontaneous or easily provoked by needle insertion, needle movement or muscle percussion. Neonates, 5-7 days old, EMG grade 0.9±0.14(6) (±SEM), and 10-12 days old, EMG grade 1.3±0.21(6), failed to demonstrate 9-AC induced, EMG myotonia. Furthermore, the neonates exhibited no clinical signs of myotonia. Young rats, 28-30 days old, developed EMG myotonia, EMG grade 3.0±0.06(6), and all showed obvious muscle stiffness within 3-5 minutes after 9-AC dosing.

The failure of neonates to develop myotonia in response to 9-AC injection might indicate that 9-AC does not block G_{Cl} in neonatal muscle. However, an alternative explanation is that the incomplete T-tubular system development in neonatal rat muscle may not support an adequate accumulation of potassium ions necessary for the generation of depolarizing after-potentials and myotonic spike-discharges. The threshold potential for the activation of sodium conductance in neonatal muscle may be more positive than in adult muscle; therefore, larger depolarizing after-potentials would be required to elicit the myotonic discharges in neonatal muscle (Bryant, S. H., Ann. N. Y. Acad. Sci., 317:314, 1979). Intracellular microelectrode studies on 5-12 day old neonate rats are in progress.

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193.1 THE IONIC BASIS OF RESTING, RECEPTOR AND ACTION POTENTIALS IN THE CILIATE, STENTOR COERULEUS. D.C. Wood, Psychobiology Program, University of Pittsburgh, Pittsburgh, PA 15260.

The ciliate protozoan, Stentor coeruleus, produces an all-or-none contraction in response to suprathreshold mechanical and electrical stimuli. Mechanical stimuli elicit graded receptor potentials. Mechanoreceptor potentials of sufficient magnitude trigger all-or-none action potentials and contractions. The ionic basis of these mechanoreceptor and action potentials was investigated in these studies by varying the external concentration of K^+, Ca^{++}, Na^+, Mg^{++} and Cl^-.

The resting potential is a curvilinear function of $[K^+]_0$ in the range of 0.1-8mM. Dependence on extracellular $[Ca^{++}]$ was also observed over the concentration range of 0.04-10mM. Changes in extracellular $[Mg^{++}]$, $[Na^+]$ and $[Cl^-]$ produced little effect on the resting potential. These data are well fit by an equation derived with the same assumptions as the Goldman equation but in which I_{Na^+} is replaced by $I_{Ca^{++}}$ and allowance is made for the double valence of Ca^{++}. The best fit parameters in this equation are: $[K^+]_i$ = 13.1mM, $[Ca^{++}]_i$ = 0mM, $[Cl^-]_i$ = 9.9mM, P_{Ca}/P_K = 0.066 and P_{Cl}/P_K = 0.072. These intracellular concentrations are in good agreement with the values of 12.5 and 9.4mM found for $[K^+]_i$ and $[Cl^-]_i$ respectively by flame photometry. At low (<2mM) $[K^+]_0$ P_K is reduced significantly below the values observed at higher $[K^+]_0$.

As in Paramecium the peak of the action potential is most affected by variations of $[Ca^{++}]_0$ but the slope of this relation is only 12.5mV/10 x change in $[Ca^{++}]_0$. Increasing $[Cl^-]_0$ concentration makes the peak of the action potential less positive and suggest that Cl^- as well as Ca^{++} carries current during the generation of the action potential. The variations of the action potential peak are well described by the Goldman model if the previous $[K^+]_i$, $[Ca^{++}]_i$ and $[Cl^-]_i$ concentrations are assumed and P_{Ca}/P_K = 7.9 and P_{Cl}/P_K = 1.0 at the peak of the action potential.

Mechanical stimuli elicit a brief (<40m sec) inward current which was studied by voltage clamping. The reversal potential of this mechanoreceptor current is most dependent on $[Ca^{++}]_0$ but the slope of this relation is again less than 1/2 that predicted by the Nernst relation. As with the action potential increasing $[Cl^-]_0$ makes this reversal potential less positive. The data are described by the Goldman model if P_{Ca}/P_K = 18.1 and P_{Cl}/P_K = 1.74.

Both the current which generates the action potential and that which generates the receptor potential are carried largely by Ca^{++} with a lesser involvement of Cl^-. Both currents are largely blocked by 2mM Co Cl_2 and 0.1mM La Cl_3. However the channels for these 2 currents are different since a) the voltage dependence of the 2 sets of channels is different and b) d-tubocurarine blocks mechanoreceptor currents but does not affect action potentials.

193.2 THE CELLULAR ORGANIZATION OF SEGMENTAL GANGLIA. Kate Skinner. Dept. of Zoology, University of California, Davis, CA 95616.

The arrangement of neurons into tracts and synaptic neuropils is being investigated in the fourth abdominal ganglion of the crayfish, Procambarus clarkii. The ganglion consists of a ventral layer of neuronal somas, several cells thick, and a ganglion "core" of neuropils. The neuropils can be distinguished from one another on the basis of density of stain and size of the component fibers. They form landmark structures within the ganglion which can be repeatedly recognized from animal to animal.

The largest volume of neuropil is occupied by the four layers of longitudinal through-tracts which alternate with the three layers of cross-ganglion commissural tracts. The two dorsal layers of through-tracts carry the largest fibers, including the giant fibers, and have the largest area in cross-section. The more ventral tracts carry smaller fibers and are more variable in route through the ganglion. The most dorsal commissure is likewise the largest, capping the entire neuropil, while the more ventral ones are divided into anterior and posterior branches and are more variable in route. These through-tracts and commissural pathways can be used to describe the routes through the ganglion of the major identified neurons.

In the ventral half of the ganglion core is a large, U-shaped synaptic neuropil arranged horizontally with the arms of the U directed anteriorly and slightly ventrally. This synaptic neuropil is a dense and distinctly dark-staining structure which can be seen in section and in the living, trans-illuminated ganglion. It is composed of small (10-30 micron), even-staining clumps which appear at the electron microscope level to be synaptic glomeruli. There are substructures within the U-shaped neuropil at three locations, which consist of horizontal rings or wreaths of glomeruli. One of these is at the midline at the level of the second peripheral roots and the other two are in the "arms" of the U at the level of the first peripheral roots. The synaptic neuropil at the lateral edges of the ganglion, between the first and second roots, appears lighter in the light microscope and is composed of larger axonal profiles (2-10 microns), which frequently contain dense core vesicles.

For light microscopy, ganglia were fixed in glutaraldehyde and osmium tetroxide, block-stained in ethyl gallate solution, and embedded in soft plastic. For electron microscopy, ethyl gallate was omitted and the selected thick sections were re-embedded for thin sectioning of specific regions of neuropil.

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193.3 THE MEDIAL GIANT FIBER NEURON: MORPHOLOGY, ANATOMY, PHYSIOLOGY T. Viancour, Rice U., Biology, Houston, TX 77001. Abbreviations: Al=Antennule, A2=Antennal, c.=contralateral, i.=ipsilateral, MGFN=Medial Giant Fiber Neuron, np=neuropile.

Results from intracellular injection of Lucifer-Yellow dye: The MGFN has three major 2nd order branches terminating within the cerebral ganglion of crayfish. The parolfactory branch arborizes to the 5th order within the i. parolfactory np. The Al branch courses ventrad through the c. parolfactory np to the entrance of the Al nerve; it sends off a few 'insignificant' 3rd order branches, and its distal termination is illdefined and amorphous, appearing to invade a bundle of large axons from the Al nerve. The A2 branch terminates along the rostral margin of the A2 lobe. Three 3rd order branches of the initial segment terminate within the i. parolfactory lobe, and another terminates in the i. optic np. Other 3rd and higher order branches are variable in position and occurrence from preparation to preparation. The MGFN dye-couples, with a high probability, to its contralateral homolog and, with a lower probability, to one ipsilateral and one contralateral optic tract fiber. Dye coupled neurons contact the MGFN as it decussates between the initial segment and parolfactory neurites ipsilaterally and the Al, A2 and axon neurites contralaterally. Adaxonal sheath glia also dye-couple to the MGFN axon permitting an estimation of 19,000 such glia per MGFN axon.

Al and A2 motor neurons backfilled with cobalt terminate in close apposition to the MGFN axon on the dorsal surface of the circumesophogeal connective at the base of the brain.

Intracellular recordings made at the decussation: Depolarizing PSPs result from Al, A2, tegumental and visual stimulation ipsilaterally and contralaterally. Antidromic spikes invade the decussation decrementally, and coupling potentials from spikes evoked in the contralateral MGFN have the same time course as the antidromic decremented spike. The coupling potential amplitude is 0.6 times that of the decremented antidromic spike.

The MGFN axon has a length constant of 4.4 mm, a time constant of 2.3 ms, input impedance of 3.7×10^4 ohms, membrane resistance of 2×10^3 ohm·cm^2, core resistance of 52 ohm·cm and a membrane capacitance of 1.1 μf/cm^2. Similar determinations are being attempted for the passive neurites, and presentation of an electro-morphological model of the MGFN is anticipated.

[Portions of this work were conducted in collaboration with R. Glantz, Rice Univ., and G. Bittner and M. Ballinger, Univ. of Texas, Austin]

193.4 EFFECTS OF ENVIRONMENTAL AND ACCLIMATION TEMPERATURE ON LONG TERM FACILITATION IN TWO CRAYFISH SPECIES. J. Roger Jacobs and Harold L. Atwood. Dept. Zoology, University of Toronto, Ontario, Canada, M5S 1A1.

Previous studies on long term facilitation (LTF) at the crustacean neuromuscular synapse have done much to clarify the presynaptic processes involved, and the conditions favouring LTF development, but little attention has been directed towards determining the ability of the intact animal to generate and exploit LTF. In the experiments reported here, it was established that LTF can develop in normal crayfish, and that LTF can be exploited to compensate for changes in neuromuscular performance brought on by sudden temperature changes.

Experiments were performed by stimulating the common excitor motoneuron innervating the 'opener' and 'stretcher' muscles in vivo and in vitro, and recording changes in the amplitude of the myogram of the stretcher muscle. The frequency of spontaneous firing in this motoneuron is suppressed at temperatures colder than that at which the crayfish is acclimated. These low temperatures do not greatly affect short term facilitation, but LTF develops to higher levels. Depression interferes only at extremely cold temperatures, or during long periods of stimulation.

After acclimation to colder temperatures, the temperature range over which LTF is produced is lowered. This adjustment is more pronounced in a northern crayfish species (Orconectes virilis) which must be active during cold periods, than in a more southern species (Procambarus clarkii), which is under less compulsion to be active during cold spells.

Tension development was also studied during periods of LTF growth. Tonically active muscles such as the stretcher appear to be capable of developing increasing tension during long periods of activation, but the degree of facilitation in tension growth during decay of LTF is highly variable. Most especially at cold temperatures, postsynaptic changes have greater influence than LTF on tension development.

193.5 IONIC DEPENDENCE OF RAPID PICROTOXIN-SENSITIVE AND SLOW PICROTOXIN-RESISTANT INHIBITORY POSTSYNAPTIC POTENTIALS IN THE LOBSTER STOMATOGASTRIC GANGLION. J. Eisen and E. Marder. Biology Dept., Brandeis University, Waltham, MA. 02254.

Inhibitory Post-Synaptic Potentials (IPSPs) are usually due to increases in Cl^- or K^+ conductance. In many preparations rapid IPSPs are mediated by Cl^-, and slow IPSPs by K^+. Both glutamate (glu) and acetylcholine (ACh) elicit increases in K^+ conductance on stomatogastric ganglion (STG) neurons, and are likely to be STG inhibitory transmitters. The 2 PD neurons and the AB neuron of the STG are electrically coupled, show simultaneous membrane potential oscillations, and make similar chemical synaptic connections in the STG. Combined IPSPs from the PD and AB cells were recorded in LP, VD, or PY cells impaled with 2 microelectrodes, while simultaneously monitering both AB and PD membrane potentials intracellularly. This compound IPSP showed rapid rise-time (7-15 msec) IPSPs which corresponded 1-to-1 with AB action potentials, superimposed upon a slow component. Both rapid and slow components of the IPSP recorded in LP, VD and 1 class of PY cells showed clear reversal potentials at 20-25 mV hyperpolarized from rest, a value typical of the reversal potential of K^+ dependent iontophoretic responses in these cells. In $2x[K^+]$ saline, E_{IPSP} was shifted by 15-19 mV, as expected for an increase in K^+ conductance. Extensive hyperpolarization with KAc or KCl electrodes had no measurable effect on E_{IPSP}, although E_{Cl^-} is easily shifted by such manipulations. Thus, it appears that both the fast and slow components of these IPSPs are mediated by an increase in K^+ conductance. 10^{-6} M picrotoxin (PTX) blocks the fast component of these IPSPs, leaving the slow IPSP. PD-AB IPSPs recorded in a second class of PY cells were complex. They showed fast components similar in all respects to the fast IPSPs described above. The slow component, however, was hyperpolarizing at all levels of membrane potential from rest to -120 mV. 10^{-6} M PTX abolished the fast IPSP, leaving the slow IPSP. A portion of the slow IPSP appeared to be sensitive to changes in external $[K^+]$. That portion resistant to changes in external $[K^+]$ may be due to a conductance decrease. These 2 classes of PY cells are likely the PE and PL classes defined by Hartline et. al. (1979 Neurosci. Ab. 5:248). The PD cells make cholinergic neuromuscular junctions, and the PTX-resistant IPSP may be due to PD-released ACh. PTX blocks glu elicited increases in K^+ conductance, and rapid IPSPs correlated with AB action potentials may be due to synaptically liberated glu. Glu is likely to be the neuromuscular transmitter used by the LP neuron. LP-PD and LP-AB IPSPs were similar to the rapid, PTX-sensitive IPSPs discussed above, and thus are likely to be glu-mediated. Support: McKnight Found. Scholars Award and NSF BNS 78-15399.

193.6 EFFECTS OF TEMPERATURE ON FACILITATION AT CRAB NEUROMUSCULAR JUNCTIONS. H.L.Atwood and P.J.Stephens. Dept. of Zoology, Univ. of Toronto, Toronto, Ontario M5S 1A1, Canada.

The stretcher muscle in the crab limb is innervated by a single excitor (E) axon. Excitatory junctional potentials (ejp's) recorded from stretcher fibers have been categorised with respect to amplitude and facilitation. Small ejp's facilitate well at high frequencies whereas large amplitude ejp's show poor facilitation (Atwood and Bittner, J. Neurophysiol. 34, 1971). The present paper reports some effects of temperature on the amplitude and facilitation of ejp's recorded from stretcher muscle fibers of crabs (Pachygrapsus) acclimated to 12°C.

In most stretcher fibers the ejp amplitude decreased as temperature was increased from 8 to 23°C. Facilitation measurements using pairs of pulses applied to the E axon at short (25 ms) intervals revealed a decline in facilitation from 8 to 10°C, a near uniform value up to 16°C, and an increase in facilitation from 17 to 23°C.

Frequency-response curves were constructed by making facilitation measurements using pairs of pulses with intervals between 10 ms and 2 s. In fibers with small ejp's, facilitation was high at short intervals and decayed exponentially with increased time interval. There was a semi-log relationship between facilitation and pulse interval, and two linear components were discerned. The initial component exhibited a more rapid rate of decay than the second. The pulse interval at which the two components intersected decreased with temperature.

In fibers with large amplitude ejp's, pairs of pulses at short intervals revealed that the second ejp was smaller than the first. Extracellular recordings of synaptic potentials showed that this anti-facilitation was due to a decrease in synaptic output. An exponential decay of anti-facilitation was observed with increased time interval. At warm temperatures the anti-facilitation observed at short intervals was followed by facilitation at longer intervals. The degree of facilitation increased with temperature. Temperature increases also produced changes in the rates of facilitation decay and changes in the pulse interval value at which the two components intersected. In some fibers that exhibited poor facilitation at low temperatures, changes in their facilitation properties at high temperatures resulted in their conversion to small ejp, highly facilitating fibers.

This work was supported by grants from the National Research Council of Canada and the Muscular Dystrophy Association of Canada.

193.7 THE EFFECT OF TEMPERATURE ON THE MINIATURE EXCITATORY JUNCTION POTENTIALS AT THE NEUROMUSCULAR JUNCTION OF THE DROSOPHILA MUTANT, SHIBIRE^{ts1}. J. H. Koenig and Kazuo Ikeda, Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

The junction potentials of the dorsal longitudinal flight muscle (DLM) of the Drosophila mutant, shibire^{ts1} (shi), have been shown to progressively diminish as the temperature is raised above 27° C, until they almost completely disappear at 30° C (Ikeda, K. et al, Nature 259: 489-491, 1976). It has also been shown that synaptic vesicles at the nerve terminals of the DLM of this mutant are common at 18° C, but are almost completely absent at 30° C (Ikeda, K. & Saito, K., Neurosci. 5: 1448, 1979). The effect of raising the temperature on the miniature excitatory junction potentials (m.e.j.p.s) in the DLM of shi was observed and compared to that of the wild-type controls. In shi flies, temperatures above 26° C induce centrally originating nervous activity to the DLM. In order to observe the effect of temperature on the m.e.j.p.s independently of the effect of previous nervous activity, the posterior dorsal mesothoracic nerve (PDMN) which innervates the DLM was cut in some flies.

In both the wild-type flies and the shi flies with the PDMN cut, the m.e.j.p.s, which ranged in amplitude from about 2 mV to under 100 μV, increased drastically in frequency as the temperature was raised from 18 to 30° C. No qualitative difference in the frequency or amplitude of the m.e.j.p.s was observed between the wild-type and shi flies at 30° C, the temperature at which the evoked junction potential and the synaptic vesicles have almost completely disappeared in shi. However, in shi flies in which nervous activity to the DLM occurred as the temperature was raised (PDMN uncut), the frequency of the m.e.j.p.s decreased to almost zero by 30° C. The amplitude of the m.e.j.p.s at 30° C did not appear to be affected.

The results suggest that the occurrence of m.e.j.p.s is not dependent on the presence of synaptic vesicles in the nerve terminal. However, a relationship between the occurrence of the m.e.j.p.s and previous activity in the nerve terminal is demonstrated. Supported by USPHS NIH grant NS-07442.

193.8 IDENTIFIED ISOLATED APLYSIA NEURONS CULTURED IN VITRO. D. Dagan* and I.B. Levitan, Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.

Events occurring on the somatic membrane of Aplysia neurons can be dissociated from synaptic events occurring in the complex neuropile by intracellular recordings from isolated neurons in tissue culture. Utilizing a variety of enzymes and several methods to induce attachment of cells to a growth substrate, the following conditions were found most favorable for culturing: Incubation of ganglia in 1% neutral protease (Strumwasser et al., Neurosc.Abstr.4,207,1978) for 15 hrs at 15°C followed by 3 hrs at 21°C. Ganglia are then desheathed and identified somata are gently separated by mechanical manipulation. The isolated neurons are transferred to either chick plasma (GIBCO) clotted with 300 U/ml thrombin (Hoffmann La Roche), or to a 2% polymerized methyl cellulose solution (DOW). Single neurons and clusters are then cultured at 20°C in an Aplysia salt solution supplemented with vitamins, amino acids, hormones and fetal calf serum. Neurons extend long processes either in 3 dimensions in the plasma clot or on the surface of acid washed glass cover slips, within 5 hours of plating. Growth rates studied by time lapse cinematography range from 4 to 40 μ/hr. Growth of individual branches of one neuron are independent, and fast retraction (70 μ/hr) of single processes observed. Somata are seen to pivot around an attachment point of a growth cone in cases of monopolar cells, indicating that the somata themselves are not attached.

Intracellular recordings utilizing voltage clamp techniques show normal input resistances and spiking activity in many of the neurons cultured for up to three weeks. We are studying the conditions necessary for synapse formation in vitro utilizing these cultures.

193.9 NEURONAL CIRCADIAN OSCILLATOR: RHYTHM IN PROTEIN SYNTHESIS IN THE EYE OF APLYSIA. D.P. Lotshaw* and J.W. Jacklet. Dept. of Biol. Sci. and Neurobiology Res. Ctr., SUNY Albany, Albany, N.Y. 12222.

The isolated eye of Aplysia exhibits a circadian oscillation in the frequency of spontaneous compound action potentials (CAP) recorded from the optic nerve. Under free-running conditions the rapid increase in CAP frequency anticipates the subjective dawn (circadian time 0 or CT 0) and later declines to a low frequency corresponding to the subjective night. Pulse applications of protein synthesis inhibitors, puromycin and anisomycin, induce phase-dependent phase shifts in this rhythm (Rothman and Strumwasser, J. Gen. Physiol., 68:359-384; Jacklet, Science, 198:69-71), and inactive derivatives of anisomycin do not (Jacklet, J. Expt. Biol., 83:3342) suggesting that protein synthesis may be involved in the underlying circadian clock. When maintained in culture medium under free-running conditions, the isolated eye exhibits a circadian oscillation in total protein synthesis as measured by ^{3}H-leucine incorporation into TCA insoluble material during 1.5 hr pulses applied at various times during the CAP rhythm. Minimal incorporation occurs following the peak CAP rate and remains constant until the CAP rate sharply declines. Incorporation then increases throughout the subjective night reaching a maximum during the rising phase of CAP activity and then declines rapidly to the minimum level. Comparison of the phase response curve (PRC) of anisomycin with the synthesis levels indicates that inhibition of protein synthesis during the decrease in protein synthesis results in phase advances. Inhibition of synthesis during the subjective night produces increasingly large delays corresponding to the increasing rate of synthesis. Repeating the PRC to 6 hr pulses of puromycin gave results similar to those of Rothman and Strumwasser. The transition from delays to advances coincides with that produced by anisomycin, occurring at the time which the CAP frequency increases to half its maximum (CT 0). The maximum delay of 8 to 9 hrs is produced when a pulse is applied shortly before the transition from delays to advances. Advances of 4 to 5 hrs were produced by pulses applied after CT 0. While the transition point in the PRC's of puromycin and anisomycin are very similar, anisomycin produces larger delays earlier in the subjective night than puromycin, 8 hr and 1 hr respectively, when applied around CT 12. This difference may be related to a more efficient inhibition of protein synthesis produced by the concentration of anisomycin used as compared to puromycin. These data suggest that the circadian clock in the eye of Aplysia has a continual requirement for protein synthesis except for the period between maximum CAP frequency and the transition to a rapidly decreasing CAP frequency. Supported by NSF BNS 11154.

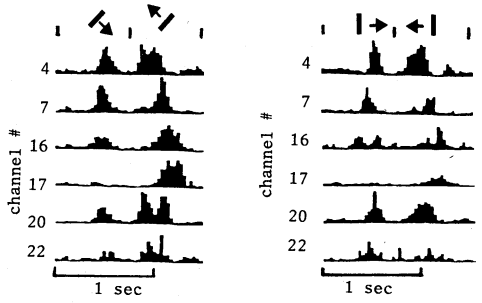
193.10 EFFECTS OF BACLOFEN AT SYNAPSE RC1-R15 OF APLYSIA CALIFORNICA ON SYNAPTIC DEPRESSION, FREQUENCY FACILITATION AND PTP. J.P. Tremblay and É. Philippe*. Lab. de neurobiologie, Dépt. d'Anatomie, U. Laval, G1K 7P4.

Baclofen [-(4-chlorophenyl)- - aminobutyric acid or lioresal] is currently used to alleviate spasticity. Because of its structural ressemblance to GABA it was initially postulated that baclofen produces its effects by activating the GABA receptor (Pierau et al., Exper. Neurol., 48, 343, 1976). More recently Curtis et al. (Brain Res., 70, 493, 1974) and Davis and Watkins (Brain Res., 70, 501, 1974) have suggested that the effect of baclofen might derive from an interaction with catecholamine receptors. Cholinergic EPSPs can be recorded in cell R15 of Aplysia californica after minimal stimulation of the right pleurovisceral connective. The synapse producing this EPSP has been called RC1-R15. Since GABA, serotonin and dopamine affect the synaptic transmission of RC1-R15, we expected that baclofen would also modify it and that its mechanism of action could be determined by using blockers of GABA, dopamine and serotonin. Perfusion of baclofen (3×10^{-5}M or 5×10^{-5}M) reduces the size of all EPSPs produced by a train of 100 stimuli at 1.5Hz. Baclofen also reduces the amount of synaptic depression observed during the first few EPSPs of that train, the depression being quantified by the ratio of the first EPSP of the train to the second EPSP. Baclofen increases the amount of facilitation observed during such a train, facilitation being quantified by the ratio of the hundreth EPSP to the first EPSP. Finally baclofen reduces the posttetanic potentiation observed for an EPSP produced 30 sec after the end of a train. The PTP is quantified by the ratio of the size that EPSP to the size of the hundreth EPSP of the train. These effects are similar to those produced by GABA, serotonin and dopamine on this model. The effects of baclofen are not antagonized by bicuculline and picrotoxin (two GABA antagonists). However its effects are partially blocked by SQ-10,631 (antagonist of serotonin) and butaclamol (antagonist of dopamine). To establish if baclofen produces its action by liberating the amines from some nerve ending or by direct interaction with aminergic receptors located on the presynaptic terminal of synapse RC1-R15, Aplysia were treated with reserpine to deplete the aminergic pools. Following a two days treatment with reserpine, baclofen failed to produce any effect suggesting that it acts by liberating dopamine and serotonin. However in these preparations treated with reserpine, GABA still produce its normal effects. This result suggest that baclofen produces its effects by liberating amines from synaptic terminals located near RC1-R15 synapse. These results also indicate that the mecanisms of action of baclofen and of GABA on the synaptic transmission of RC1-R15 are different.

194.1 PARALLEL UNIT RECORDING IN VISUAL CORTEX. Michael Kuperstein
Dept. Psychol., M.I.T., Cambridge, Ma. 02139

With the development of a new multichannel microelectrode, simultaneous unit recordings can be made from areas of the brain accessible within 2 mm of the surface. The electrode named PRONG (parallel recording of neural groups)has 24 independent recording sites. They are spaced at 85 um intervals along the edge of the PRONG's needle shaped tip. The fixed sites are arranged in one of two ways for a given electrode: with either all sites along one edge spanning 2 mm or a 2 X 12 array along both edges spanning 135 um X 1 mm. The PRONG is fabricated in 4 patterned layers using photolithography. A 17 um thick molybdenum foil is the substrate and layers of photoresist, gold and photoresist are applied and patterned in succession. The gold layer is patterned into recording sites, leads and connector pads, while the photoresist insulates the gold layer from the substrate and the brain leaving the recording sites exposed. The area of each site is 120 um^2 and each site is plated with platinum black. For implantation the PRONG is clamped into a connector and fixed to a microdrive. The input capacitance is 20-30 pf, the shunt capacitance is 3-4 pf and the cross channel capacitance is .6 pf.

Unit recordings with the PRONG in cat visual cortex show two features: single channels pick up neural activity similar to single microelectrodes and visually evoked responses recorded by single channels show the classical receptive field specificities. Below are examples of simultaneous histograms from 6 channels correlated to moving light bars of different orientations. These results suggest that the PRONG is a practical experimental tool which might be productive in analyzing local circuitry in visual cortex and other brain areas. (Supported by NSF grant #BNS 76-82543 and NIH grants 5 R01 EY00676 and #1 T31 GM07484.)



194.2 THE NUMBER OF NEURONS, GLIAL CELLS AND SYNAPSES IN THE STRIATE CORTEX OF MACAQUE MONKEY: A STEREOLOGICAL ANALYSIS. J. O'Kusky* and M. Colonnier. Lab. de neurobiologie, Pavillon Notre-Dame, 2075 av. de Vitré. Québec. Qué. G1J 5B3.

The diameter of the nuclei of neurons and glial cells was measured in individual laminae of macaque visual cortex in toluidine blue, 1.0 um sections using a semi-automatic image analyzer. Their numerical density was determined by using the method of size-frequency distribution (Weibel '69, Int. Rev. Cytol: 26,266). Under each mm^2 of surface there is an average of about 330,000 neurons, each lamina having its own characteristic density confirming the visual impression which underlies the classical description of laminae and sublaminae. The number of glial cells within the same volume of tissue is of the order of 119,000, giving a glia-neuron ratio of 0.36. This ratio is considerably less than that found in whale cortex and thus supports the hypothesis that the glia-neuron index does not increase with phylogenetic development but rather with brain size (Hawkins and Olszewski '57, Science: 126,76). The surface of the striate cortex was reconstructed from frozen Nissl sections cut in the frontal plane. The sum for both hemispheres is about 1200 mm^2. There are thus about 4.0×10^8 neurons and 1.4×10^8 glial cells in the total striate cortex.

The numerical density of synapses, determined by measuring the length of synaptic membranes and treating them according to the formula for disks as in Mayhew '79 (J. Anat: 129,95), is estimated at approximately 6.0×10^8 under each mm^2 of cortical surface. There are thus about 7.2×10^{11} synapses in the sum of right and left striate areas. From these combined figures, a monkey striate neuron receives on average 1800 synapses.

Our data on individual laminae show that there are 9.3×10^7 neurons and 1.0×10^{11} synapses in layer IVC, the main recipient of the geniculate input. From the literature, the number of thalamocortical terminals in that lamina is most reasonably estimated at 10 to 25% of the total number of synapses, giving $1.0 - 2.5 \times 10^{10}$ synapses. If they contacted only neurons in that lamina, each neuron would receive on average from 100 to 270 geniculocortical synapses. Taking into account the apical dendrites of underlying pyramidal cells and the dendrites of all cells in the immediately adjoining sublaminae, our figures show that the terminals might contact at most 2.0×10^8 cells for an average of 50 to 125 contacts per neuron.
Supported by grant MA-3735 of the MRC of Canada.

194.3 VISUALLY RESPONSIVE UNITS IN STRIATE CORTEX OF BLIND MONKEYS. Richard K. Nakamura, Robert Desimone*, Stanley J. Schein, and Mortimer Mishkin, NIMH & NEI, Bethesda, MD 20205.

We have previously reported that chronic blindness can be produced in monkeys by a large cortical removal that preserves all areas known to be necessary for visual discrimination learning (Neurosci. Abs. 5:800, 1979). The large lesion spared the visual cortex (striate, prestriate, and inferior temporal) but included all other cortical areas. The lesion was placed in only one hemisphere, the other hemisphere having been left intact but visually deafferented by optic tract section and forebrain commissurotomy.

To investigate the basis of the blindness we have begun single-unit recordings in the blind animals. Striate cortex was studied in five monkeys prepared as above to produce chronic blindness, except that the optic tract was left intact in two of the animals to provide control hemispheres. The recordings were made while the monkeys were anesthetized with nitrous oxide and immobilized with pancuronium bromide. Implanted wells and head holders were used to minimize possible sources of pressure or pain.

We isolated 244 units from the hemispheres made 'blind' by the ablation, and 83 units from the 'seeing' intact hemispheres. Of the units from the blind hemispheres, 53% were moderately or highly responsive visually as contrasted with 95% of those from the seeing hemispheres. The loss of visual responsiveness was related to a consistent histological finding of inadvertant damage to optic radiations representing primarily the lower visual field, in that there was a strong positive correlation between a cell's visual responsiveness and the vertical position of its receptive field. For example, at more than 5^o below the horizontal meridian, only 12% of the units were moderately or highly responsive, whereas at more than 5^o above the horizontal meridian, 71% of the units were moderately or highly responsive.

There was no difference between the blind and seeing hemispheres either in the proportion of responsive cells with oriented receptive fields (69% and 68%, respectively) or in the proportion of oriented cells with complex-type receptive fields (71% and 63%, respectively).

We conclude that 1) although many units respond poorly or not at all in the striate cortex of the blind hemispheres, there appear to be enough moderately or highly responsive units to support vision, particularly in the upper fields; 2) moreover, visual information is not only reaching striate cortex but is being processed there as indicated by the normal proportions of cells with oriented and with complex-type receptive fields; 3) the loss of visual processing or disconnection of the efferent visual pathway responsible for the blindness must be occurring at some point beyond striate cortex.

194.4 ROLE OF VESTIBULAR INPUT IN VISUAL CELL SPECIFICITY. S. Reinis, R.H. Lahue* Jr., J. P. Landolt and K. E. Money* Dept. Psychol., Univ. of Waterloo, Waterloo, Ont. N2L 3G1 and DCIEM, Downsview, Ont., Canada.

Receptive field characteristics of complex cells in area 18 of the visual cortex were examined in cats as a function of head position. Properties of cells after 30^o head tilt to the right or to the left were compared with those obtained in a horizontal head position. Of 25 cells, almost all exhibited changes in receptive field size and two thirds exhibited changes in relative receptive field position in the visual field after head tilt. In one third of the cells changes in the orientation of the major axis of the receptive field corresponded to the degree of head tilt, in the remaining cells the change in orientation either exceeded or fell short of the degree of head tilt. Directional preferences for stimulus movement also altered in some cells following head tilt. Spontaneous firing of the studied cells depended on the position of the head as well. Intravenous injection of deuterium oxide changed the receptive field size in all studied cells, and increased substantially the rate of their spontaneous firing. In contrast to the previous literature, a rather complex visual-vestibular interaction was demonstrated.

194.5 NEURONAL PROPERTIES IN AREA 19 OF THE CAT. G.A. Orban, J. Duysens and H.W. van der Glas*. Laboratorium voor Neuro- en Psychofysiologie, Katholieke Universiteit te Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

Single unit recordings were made in area 19 of anesthetized and paralyzed cats. Shifts in receptive field (RF) position and histological reconstruction allowed us to attribute neurons to area 19. Eccentricities between 5 and 40° were explored. RF organization and orientation selectivity were assessed with hand-held stimuli. Direction selectivity and velocity sensitivity were evaluated using computer-driven stimuli according to the multihistogram technique. 75% of the area 19 neurons were end stopped to various degrees. About two-thirds of the cells could be fitted in the A, B, C, S scheme (Kennedy, H. and Orban, G.A., J. Physiol., 298:24-25P, 1980) since they responded to light slits, bars and edges of optimal length. The remaining cells responded only to bright or dark rectangles (patterns which are wider than their length).

The velocity-response curves of area 19 neurons were chiefly of two types : neurons responding over a wide range of velocities, including slow ones (broad band type : 50%) and neurons responding better to fast movements (high pass type : 30%). The response level of the neurons was low. Most of the cells were not direction selective or only direction asymmetric.

Compared to area 18 neurons of similar eccentricities, areas 19 neurons show more end stopping, less direction selectivity, lower response levels and stronger responses to slowly moving stimuli. These differences marked a clear functional separation between areas 18 and 19 in penetrations crossing the 18-19 border.

194.6 SOME CONNECTIONS OF STRIATE CORTEX (AREA 17) IN THE GREY SQUIRREL. C. G. Cusick*, T. P. Pons*, and J. H. Kaas. Depts. of Psychology and Anatomy, Vanderbilt Univ., Nashville, TN 37240

The squirrel is a good experimental animal for studies of visual connections because its visual system is well-developed with architectonically distinct subdivisions at both cortical and subcortical levels. Efferent and afferent connections were investigated by injecting horseradish peroxidase (HRP) or HRP conjugated to wheat germ agglutinin (WGA-HRP) into striate cortex in six grey squirrels. Standard histochemical procedures (diaminobenzidine, benzidine dihydrochloride, and tetramethyl benzidine reactions) were used to reveal retrogradely labeled cell bodies and anterogradely labeled terminal fields of axons. In some of the cases, connections were also revealed with autoradiographic methods after combined injections of ^{3}H-proline and HRP. Subcortical targets of Area 17 projections included the dorsal lateral geniculate nucleus, the rostro-medial pulvinar, the ventral lateral geniculate nucleus, the reticular nucleus of the thalamus, the pretectum, pontine nuclei, and the superficial layers of the superior colliculus. The connections to at least the dorsal lateral geniculate nucleus and the superior colliculus were homotopic. The locations and concentrations of labeled cells in the thalamus indicated that most neurons in the dorsal lateral geniculate nucleus, and a few of the cells in the pulvinar project to striate cortex. Ipsilateral cortical connections included homotopic and reciprocal connections with Area 18 (V-II). Zones of reciprocal connections were also observed in cortex lateral to Area 18. Finally, in some of the cases with injection sites confined to Area 17, labeled cells were also found in cortex anteromedial to Area 17 in "limbic" cortex. In all cortical regions projecting to Area 17, labeled cells were found in both supragranular and infragranular layers. Efferent projections of Area 17 terminated in both infragranular and supragranular layers. Overall, the connections of striate cortex of the squirrel are similar to the patterns observed in more commonly studied mammals. However, species differences do appear to exist. Area 17 projections in monkeys, for example, include the superior pulvinar and two divisions of the inferior pulvinar. Projections in squirrels were observed in only one division of the pulvinar complex.

Supported by NIH Grants EY-02686 and EY-05380.

194.7 CONNECTIONS OF THE DORSOLATERAL VISUAL AREA (DL) OF EXTRASTRIATE VISUAL CORTEX OF THE OWL MONKEY (AOTUS TRIVIRGATUS). R. E. Weller* and J. H. Kaas, Depts. of Psychology and Anatomy, Vanderbilt Univ., Nashville, TN 37240

Electrophysiological mapping methods have been used to subdivide extrastriate cortex of owl monkeys into a number of separate representations of the visual hemifield (Allman and Kaas, Sci., 191: 572, 1976). These include the second visual area, V II, the Dorsomedial Visual Area (DM), the Medial Visual Area (M), the Dorsointermediate Visual Area (DI), the Middle Temporal Visual Area (MT), and the Dorsolateral Visual Area (DL). To date, connections of many of the identified extrastriate visual areas have been described. A puzzle has been that none of these areas provide input to the large region of visually responsive inferior temporal cortex (IT), while in macaque monkeys, part of extrastriate cortex has been found to project to IT. Our present ongoing investigations of the connections of DL in the owl monkey lead to the conclusion that the major source of visual input to the IT region is DL. In these studies horseradish peroxidase (HRP) or HRP conjugated wheat germ agglutinin, and/or ^{3}H-proline was injected into DL of one hemisphere of seven owl monkeys. Since DL is not architectonically distinct, the injection sites were defined by location relative to the clear architectonic borders of Areas 17 and MT, and the estimated width of Area 18. All injection sites were judged to be in DL, but some involvement of Areas 18 and MT occurred in all but two cases. The results indicate a major projection from the region of DL to IT. This projection is not the result of involvement of Areas 18 or MT, since they do not project to IT. Other connections of DL are less certain because they are similar to those of adjoining visual areas, but injections of ^{3}H-proline judged to be confined to DL in two owl monkeys produced label in ipsilateral MT, V II, and the frontal lobe. Callosal connections of DL were with DL and IT cortex. The projection of DL to IT cortex may account for a major feature of the responsiveness of neurons in IT, since most neurons in both visual areas have receptive fields including central vision.

Supported by NEI Grants EY-02686 and EY-07007.

194.8 AREAL BOUNDARIES AND TOPOGRAPHIC ORGANIZATION OF THE VENTRAL POSTERIOR AREA (VP) OF THE MACAQUE MONKEY. W. T. Newsome, J. H. R. Maunsell and D. C. Van Essen. Div of Biology, California Institute of Technology, Pasadena, CA, 91125

In a previous report (Van Essen, et al., Neurosci. Abst.,1979) a marked asymmetry was found in the projections of striate cortex in the macaque monkey. Whereas dorsal striate cortex projects to three cortical targets - V2, V3 and the Middle Temporal area (MT) - ventral striate cortex projects only to V2 and MT. We have studied the cortex adjacent to the ventral half of V2 and have found that it contains a well defined visual area which we have called the Ventral Posterior area (VP) because of its apparent homology with VP in New World primates (Newsome and Allman, J. Comp. Neurol., in press). VP has a long, narrow shape (25-30 mm by 2-3 mm) and contains a topographic representation of only the upper half of the visual field. Electrophysiological mapping experiments have shown that VP shares a common representation of the horizontal meridian with V2 along its posterior boundary, whereas its anterior boundary is formed by a representation of the superior vertical meridian. Thus the representation of the upper visual quadrant in VP forms a mirror image to that of ventral V2, much as the representation of the lower visual quadrant in V3 forms a mirror image to that of dorsal V2. However, because of the difference in connections with V1, and also a difference in myeloarchitecture, it seems most reasonable to regard VP as a visual area distinct from V3. The representation of the upper visual quadrant in VP emphasizes the center of gaze to about the same degree as is found for V1 and V2. A narrow band of callosal inputs coincides precisely with the vertical meridian representation along the anterior boundary of VP and thus serves as an effective guide in locating VP. ^{3}H-proline injections into ventral V2 have shown that VP can be independently defined by a topographically organized projection which it receives from V2. Occasionally V2 injections give rise to multiple sites of transported label in VP, suggesting that the topographic organization of VP is not always simple. Focal HRP injections in V2 demonstrate a reciprocal projection from VP to V2.

194.9 THE SPATIAL ORGANIZATION OF CONNECTIONS BETWEEN V1 AND V2 IN THE MACAQUE: PATCHY AND NON-PATCHY PROJECTIONS. J. H. R. Maunsell, W. T. Newsome and D. C. Van Essen. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Several recent reports have demonstrated an unexpected complexity in the organization of projections from V1 to V2 in primates, in that discrete injections of labeled amino acids in V1 often reveal a patchy pattern of terminations in V2. We have analyzed the interconnections of V1 and V2 in the macaque in greater detail using ^{3}H-proline as an anterograde tracer and horseradish peroxidase (HRP) as both an anterograde and retrograde tracer. Projections from V1 to V2 originate from cells in layers II, IVb, and, to a lesser extent, III and IVa; they terminate in all layers of V2, but predominantly in layer IV. Projections from V2 to V1 originate from cells in layers II, III, V and VI and terminate mainly in layers I, II, IVb, and V.

Focal tracer injections in V1 (1-2 mm in extent for proline, 0.1-0.3 mm in extent for HRP) resulted in anterograde labeling over a variable extent in V2, ranging from 2-4 mm in width, and 3-8 mm in length. In most cases (10 of 12) the projection was distinctly patchy, with a center-center spacing between patches of 1-3 mm. Cells in V2 labeled by retrograde transport were largely coextensive with the region of anterograde projections, but the two distributions were not identical.

Markedly different results were obtained following HRP injections in V2. In all 7 cases the projections from V1 originated in a roughly circular zone 3-4 mm in diameter, with most cells situated in a central core 1.5-2 mm across. Within this central region cells were usually distributed relatively uniformly, but in one case the pattern was clearly patchy, with a center-center spacing of 0.75-1 mm between patches. The distributions of anterograde and retrograde labeling were very similar. These observations are consistent with the notion that V2 contains a "repetitive" representation in which most points in the visual field are represented at multiple, distinct loci within V2.

The internal connections of V1 and V2 revealed by HRP injections differ markedly in their spatial and laminar distribution. In V1 layers II and III are densely labeled, but only within 1-3 mm of the injection site, deeper layers are labeled more sparsely but over a greater extent (up to 10 mm in layer VI). In V2 the interconnections are more irregular and extensive, with distinct patches of anterograde and retrograde labeling up to 5 mm from the injection site.

194.10 SPECTRAL PROPERTIES OF CELLS IN THE PRESTRIATE CORTEX OF MONKEY. S.J. Schein, R.T. Marrocco and F.M. de Monasterio*. Clinical Branch, National Eye Institute, NIH, Bethesda, Maryland 20205.

Recent studies of monkey prestriate cortex have reported an abundance of both color-opponent and color-biased cells in an area termed V4. The mechanisms underlying color-opponency and biasing in this broadly defined area are not well understood. We report here preliminary findings on the spectral properties and the distribution of cell types encountered in this area.

Extracellular recordings were made from anesthetized (N_2O) and paralyzed rhesus and cynomolgus monkeys. We made horizontal penetrations into the anterior bank of the lunate sulcus, the posterior bank of the superior temporal sulcus, and points in between. Penetrations were confined to the region within 10 mm of the tip of the inferior occipital sulcus. Averaged responses to moving and flashing stimuli were obtained on neutral and chromatic backgrounds photopically matched; the narrow-band test stimuli were equal-quantum or photopically matched.

Of 152 cells, 21% were visually unresponsive. The remaining cells were classed as color-opponent (CO, 3%), color-biased (CB, 19%), and non-color-coded (NC, 57%). Two varieties of CO cells were observed. Whereas both showed excitation to the onset of some wavelengths and opposite excitation to the offset of others, one variety also showed typical spectrally-opponent inhibition. CO cells were of the "red-green" or "magenta-green" opponent type. CB cells were strongly excited by some lights and weakly or not at all by others, including white. Most were red-biased while a few were blue- or magenta-biased; so far, no green-biased cells have been encountered. They do not have concealed color-opponency since an (inhibitory) opponent response was not disclosed by neutral or chromatic backgrounds. NC cells had roughly equal responses to photopically-matched lights on neutral backgrounds, and selective chromatic adaptation did not disclose a color-opponent response. The spectral sensitivity of CO and CB cells was not unusually narrow-band but comparable to that observed at more peripheral centers. All cells had spatially-delimited receptive fields (typically smaller than 4-5° on the side) within the central 10° of the visual field: many were orientation-biased or directionally-biased. CO and CB cells were found adjacent to NC and visually-unresponsive cells of the same penetration. Also, any given penetration produced a mixture of cell types.

Our results do not lend support to the notion that the area termed V4 is primarily devoted to color processing. Rather, they suggest that V4 may be functionally heterogenous, subserving luminance, movement and orientation, as well as color information.

194.11 QUANTIFICATION OF COLOR VISION ABNORMALITIES. D. B. Reingold*, F. C. Chu*, R. D. Gunkel*, and D.G. Cogan*. Nat. Eye Institute, National Institutes of Health, Bethesda, MD 20205.

Recently we described a method for evaluating color vision, based on outlining the portion of a chromaticity diagram perceived as colorless, or neutral. Enlargement of this "neutral area" towards a particular color indicates some difficulty in detecting that color.

Colors of various hues and saturation are presented sequentially to each eye, and the chromagram, or outline of the neutral area, is mapped. We digitize the data, and curve fit the C.I.E. x,y coordinates of each threshold point using a least squares technique. The parameters describing these curves are derived for each subject or group of subjects. We have obtained normal values for these curves, and applied the method to the study of acquired color vision defects.

194.12 COLOR DISCRIMINATION BY GROUND SQUIRRELS: VARIATION WITH STIMULUS SIZE. Earl Kicliter. Lab. of Neurobiology and Dept. of Anatomy, Univ. of Puerto Rico Sch. of Med., San Juan, PR 00901

An aspect of color vision which has received little attention in animal psychophysics is the relation between spectral and spatial properties of a color stimulus. These relation(s) may reveal some organizing principles of both spatial and color vision since it appears that these two stimulus dimensions are processed together cortically. As a first step I have investigated the color discrimination of ground squirrels (Spermophilus tridecemlineatus) as a function of stimulus size. The color discrimination test employed (Kicliter and Loop, Vision Res. 16: 951-956, 1976) is based on pseudoisochromacy and contains internal controls against the subjects' use of brightness cues. Three ground squirrels were trained on the color discrimination task in a darkened two-choice visual discrimination apparatus with the stimuli located approximately 2.0 cm from the animal. The stimuli for original training measured 6.5 X 5.0 cm and subtended approximately 70°of visual angle. After training to criterion performance the animals were tested on the same color discrimination at both 6 and 12 cm viewing distances with targets measuring 10 X 10 mm to 0.35 mm diameter. A staircase method was employed and animals were tested for 200-300 trials with each of the different sized targets. The target size necessary for 80% correct discrimination subtended from 0.2 - 1.0°for these three subjects. Each subject had similar thresholds at 6 and 12 cm viewing distances. I also determined spectral sensitivity over the range 450 - 640 nm for each subject under dimly light-adapted conditions (background intensity approximately 5 cd/m^2). For the spectral sensitivity measures the 70°stimulus was used. Goodness of target size discrimination was directly related to spectral sensitivity and inversely related to number of trials required for criterion performance on initial learning of the pseudoisochromatic color problem. That is, the animal most sensitive in the spectral sensitivity test had the smallest threshold for target size. This animal required 700 trials to criterion versus 1000 and 1100 for the other two subjects.

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194.13 RESPONSES OF INFERIOR TEMPORAL NEURONS TO COMPLEX VISUAL STIMULI. R. Desimone*, T.D. Albright*, C.G. Gross and C. Bruce. Dept. Psychology, Princeton Univ., Princeton, N.J. 08544

The inferior temporal visual area (IT) of primates is known to play an important role in visual perception and memory. We have shown that, unlike neurons in striate or prestriate cortex, IT neurons have very large, bilateral receptive fields which include the center of gaze. In the present study we found that most IT neurons are selective for complex stimulus features rather than the size, orientation, color, or direction of movement of simple slits or edges. The recordings were made in the middle part of IT in immobilized macaques, anesthetized with nitrous oxide. We studied each unit with bars, slits, edges, shadows, and a set of fifteen complex three-dimensional objects and transformations of these objects. The transformations included photographs and slides of different sizes and orientations, scrambled photographs, high contrast cutouts, and modified versions of the objects themselves.

Two-thirds of the units responded strongly and consistently enough to be studied. Of these, most responded selectively to one or a small subset of the three-dimensional objects. Thirty-five percent were selective for an object, but responded weakly to slits and edges. When tested with modified representations of the object, such as scrambled photographs, these units did not appear selective for the object per se, but rather appeared selective for such properties as the object's texture, shape, size, or color. Ten percent of the units were selective for one object, but responded equally well to a slit or edge which shared a common property such as size, color, or orientation. About fifteen percent of the units responded best to an object, but we were unable to discover any simple stimulus feature which could explain their response. These included units which responded best to faces, hands, or body shapes. Thirty-five percent of the units were classified as non-selective, since they did not respond selectively to any of the objects or the bars; however, they sometimes responded better to each of the objects than to a bar, slit, or edge.

194.14 ELECTROCORTICAL EVIDENCE FOR A TEMPORAL LOBE VISUAL FUNCTION IN MAN. Richard A. Roemer, Charles Shagass*, and William Nixon*. Eastern Pennsylvania Psychiatric Institute and Temple University Health Sciences Center, Philadelphia PA 19129.

We studied patterns of functional linkage between different brain areas by means of an electroencephalographic (EEG) measure, the "cortical coupling" index (Callaway and Harris, Science, 183, 873, 1974). Evidence is presented for systematic changes of cortical coupling patterns associated with simple changes in visual activity. EEG records from 12 scalp leads were obtained in 80 paid volunteers. Two and one half minutes of EEG recordings were made while subjects sat in a dark room with their eyes closed and then while fixating on a central point embedded in a stationary checkerboard pattern of low luminance (1.2 ft L.).

The work of Callaway and colleagues has suggested that patterns of functional relationships between different brain areas may be revealed by the cortical coupling relationships. This index, derived from information processing theory, calculates the extent to which activity in different "channels" (or in our case, different scalp areas) may be related. From a computational point of view, the coupling measure is more economical than other procedures, such as cross-correlation or coherence analyses, that could be used to evaluate such brain relationships.

Marked differences in patterns of cortical coupling were observed between the eyes closed and open conditions; these differences were primarily related to the lead pairs which included temporal scalp areas. Summary results of visual activity include: (1) increased temporal-occipital coupling both within and between hemispheres; (2) reduced coupling between frontal-central and temporal-central areas; and (3) increased interhemispheric coupling between homologous brain areas.

These findings are compatible with the visual functions in the temporal lobe which have been observed in primates and subprimates.

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197.1 THE ORGANIZATION OF AXONS IN THE OPTIC TRACT OF THE CAT. F. Torrealba*, C.A. Mason, E.H. Polley and R.W. Guillery. Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL, 60637.

Previous reports, that there is at least a rough visuotopic order in the arrangement of axons in the optic tract, have been confirmed by making small retinal photocoagulation lesions and by localized injections of ^{3}H proline into the retina. Axons from the two eyes are ordered in the tract so that in a frontal section the upper contralateral hemifield is represented dorsolaterally, the lower field ventromedially, central areas dorsomedially, and peripheral areas ventrolaterally. A segregation of fiber diameter classes previously described (e.g., Bishop, P.O. et al., J. Physiol., 121: 415-432, 1953) has also been confirmed. In a 1µm coronal section one sees the coarsest fibers confined to the ventrolateral half of the tract, where they are mingled with many much finer axons. In the dorsomedial parts of the tract the axons are predominantly intermediate in size, mingled with few fine fibers. Cutting or radioactively labelling all of the axons in one optic nerve shows that whereas crossed and uncrossed axons are mingled in most of the tract, there is a small dorsomedial crescent that is made up of crossed axons only.

Our first interpretation, that this "monocular crescent" of the tract might represent the monocular crescent of the visual field, is disproved by the fact that the axons in the crescent are relatively fine, and by lesions of the relevant part of the nasal retina, which produce coarse and fine degeneration in the ventral but not the dorsal parts of the tract. A second possibility is that this monocular component of the tract represents crossed axons arising in the temporal retina. Lesions limited to the temporal retina do produce degeneration in the contralateral tract, but the degenerating axons are not seen in the monocular crescent of the tract if the lesions are clear of the area centralis. Many of the fibers in the crescent appear to arise from the region of the area centralis. The particular population of ganglion cells giving rise to these crossed axons that are not accompanied by any visuotopically matching uncrossed axons remains to be defined.

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197.2 TWO KINDS OF TOPOGRAPHIC PROJECTION IN THE CHICK VISUAL SYSTEM. D. Ehrlich* and R.F. Mark* (SPON: M.J. MUSTARI). Department of Behavioural Biol., R.S.B.S., ANU, Canberra, Australia.

In vertebrates the primary visual projections to the optic tectum are retinotopically organised so that the axons of ganglion cells from adjacent points on the retina terminate beside one another in the brain. Scholes (Nature 278: 620-624, 1979) has shown that in the cichlid fish the arrangement of axons travelling in the optic nerve also preserves the neighbourly relations of ganglion cells over most of the retina but the mapping of the polar coordinates of the retina is transformed into a Cartesian mapping in the nerve. Fibers from cells on either side of the position of the choroidal fissure take diverging pathways across the retina and add on to opposite sides of the nerve at the optic nerve head, becoming separated in the nerve even though their parent ganglion cells are close together in the ventral retina. The reformation of a map on polar coordinates is seen as a reordering of fiber bundles to reunite the separated fascicles just as the optic nerve enters the optic tectum.

By the use of small lesions made through the full thickness of the retina with a laser and staining degenerating nerve terminals, we have mapped the central projections of the optic nerve in chickens. Lesions placed near the optic nerve head axotomise a sector of ganglion cells radiating out from the lesion to the peripheral retina. There appear to be two kinds of topographic projection, exemplified by those to the optic tectum and to the ventral lateral geniculate of the thalamus. The projection to the tectum is retinotopically organised, showing radial patterns of degenerating terminals over the surface corresponding to the radial sectors of axotomised retinal ganglion cells. The projection to the ventral geniculate is Cartesian in that radial lesions of the retina produce side-by-side rostro-caudal slabs of terminal degeneration. Retinal sectors on either side of the pecten, the position of the choroidal fissure, are represented at opposite sides of the nucleus just as they are represented at opposite sides of the optic nerve in the cichlid fish. The projection to the tectum, representing retinotopic order over the whole field, is likely to subserve spatial aspects of vision. That to the ventral lateral geniculate, which appears to follow the nearest neighbour relations in the retina and the developmental constraints of the position of the choroidal fissure, may reflect the packing of fibers in the optic nerve. It may have no spatial significance since it does not undergo the re-transformation required to transmit faithfully the geometry of the retina and visual field.

197.3 CENTRAL MORPHOLOGY OF PHYSIOLOGICALLY CHARACTERIZED AXONS FROM SINGLE RETINAL GANGLION CELLS IN THE CAT. Douglas B. Bowling* and Charles R. Michael. Dept. of Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Since the axons of retinal ganglion cells are the sole channels carrying information from the eye, the organization of their branches and terminations is of fundamental importance in visual processing. To examine the central projections of single optic-tract fibers we have impaled the axons themselves, near their sites of termination, and injected them iontophoretically with the marker enzyme horseradish peroxidase. This method has revealed the thalamic and midbrain ramifications of single physiologically characterized axons. The axons we have studied were Y-like in that they had high conduction velocities and nonlinear spatial summation in their receptive fields. Individual axons sent collaterals to three nuclei: the LGNd, the MIN, and the SC. In each nucleus the axons' collaterals formed arborizations with different and characteristic patterns: converging to single loci in the LGNd, diffuse and radiating in the SC, and spread out in thin, bowl-shaped laminae in the MIN. In the geniculate the single axons from the contralateral eye terminated in more than one lamina (A, C, and C_2) while ipsilateral afferents terminated in only a single lamina (A_1). In lamina A and A_1 the synaptic terminals were distributed in broad-based columns that spanned the thickness of the laminae. In the planes of the laminae, the distributions of the terminals were basically circular with a constant lateral spread of 300-400 µm. In the MIN the bowl-shaped terminal distributions were also about 300-400 µm across but were oriented perpendicularly to the retinotopic map. Their lateral spread was about 50 µm. In the SC the terminals were grouped into irregular patches which were distributed as an ellipse (1 x 2 mm) with its major axis oriented approximately medio-laterally. In the geniculate complex single axons often had two or more long branches which traveled by different paths to converge on the same retinotopic location. It would be interesting to know what functional significance, metabolic constraint, or mode of development these multiple and somewhat indirect pathways may reflect. Supported by NIH Grant EY00568 and NIH Training Grant NS07102-03.

197.4 MORPHOLOGY AND CENTRAL PROJECTIONS OF DIFFERENT TYPES OF RETINAL GANGLION CELLS IN CAT AND OLD-WORLD MONKEY (M. FASCICULARIS). A. G. Leventhal, R. W. Rodieck, B. Dreher . Dept. of Ophthalmology, University of Washington, Seattle WA 98195.

Labeled ganglion cells were studied in whole-mount retinas of animals that received electrophoretic injections of horseradish peroxidase (HRP) in various regions of the brain. The histochemical procedures used allowed the cell bodies, axons and dendritic fields of many of the labeled cells to be visualized.

In the cat, the morphological types we have labeled include the previously recognized types: alpha, beta, gamma and at least three other distinct types. Alpha cells project to laminae A, A_1 and C complex of the dorsal lateral geniculate nucleus (LGNd), superior colliculus (SC) and pretectal complex (PC). Beta cells project to laminae A, A_1 and C complex of the LGNd and to PC. Gamma cells project to the SC, PC and ventral LGN. Other types project to the C complex of LGNd, retinal recipient zone of pulvinar complex, ventral LGN, SC and PC.

The A laminae of the cat LGNd are reported to receive retinal afferents only from the functional types Y and X; our findings show that they receive retinal afferents only from the morphological types alpha and beta, thus lending further support to a correspondence between these functional and morphological types.

In the monkey, injections were made into the parvocellular and magnocellular laminae of the LGN and into SC and PC. At least six morphological types have been observed. Three of these have been termed A cells, B cells and C cells. A cells have large somas, coarse axons and large, distinctive dendritic fields. B cells have small to medium size somas, fine to medium gauge axons and small, distinctive dendritic fields. C cells have small to medium size somas, fine axons and large, distinctive dendritic fields. Some of the remaining types have dendritic fields that are much larger than those of A or B cells and somas as large as those of A cells. The morphology of some if not all types varies gradually with distance from the fovea. Characteristics that vary include soma size, dendritic field size, thickness of dendrites and number of dendrites. For example, B cells in the foveal region are typical midget ganglion cells, while B cells in the far periphery have larger somas, larger dendritic fields and more than one principal dendrite. In all parts of the retina, however, adjacent B cells closely resemble each other.

The magnocellular laminae receive their major retinal inputs from A cells while the parvocellular laminae receive their major inputs from B cells. SC receives inputs from C cells and some A cells. Other types of ganglion cells also project to the LGN and SC. PC receives retinal inputs from C cells, A cells and other cell types.

A cells project to the magnocellular laminae, which reportedly contain only relay cells with Y-like properties; B cells project to the parvocellular laminae, which reportedly contain only relay cells with X-like properties. Thus, A and B cells presumably have Y-like and X-like properties, respectively.

Although A and B cells in the monkey differ morphologically from alpha and beta cells in the cat, the relative differences between the different cell types in the two species parallel each other. Supported by NIH Grants EY 0923, EY 05212 and the E. K. Bishop Foundation. Dr. Rodieck is a Research to Prevent Blindness - James S. Adams Scholar.

197.5 FUNCTIONAL ORGANIZATION OF THE TREE SHREW LATERAL GENICULATE NUCLEUS. Janet Conway, Peter H. Schiller and Lisa Misler, Dept. Psychology, MIT, Cambridge, MA 02139

The tree shrew (Tupaia glis) has a dorsal lateral geniculate with six well-defined layers. Ipsilateral retinal fibers terminate in the most medial layer (layer 1) and in layer 5 and contralateral fibers project to layers 2, 3, 4, and 6. Unlike other primate and prosimian species, there are no striking differences in the size of cells in the different laminae of the tree shrew LGD.

Tree shrews weighing 130-240 grams were paralyzed with a continuous infusion of Pavulon, artificially respirated with a 70/30% mixture of NO2 and O2, and catheterized to prevent urinary retension. A maxillary clamp fixed the head and allowed for unrestricted vision during recording. Visual stimuli generated by an optic system were back-projected onto a hemisphere positioned around the head. Horizontal electrode penetrations were made through a burr hole. Small lesions (3 microamp for 3 sec.) were made during penetrations to identify recording sites. In some experiments HRP injections (.5 microliter) were made in different locations in Area 17. Separate brains were processed for cresyl violet, Merker silver nissl, acetylcholinesterase activity, Golgi Kopsch, and TMB neurohistochemistry.

Our neurophysiological results revealed a pattern of activity which was quite consistent from animal to animal. Y type cells with transient response properties were found only in layer 6, adjacent to the optic tract. Some of these cells discharged to both stimulus onset and offset. In the remainder of the LGD all cells appeared X like, with sustained response properties. Layers were specific in that cells in each lamina gave either only ON or only OFF responses. We found three OFF layers and one ON layer for the contralateral eye and one OFF layer and one ON layer for the ipsilateral eye. The cells in layers 1 and 5, the ipsilateral layers, had their receptive fields limited to an area near the vertical meridian. In the anterior LGD, layer 1 was not present and layer 5 is present, as our histology shows, only in the dorsal part of the nucleus. (This work was supported by NSF grant #BNS 76-82543 and NIH grant 5 ROI EY00676).

197.6 PROPERTIES OF X AND Y CELLS IN THE LATERAL GENICULATE NUCLEUS OF THE MACAQUE MONKEY. R. Shapley* and E.Kaplan* (SPON: J.GORDON) Rockefeller University, New York, N.Y. 10021.

Visual cells in the lateral geniculate nucleus (LGN) of macaques may be classified as X or Y on the basis of linearity of spatial summation, as in the cat LGN. The four parvocellular layers of the macaque LGN are composed, with rare exceptions, only of X cells. These parvocellular X cells are of three main types: 1) color-opponent cells with an obvious neutral point near 580 μm. 2) non-opponent cells. 3) "Blue" cells which receive excitation from short wavelengths and inhibition from long wavelengths. The "Blue" cells are much less sensitive to grating patterns than any of the other parvocellular X cells, and their spatial resolution is also much less. In the magnocellular laminae there are X cells and Y cells. The monkey Y cells are very similar to cat Y cells; their responses at the modulation frequency (of contrast reversal or drift) have poor spatial resolution, while their frequency-doubled responses to contrast reversal have a spatial resolution comparable to that of X cells. Almost all monkey X cells (parvo- and magnocellular) showed less spatial tuning than their feline analogues, indicating less center-surround spatial antagonism in the monkey at the level of the LGN.

197.7 MORPHOLOGY OF PHYSIOLOGICALLY IDENTIFIED NEURONS IN THE C LAMINAE OF THE CAT'S LATERAL GENICULATE NUCLEUS. L.R. Stanford*, Michael J. Friedlander, and S. Murray Sherman. Dept. of Neurobiology and Behavior, State Univ. of New York at Stony Brook, Stony Brook, NY 11794.

Single unit recordings from neurons in the C laminae of the cat's lateral geniculate nucleus allowed us to classify a number of these cells as W-cells. Both X- and Y-cells have also been recorded in these deeper laminae of the LGN. A neuron was considered to be a W-cell if the following criteria were met: 1) the latency of the postsynaptic response to optic chiasm stimulation was greater than 3 msec. and 2) a "sluggish" response occurred with visual stimulation. All of the neurons meeting the above criteria (and therefore classified here as W-cells) have been encountered in the C laminae of the lateral geniculate nucleus.

The receptive fields of some of the W-cells found thus far have had antagonistic center-surround organization. When tested with a counterphased sine wave grating these cells display linear properties similar to those seen in geniculate X-cells. The responses of W-cells to this stimulus differed from X-cells in their marked preference for low spatial frequencies. Other W-cells responded only to slowly moving stimuli; some of these cells were directionally selective. Motion sensitive W-cells did not respond to a counterphased grating for contrasts up to 0.6.

After determining that a neuron, by our criteria, was classifiable as a W-cell, we impaled and iontophoretically injected the cell with HRP. The W-cells successfully injected and recovered thus far have had small to medium sized perikarya (150 to 250 μm^2). The dendrites of these W-cells are fine and relatively appendage-free (although occasionally spines and small clusters of grape-like appendages are seen). These W-cell dendrites are oriented primarily parallel to the laminar borders of the LGN and therefore perpendicular to the projection lines through the nucleus. The axons of the injected W-cells can be traced into the optic radiation. Guillery[1] has described cells in the C laminae of the cat LGN that have small to medium-sized cell bodies and dendritic arborizations parallel to the plane of lamination. It thus appears that Guillery's Class 4 cells may be equivalent to geniculate W-cells. Experiments are now in progress to test this hypothesis and to further describe the morphological characteristics of physiologically identified W-cells. Most C laminae Y-cells have morphology similar to those in the A laminae[2], although occasional unusual morphological variants were seen. To date, no C laminae X-cells have been recovered.

1. Guillery, J.C.N. 138:339 (1966).
2. Friedlander et al, Soc. Neurosci. Abstr., 5, 785, 1979.
(Supported by USPHS Grant EY 03038.)

197.8 SYNAPTIC MORPHOLOGY OF X- AND Y-CELLS IN THE CAT'S DORSAL LATERAL GENICULATE NUCLEUS. James R. Wilson, Michael J. Friedlander and S. Murray Sherman. Dept. of Anatomical Sciences and Dept. of Neurobiology and Behavior. SUNY at Stony Brook, NY 11794. (SPON: J. Cabot).

Neurons in laminae A and A1 of the cat's dorsal lateral geniculate nucleus were recorded extracellularly and classified as X- or Y-cells by previously used criteria including latency to optic chiasm stimulation, response to gratings, and receptive field size. These neurons were subsequently impaled, intracellularly re-identified, and then injected with horseradish peroxidase. The brain was perfused, vibratomed, reacted with diaminobenzidine, and embedded in Epon 812 between plastic slides. Each injected neuron was morphologically identified and drawn prior to final trimming and thin sectioning. The thin sections were stained and then examined under the electron microscope. To date, only one X-cell and one Y-cell have been ultrastructurally analyzed. The X-cell and Y-cell each had retinal terminals (RLP profiles) which formed synapses close to the cell soma, either on spines or large dendrites; on more distal and smaller dendrites were synaptic terminals which were probably from cortex (RSD profiles). However, a qualitative assessment of these neurons indicates that the X-cell had a larger number of flattened-vesicle terminals (F profiles) associated with the area of RLP terminals and a lesser number of RSD terminals making synapses onto its peripheral dendrites compared with the Y-cell. Also, collaterals from the X-cell and another Y-cell (which is still under analysis) gave rise to terminals which were small to medium size, contained round vesicles, and made asymmetrical synapses which were usually onto large, proximal dendrites. We are continuing our analysis of geniculate X- and Y-cells, but our present sample suggests that these two physiological cell types have different synaptic morphology.

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197.9 LAMINAR DIFFERENCES IN RECEPTIVE-FIELD PROPERTIES OF LATERAL GENICULATE NEURONS IN A PROSIMIAN PRIMATE (GALAGO CRASSICAUDATUS). T.T. Norton and V.A. Casagrande. Dept. of Physiological Optics, Sch. of Optometry/The Medical Center, Univ. of Alabama in Birmingham, Birmingham, AL 35294 and Depts. of Anatomy and Psychology, Vanderbilt University, Nashville, TN 37232.

Several recent studies have suggested a correlation between the size of retinal ganglion cells (i.e., small, medium and large) and functional subdivisions into W, X and Y cells respectively. Moreover, recent studies in monkey lateral geniculate nucleus (LGN) have shown that X cells occur in the parvocellular layers and Y cells occur in the magnocellular layers. The object of the present investigation was to assess the degree of correlation between LGN receptive-field properties and soma size in Galago, a primate which shows a unique laminar segregation into pairs of layers containing small, medium and large cells. LGN units were studied in paralyzed Galagos anesthetized with nitrous oxide. Our main finding is that there are basic laminar differences in unit properties in Galago LGN. However, these differences are not identical to those reported for other primates. Specifically, we find the following: neurons recorded in the magnocellular layers (1 and 2) have short latency responses to optic chiasm shock (OX latency), short latency responses to antidromic activation, relatively large receptive-field center diameters and respond transiently to visual stimuli. These findings are consistent with previous reports suggesting that the magnocellular layers in primates contain Y cells. However, when these units were studied with a variety of other measures, including rapidly moving stimuli, spatio-temporal maps to flashing spots and a bipartite field, their variable response profiles suggested that the magnocellular layers may contain a mixture of X and Y cells. In contrast, similar tests of units within the medium-size layers (3 and 6) indicate that most of these cells belong to a homogeneous population with X-like properties. Finally, units in the small-cell layers (4 and 5) typically show very long OX latencies but have heterogeneous response properties. Some of these units resemble X and Y cells, while others exhibit properties not typically associated with the primate LGN, such as direction selectivity, and still others were not driven by any of the visual stimuli we presented. In each of the six LGN layers, both ON-center and OFF-center units were found intermixed. Taken together, our results suggest that cell size segregation in the Galago LGN, as in other primates, reflects differences in laminar function. Supported by grants EY02909, EY01778 and 1 K07-EY00061.

197.10 DEVELOPMENT OF NEURONAL RESPONSE PROPERTIES IN THE CAT LATERAL GENICULATE NUCLEUS DURING MONOCULAR LID SUTURE. Stuart C. Mangel*, James R. Wilson, and S. Murray Sherman. Dept. of Physiology, Univ. of Virginia, Charlottesville, VA 22908

Following neonate monocular lid-suture, previous physiological recordings in the adult cat's dorsal lateral geniculate nucleus (LGNd) have revealed two findings. Fewer Y-cells are encountered in deprived as compared to nondeprived laminae and deprived X-cells exhibit lower spatial acuity on average than nondeprived X-cells. We further studied the development of these effects by examining neuronal responses in the LGNd at 8, 12, 16, 24, and 50-60 weeks of age.

Conventional single unit, extracellular recording was limited to laminae A and A1. The eyes were refracted and covered with contact lenses having 3 mm artificial pupils. Each cell was classified using standard tests, including latency to optic chiasm stimulation, linearity of spatial summation and response to a large, fast-moving disk of opposite contrast to the receptive field center. After hand-plotting, further visual stimuli were generated on an oscilloscope and consisted of counterphased, sine-wave gratings with a mean luminance of 33 cd/m^2 and contrast from 0 to 0.6.

Within the central 10° of visual field no significant difference in spatial acuity between nondeprived and deprived X-cells was observed until 24 weeks of age. In addition, at 24 weeks of age, X-cell spatial acuity was significantly reduced in deprived lamina A1 whereas mean spatial acuity in deprived lamina A and in the nondeprived lamina A were indistinguishable. However, a spatial acuity deficit was observable in both deprived A laminae in cats 13 months of age.

The average X-cell latency to optic chiasm stimulation was greater in deprived laminae than in the nondeprived laminae at all ages studied. Y-cell latency, however, appeared to be unaffected by deprivation.

At eight weeks of age, the proportion of Y-cells encountered in the A laminae was approximately 30 percent in both nondeprived and deprived laminae. However, by twelve weeks, Y-cell proportions are significantly lower in deprived laminae. No obvious differences in the development of this effect were observed between layers A and A1.

These data indicate that following neonate monocular lid-suture the onset of the deprivation effect on Y-cells precedes the spatial acuity effect on X-cells by approximately two to three months. In addition, a spatial acuity deficit of X-cells is observed at an earlier age in deprived lamina A1 as compared to deprived lamina A.

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97.11 ABNORMALITIES OF TEMPORAL RETINA IN SIAMESE CATS. M.H. Rowe, D. Murakami and M.A. Sesma. Dept. Psychol., Univ. Calif., Riverside 92521

Ganglion cell axons arising in temporal retina of the siamese cat have an anomalous pattern of decussation at the optic chiasm, resulting in a substantial reorganization of the central visual pathways. We have compared the soma size and axonal conduction velocity of ganglion cells in temporal and nasal retina in both siamese and normal cats in an effort to identify some of the factors which might underlie these abnormalities.

In normal cats, X-cells in temporal and nasal retina identified following injections of HRP into LGN laminae A_1 and A, respectively, differ markedly in soma size: temporal X-cells are larger ($\bar{x}$ = 21μm) than nasal X-cells ($\bar{x}$ = 18 μm), and Y-cells also show a tendency to be larger in temporal retina ($\bar{x}$ = 32.1 μm) than in nasal retina ($\bar{x}$ = 30.5 μm). Similarly, in field potentials recorded at the optic disc in response to optic chiasm stimulation, the amplitude of the t_2 component is larger for temporal retinal axons than for nasal retinal axons, and the latency of this component is shorter in temporal retina, suggesting that the axons as well as the somas of temporal X-cells are larger than those of nasal X-cells.

In the siamese cat medium sized cells projecting to lamina A from nasal retina average 1μm smaller than either the medium sized cells in temporal retina which project to the normal (ipsilateral) segment of A_1 or those that project to the abnormal (contralateral) segments of A_1. In field potentials recorded at the optic disc in response to stimulation of the optic chiasm or optic tracts, the amplitude of the t_2 component relative to the t_1 component is noticeably reduced, and the reduction seems more pronounced among ipsilaterally projecting axons. Consistent with earlier findings, both the HRP and field potential analyses indicate that very few large cells in temporal retina project to the normal segment of A_1. Many of these results have also been seen in white, non-siamese cats in which retinal pigment is largely absent.

198.1 LONG-TERM, COMPLEX FIXED ACTION PATTERNS PERSIST IN THE ISOLATED UNSTIMULATED VENTRAL NERVE CORD OF LIMULUS. Winsor H. Watson III Zoology Department, Univ. of New Hampshire, Durham, NH 03824.

The gill appendages of Limulus are utilized for several different activities, all of which commonly occur in long-term patterns. The most common pattern observed consists of 1 minute bouts of gill cleaning, alternating with 1 minute periods of ventilation. Isolated, unstimulated abdominal nerve cords of Limulus display patterns of motor output characteristic of rhythmic gill ventilation and of gill cleaning, and these also occur as organized long-term patterns.

In the intact animal there are two different mirror-image arrangements of the gill-plates during cleaning. Both are also expressed by the isolated CNS and occur with the same rough alternation as in intact animals. The cardioinhibition associated with gill cleaning also appears to be part of the endogenous central motor program generated by the isolated CNS. Similar patterns of cardioregulatory nerve discharge are observed in both instances. Thus, all the patterns of gill-plate movement and the associated changes in heart rate observed in intact animals, except those involving swimming, have underlying motor programs that are expressed by isolated nerve cords in the absence of stimulation or of sensory feedback.

198.2 MODIFICATION OF SENSORY EVOKED BEHAVIORAL AND NEURAL RESPONSES DURING DIFFERENT BEHAVIORAL STATES IN APLYSIA. Behrus Jahan-Parwar, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, USA.

Chemical cues from food (seaweed) arouse Aplysia and elicit locomotor and feeding behaviors (Am. Zool., 1972). Chemical cues from an egg-laying Aplysia and/or an egg mass also attract Aplysia and elicit aggregation, mating and/or egg-laying in this hermaphroditic organism (The Physiologist, 1976). In the presence of both food and sex attractants Aplysia displays a behavioral choice which appears to depend on its behavioral state. In the egg-laying state the food induced motor responses are suppressed. These responses are also suppressed in functional males if they are actively engaged in mating but not in their female partners. As a result, the food aroused mating females frequently end up carrying their male partners to the food site. These observations suggest a hierarchical organization of chemosensory evoked behaviors in Aplysia. A behavioral choice in the presence of different ethologically significant chemosensory stimuli suggests altered sensitivity to stimuli eliciting lower order behaviors during higher order behavioral states. Behavioral state-dependent response modification is not restricted to chemosensory responses. In a food arousned Aplysia, mechanosensory responses are also modified. Tactile stimulation of a tentacle elicits a withdrawal reflex in the unaroused state (96%, N=200) and a characteristic orienting reflex in the food aroused state (92%, N=200). Tactile response modification following food stimulation can also be obtained at the level of the 4th order neurons (4° Ns) along the activated neural pathways (Comp. Biochem. Physiol., 1980). Before stimulating with food chemicals, tactile stimulation of a tentacle produces a two component inhibitory response in the 4° Ns, but after food sensory stimulation which strongly activates the 4° Ns, an identical tactile stimulation results in an excitatory (spiking) response. Preliminary data suggest that interaction of an electrogenic pump (Comp. Biochem. Physiol., 1979) and the two component tactile synaptic input may be partially responsible for the motivational state-dependent modification of sensory integration in the 4° Ns. The firing of the 4° Ns in food aroused Aplysia may be significant because these cells appear to be motor neurons mediating foot movement (J. Neurophysiol., 1978). They also appear to make synaptic connections with many other neurons which also appear to have motor functions (Brain Res. Bull., 1979) and thus may be capable of coordinating the body movements required for orienting. These data suggest that behavioral and neural responses to a particular sensory stimulus obtained during one behavioral state can be altered during another behavioral state. Since a single sensory stimulation can rapidly alter the behavioral state of an organism, the interpretation of the neural responses to the commonly used random or repetitive sensory stimulations may be complicated unless the behavior or some other indication of the total organismic response to these stimuli is monitored. (Supported by grants: NS 12483, NS 14388 and BNS 77-24174)

198.3 MECHANICS AND COORDINATION OF FEEDING AND REJECTION IN PLEUROBRANCHAEA. R.P. Croll and W.J. Davis, Thimann Labs, Univ. of Calif. at Santa Cruz, Santa Cruz, CA 95064

The buccal mass of Pleurobranchaea is capable of producing two distinct cyclic behaviours. One of these, i.e. feeding, can be elicited by presentation of food homogenates to the rhinophores, tentacles or oral veil. The other behaviour, rejection of inappropriate substances from the buccal cavity, can be reliably elicited either by injecting alcohol or detergent, or by introducing a non-palatable object, such as a string, into the mouth. Although both behaviours have previously been reported (Davis and Mpitsos, 1973; MacLellan, 1978, 1979) no detailed descriptions of the muscular coordination or mechanics have appeared.

Cinemagraphic analysis of buccal movements in unrestrained, whole animals and electromyograms from exposed buccal masses reveal that a systematic difference in muscular activity underlies these two behaviours. For instance, during feeding the circumferential buccal muscle 5 (Davis and Mpitsos, 1973) contracts vigorously during retraction of the radula, thus insuring a firm hold on the food. Muscle 4, which everts the radula, then fires briefly in antiphase with muscle 5. During rejection muscle 5 is silenced during retraction of the radula. Activity in muscle 4, on the other hand, is greatly enhanced and serves to expel inappropriate substances from the mouth. Although changes in the activity patterns of these two muscles are the most dramatic, the activity in certain other muscles is also slightly inhibited or enhanced during either of the two behaviours. However, phase relationships between active muscles are preserved. This suggests that the same neural oscillatory network is responsible for both types of movements and that excitation or suppression of specific elements in the network underlies the differences in the motor patterns.

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198.4 PERISTALTIC SWALLOWING IN NAVANAX: FUNCTIONAL ORGANIZATION OF CIRCUMFERENTIAL MUSCLE AND MOTOR UNITS. M.S. Cappell, D. C. Spray, D. H. Hall and M.V.L. Bennett. Division of Cellular Neurobiology, Dept. Neuroscience. A. Einstein Col. Medicine, Bronx, New York 10461.

Cinematographic analysis of feeding by a dissected animal with a window through the body wall (Susswein et al., Soc. Neurosci. Abstr. 5: 262, 1979) shows that Navanax can swallow small prey by a peristaltic wave of circumferential constriction of the pharynx from anterior to posterior. Several features of the circumferential muscle and motoneuron organization appear important in the generation of peristalsis: Pharyngeal circumferential muscle consists of discrete bands which are highly consistent in total number and pharynx position. Circumferential bands are regionally specialized and are classifiable into anterior and posterior sphincters with three intervening groups dorsolaterally and four intervening groups ventrally based on differences in size and shape. Borders between band groups are generally distinct, with a consistent number of bands per group. Motor fields were mapped in terms of identified bands, by four techniques (Cappell et al., Soc. Neurosci. Abstr. 5: 243, 1979). The borders of specific circumferential motor fields coincide with the borders of specific muscle band groups. Although most motoneurons innervate a single band group; a few innervate several adjacent band groups. No motorneurons innervated parts of adjacent band groups. Circumferential motoneuron fields exhibit little variability between animals. Most circumferential motorneurons innervate exclusively dorsolaterally or ventrally while others innervate both regions, permitting independent or coordinated activity. The consistency and correspondence of motor fields and muscle band arrangements should permit precision in the generation of peristalsis and facilitate progress in its cellular analysis. MSC is supported by NIH grant 5T 32GM7288.

198.5 ARE SUBGENUAL ORGANS "EARS" FOR HISSING COCKROACHES? Margaret C. Nelson, Sect. Neurobiol.& Behav., Cornell Univ., Ithaca, NY

Male Madagascar cockroaches (Gromphadorhina portentosa) produce sound signals in the form of amplitude-modulated hisses. The presence of females elicits stereotyped courtship hissing; receptive females respond with stereotyped receptive behavior that may lead to copulation (Nelson and Fraser, Soc.Neurosci.Abs.3, '77). This receptive behavior depends on auditory signals, but the basis for sound reception has been unknown (Nelson & Fraser, Behav. Ecol.& Sociobiol. in press; Nelson, Soc.Neurosci.Abs.5,'79). I now report that the tibial subgenual organs of all 6 legs may be responsible for detection of auditory signals in G. portentosa.

The subgenual organ (SGO) of cockroaches typically consists of a distal organ, an accessory organ, and a subgenual organ proper. In G. portentosa the SGO contains 40-50 chordotonal sensilla (as compared to 15-20 in Periplaneta, which does not produce sounds). It is part of a tibial sensory complex that includes campaniform sensilla, spines, and hairs from the proximal tibia; the axons of all these run in the tibial sensory nerve, N5r8. The fibers from this branch of N5 enter each thoracic ganglion to form several sets of bifurcating projections in cobalt backfills.

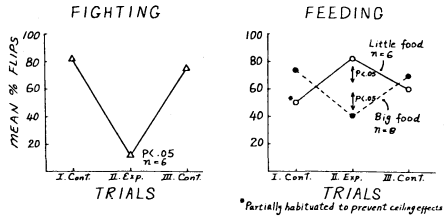
Involvement of the SGO in detection of acoustic signals was studied by recording from *1)* auditory interneurons in the thoracic connectives, and *2)* primary afferents in N5r8.
*1)*Several interneurons in the thoracic nerve cord (TNC) are driven by acoustic stimuli in the 3-7 kHz range (Nelson, Soc.Neurosci. Abs.5,'79); their activity was used to assay the contribution of various sensory fields to auditory reception. The only part of the body found to have input to these cells was the tibia: removal of a single tibia from any leg decreased activity recorded from the TCN upstream and abolished activity when the relevant thoracic ganglion had been isolated from the rest of the CNS and the opposite leg. Localized lesions at the level of the SGO sparing the tibial branches of N5r8 reduced or abolished the response, while localized lesions of the tibia distal to this region had no effect. Injection of μl amounts of vaseline into the bloodspace at the "knee" of the tibia also abolished the response.
2) Primary afferents sensitive to acoustic stimuli could be detected in N5r8 in the femur. These afferents showed essentially the same frequency range as the interneurons described above. Local destruction of the SGO sparing the other tibial branches of N5r8 greatly reduced the response; destruction of the campaniform sensilla had no effect on the auditory response.

These experiments thus strongly support the hypothesis that the SGO's mediate sound reception in G. portentosa.

198.6 ADAPTIVE CONTROL OF ESCAPE BEHAVIOR DURING FEEDING AND FIGHTING IN CRAYFISH. K. Bellman* and F.B. Krasne, Dept. Psychol., UCLA, Los Angeles, CA. 90024.

All nervous systems must be organized to cope adaptively with conflicting demands presented by the environment; the relative simplicity of nerve circuitry in invertebrates invites attack on the mechanisms involved in the resolution of such conflict. As a prelude to such investigation we have studied the effects of engagement in fighting and feeding on crayfish escape behavior.

Tail-flip escape responsiveness was evaluated by gradually approaching and touching crayfish with a fish net (an "escape trial") that on other occasions was used to capture them.* All testing was in groups of 3 successive escape trials: on the first and third, which served as controls, the net was presented alone; on the second it was presented while the animal was feeding or fighting with a conspecific. When escape was tested during fighting, tailflip probability was severely depressed; this held whether or not the animals' claws were locked together. Without such suppression of escape, animals would be unable to engage in sustained fighting. When escape was tested during feeding, the result depended on food size. Feeding on large pieces of food that could not readily be moved depressed probability of escape; but feeding on small bits of food enhanced it,** and when the animals flipped they carried the food with them. This makes adaptive sense. But it is a more complex result than might have been anticipated for crayfish, and it tends to rule out simple control schemes in which activity of feeding circuitry automatically and uniformly inhibits — or facilitates — escape mediating circuitry. Supported by NIMH NRSA MHO 7836 & NIH NS 8108.



*This sort of stimulus typically evokes non-giant mediated tailflips; experiments on giant-mediated responses are in progress.

**Facilitation of escape by possession of food in the presence of conspecifics was previously discovered by G. Hagiwara and J. Wine (in prep).

198.7 HETEROGENEITY OF THE EFFECT OF TEMPERATURE ON IDENTIFIED GRASSHOPPER NEURONS. Thomas W. Abrams and Keir G. Pearson. Dept. Physiology, Univ. of Alberta, Edmonton, Alberta, Canada.

Like many poikilotherms, grasshoppers show an increase in their level of locomotory activity at warmer body temperatures. We have tried to identify the changes in neuronal properties which are responsible for the increased behavioral activity.

We have investigated the effects of temperature on a number of interneurons and motoneurons in the thoracic CNS, including neurons involved in jumping behavior. Cells were penetrated with microelectrodes and measurements were made while the temperature was varied from 14°-35° C. We find that two groups of opposing effects combine to determine how the activity of central neurons changes with temperature; the relative importance of these different effects varies amongst neurons causing different cells to have different temperature sensitivities.

All interneurons and motoneurons tested showed a slight increase in voltage threshold (relative to V_{rest}) with warming, and a marked increase in threshold for injected currents. Thus the fact that warm animals are behaviorally more active is not due to a reduction in the threshold of the central neurons producing the behavior.

In contrast, the effect of temperature on repetitive firing properties was heterogeneous amongst the neurons studied. Generally there was a tendency towards a steepening of frequency-current (f-I) curves at higher temperatures; with sufficient current the initial firing rate is faster in warm neurons. However, the extent of the change in the f-I curve slope varied greatly; at least one flexor motoneuron showed almost no change in firing rate with temperature. Furthermore, in an auditory interneuron, the G neuron, there was greater adaptation during injected current pulses at higher temperatures; after the initial spikes, G fired more slowly when warm.

The combined effects of temperature on threshold, on the f-I relationship and on adaptation tend to cause the G neuron to be less responsive to injected currents when warm. Yet G's responsiveness to white noise stimuli increases with temperature. This is due to increased input from auditory receptors. The responsiveness of these afferents was extremely temperature sensitive, more so than any of the central neurons. This was also true of a tibia proprioceptor. We suggest that increased firing in sensory afferents may be the main factor responsible for increased behavioral activity in warm animals.

198.8 CORRELATION OF STRUCTURE AND FUNCTION OF DENDRITES IN IDENTIFIED MULTIMODAL INTERNEURONS IN THE CRAYFISH BRAIN. R.M. Glantz, M. Kirk and T. Viancour. Dept. Biology, Rice University, Houston Tx 77001.

Approximately 100 interneurons in the crayfish brain have been impaled with microelectrodes containing the fluorescent dye Lucifer-Yellow. Sensory receptive fields were mapped, and monosynaptic and polysynaptic inputs were distinguished by electrical shocks to the afferent roots. Each cell was dye filled and the dendritic morphology examined in whole mounts of the brain. The results have allowed us to uniquely identify 9 different interneurons by their sensory fields, the location of the soma, axon and principal neurite and the distribution of their dendrites. In addition to the identified cells, 45 additional neurons have each been dye filled and characterized physiologically on a single occassion. The data were analyzed to determine if the presence of a dendrite in a sensory neuropile of the brain was a sufficient or necessary condition to predict the functional (modality) and or synaptic input to the cell.

The results suggest that certain limited, functional inferences can be drawn on the basis of the dendritic geometry of a multimodal interneuron. If an interneuron projects a dendrite to a hemineuromere of the brain (left or right half of the proto-, deuto- or tritocerebrum) it will receive functional input (in 95% of our observations) from a sensory root to that hemineuromere. Most of these inputs (86%), will at least contain a monosynaptic, primary afferent component. If an interneuron does not project a dendrite into a hemineuromere, then 90% of the time it will not derive monosynaptic input from the corresponding sensory roots. The preponderance (83%) of the exceptional instances are associated with the contralateral antennal nerve. Cobalt backfills of the antennal nerve reveal groups of primary afferents which project outside of the ipsilateral tritocerebrum (Viancour, In Preparation). If a neuron does not project a dendritic branch to the protocerebrum it will not exhibit visual input. For other polysynaptic inputs the dendritic location is neither a necessary nor a sufficient predictor of input.

The results imply that the organization of the sensory neuropiles of the brain is quite simple and that both the modality and synaptic input of multimodal interneurons is essentially predictable from the distribution of interneuronal dendrites. (Supported by N.S.F. Grant No. BNS 7910335)

199.1 A SIGNIFICANT CORRELATION BETWEEN SIZE OF CANARY BRAIN NUCLEI CONTROLLING SONG AND SIZE OF SONG REPERTOIRE. F. Nottebohm, Rockefeller University, New York, N.Y. 10021.

Male canaries learn to sing by reference to auditory information (Marler and Waser, 1977; Waser and Marler, 1977). Two telencephalic nuclei, hyperstriatum ventrale, pars caudale (HVc) and nucleus robustus archistriatalis (RA) have been shown to be the two highest stations of the efferent song control pathway. Effects on song of unilateral destruction of HVc and RA suggest that the left HVc and RA are dominant for song control (Nottebohm, Stokes and Leonard, 1976; Nottebohm, 1977). The purpose of the present study was to search for possible correlations between the size of HVc and RA and the size of the song repertoire. A second intent was to look for right-left size differences in these nuclei in adult males, in hopes of discovering a clue to the functional differences between the two sides.

Twenty-five 1-3 year-old male canaries of the Rockefeller University closebred Waserschlager strain were used. They had all been exposed to other birds in our canary colony and had breeding experience. They were sacrificed after their song repertoire was recorded. These birds were perfused with 10% formalin in .9% saline. Their brain and testis were then removed. The testis were weighed. The brains were stored for a week in the perfusing solution, then transferred to 30% sucrose in 10% formalin until they sunk. At this time the brains were weighed, then embedded in gelatin-albumin. Frozen sections were cut at 50 μ, 50 μ and 25 μ intervals; one series of 50 μ sections was mounted and stained with cresyl violet. A microprojector was used to draw the total area of sections through HVc and RA, permitting a reconstruction of the volume of these nuclei.

Song repertoires ranged from 23 to 47 different syllable types per bird, with a mean of 31.6 (S.D.=6.4). There were no significant age differences in size of syllable repertoire or in size of HVc and RA. Syllable repertoire showed no significant correlation with brain weight (r=.135), testis weight (r=.042) or volume of nucleus rotundus (r=.087), a thalamic nucleus not involved in vocal control. The size of the syllable repertoire did correlate significantly with total (right plus left) HVc volume (r=.449, $P < .02$) and total RA volume (r+.380, $P < .05$). There was no significant difference between the volume of the right and left sides of HVc and RA.

The vocal control pathways of the songbird brain consist of well defined nuclei. The learned behavior they control is easily quantifiable. Clearly there is no one-to-one relation between the size of a nucleus and that of the song repertoire. But even after this caveat, this material seems well suited for establishing whether the amount of brain space available for a learned skill and the amount of learned skill are related. The answer is YES.

199.2 MEDULLARY NUCLEI FOR VOCAL CONTROL IN PARAKEETS. K.R. Manogue* and F. Nottebohm (SPON: T.J. DeVoogd). Rockefeller U., N.Y.10021.

In parrots, order Psittaciformes, the proximal tracheosyringeal (ts) branch of each hypoglossus nerve courses alongside the trachea to reach a common anastomosis on the midline. This anastomosis gives rise distally to two nerves, each of which innervates the muscles of one syringeal half. Section of either right (R) or left (L) ts nerve proximal to the anastomosis results in only minor and temporary changes in vocal patterning. Section of either R or L ts nerve distal to the anastomosis results in gross, long lasting vocal deficits. Thus parrots, unlike canaries and other oscine songbirds, show no hypoglossal dominance for song control, and both syringeal halves are equally important for vocal patterning. In addition, parrot phonation is thought to involve the larynx and tongue (Nottebohm, 1976). The absence in parrots of neural or muscular asymmetries in the peripheral control of phonation is similar to the human condition. We would like to know whether any asymmetries occur centrally. As a first step we here describe in parakeets, Melopsittacus undulatus, medullary motor nuclei presumed to partake in vocal control. These nuclei were identified by placement of horseradish peroxidase crystals on the proximal end of cut nerves supplying the motor innervation of the syrinx, larynx, or tongue, and sacrificing the birds 24-48 hrs later.

We reacted 50μ frozen sections of the fixed brains with the chromogens tetramethylbenzidine and diaminobenzidine, and obtained the following results. 1) The rostral pole of each hypoglossal nucleus (nXIIling) innervates the tongue via the lingualis branch of the hypoglossal nerve; each lingualis nerve was composed of axons from the ipsilateral nXIIling only. 2) The much larger caudal portion of each hypoglossal nucleus (nXIIts) innervates the syringeal muscles via the ts branch of the hypoglossal nerve. Proximal to their anastomosis, R and L ts nerves are composed of axons from ipsilateral nXIIts motorneurons only. Distal to the anastomosis both the R and L ts nerves contain axons arising from both R and L nXIIts. Thus, motorneurons in each, R or L, nXIIts project bilaterally to the muscles of the syrinx. 3) A distinct crescent of motorneurons arching ventrolaterally to nXII innervates the larynx; each laryngeal nerve is supplied by ipsilateral motorneurons only.

What comes as a surprise in this initial set of observations is that muscles on each right or left syringeal half receive bilateral medullary control. We also know that each nXIIts receives bilateral input from telencephalic and mesencephalic vocal control nuclei (see companion abstract by Paton, Manogue and Nottebohm). The significance of such bilateral projections remains unclear.

199.3 TELENCEPHALIC EFFERENT PATHWAYS FOR VOCAL CONTROL IN PARAKEETS. J.A. Paton, K.R. Manogue*, and F. Nottebohm. Rockefeller University, New York, N.Y. 10021.

Vocal learning in birds has evolved independently at least three times, in oscine songbirds, in hummingbirds, and in parrots and their allies, order Psittaciformes. Has the brain of these three orders of birds arrived at a similar solution for this task? We used a combination of retrograde anatomical techniques and antidromic electrical stimulation to map neural pathways connecting the telencephalon to motor neurons innervating the syrinx (vocal organ) of parakeets, Melopsittacus undulatus. We found that this pathway contains nuclei homologous to those already identified in songbirds (Nottebohm et al., 1976), but with bilateral rather than unilateral connections.

The motor nucleus of the syrinx (nXIIts) was located by electrically stimulating the tracheosyringeal nerve (ts) and searching for evoked potentials in the brainstem. Horseradish peroxidase (HRP) or HRP conjugated with wheat germ agglutinin was then iontophoresed into one side of the motor nucleus. After 24 to 48 hrs the animal was sacrificed and perfused with a 2.5% glutaraldehyde fixative. The brain was removed and sunk in a solution of 30% sucrose in fixative and embedded in gelatin-albumin, to be cut frozen into 50u sections. The sections were reacted using either the tetramethyl benzidine or diamino benzidine methods.

Well defined unilateral injections in nXIIts led to bilateral labelling of cells in two regions: 1) an area dorsomedial to the auditory nucleus of the tectum, probably corresponding to pars dorsalis medialis of nucleus intercollicularis in songbirds; 2) an area of large, loosely packed cells in the archistriatum, probably corresponding to nucleus robustus archistriatalis (RA) in songbirds. In an earlier experiment lesion of this putative RA in parakeets caused bilateral anterograde degeneration of fibers and terminals in nXIIts (D.B.Kelley and F.Nottebohm, unpub. obs.).

The afferents to RA were labelled by extending the previous paradigm one step. The nXIIts motor nucleus was located as before. The recording electrode in nXIIts was then used to stimulate RA antidromically while another electrode was advanced through the telencephalon. When the putative RA was found, judging from evoked potentials, HRP or HRP-WGA was injected. This procedure was then used to identify inputs to this nucleus.

The bilateral projection of RA to nXIIts raises interesting questions since each nXIIts, in turn, projects to the ipsi- and contralateral syringeal muscles (see abstract by Manogue and Nottebohm).

199.4 INTERHEMISPHERIC PROJECTIONS OF TEMPORAL LOBE CORTICAL AREAS IN THE RHESUS MONKEY. S. Demeter and G.W. Van Hoesen, Depts. of Neurol. and Anat., Univ. of Iowa, Iowa City, IA 52242.

The origins of axons which form the interhemispheric commissures of the primate have been delineated by others with retrograde tracing methods. With the exception of the sensorimotor cortices, less precise information is available about the trajectories of commissural axons and the topography of their terminations. Moreover, current knowledge is based largely on ablation-degeneration methods.

Therefore, we studied this problem in 14 rhesus monkeys (Macaca mulatta) using autoradiographic and horseradish peroxidase (HRP) tracing methods. Solutions of either tritium labeled amino acids (leucine, lysine and proline) or 20% HRP were injected into the various cytoarchitectural fields of the temporal lobe (Bonin and Bailey areas TE, TF, TG and TH and Brodmann areas 28 and 35). Autoradiographic cases were processed by standard methods following seven-day survivals. HRP cases were processed by the Mesulam tetramethylbenzidine procedure following two-day survivals. Injection sites and retrograde and anterograde labeling were assessed with brightfield and darkfield microscopy.

All areas studied gave rise to axons that crossed in the anterior commissure and/or hippocampal commissure (psalterium). Three cortical tiers were recognized in this regard. Cortices in close relation to the hippocampus such as area 28 were interconnected exclusively through the hippocampal commissure while cortices in more distant relation to it such as areas TG and TE were interconnected exclusively through the anterior commissure. Intermediately related cortices such as area TF were interconnected through both commissures.

Topographically, three categories of terminal labeling were recognized. Homotopic terminals were observed contralateral to all areas studied. Non-homotopic terminals contiguous with the homotopic terminals and within the same cytoarchitectural field were observed in most cases. Non-homotopic terminals in different cytoarchitectural fields were observed in some cases. All non-homotopic projections were mirrored by more prominent projections to the corresponding, ipsilateral areas.

HRP retrograde tracing studies provided support for these observations. Labeled neurons were observed in the contralateral homotopic area, in contiguous, non-homotopic areas within the same cytoarchitectural field and in non-homotopic areas in different cytoarchitectural fields.

The parahippocampal cortices, in particular, projected to and received afferents from widespread cortical areas of the contralateral hemisphere.

(Supported by grant NS 14944.)

199.5 BEHAVIORAL AND BIOCHEMICAL ASYMMETRIES IN THE RAT FOLLOWING UNILATERAL CORTICAL SUCTION LESIONS. G.D. Pearlson. Dept. of Psychiatry & Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Previous research has demonstrated asymmetries in both behavioral and biochemical response to ischemic lesions in the rat cerebral hemisphere, (Science 205: 707, 1979). Because this method is difficult to execute and vascular lesions are by their nature variable, we used suction lesions in a series of experiments in an effort to produce technically easier lesions with higher comparability.

Suction lesions were made in either the right or left cerebral hemisphere of male Sprague-Dawley rats of the same age in the lateral fronto-parietal cortex just anterior to the middle cerebral artery and above the rhinal fissure. The site chosen was located approximately 1.15 to 1.20cm ant.to ear bar zero. Marked spontaneous hyperactivity, as measured by total daily revolutions of an exercise wheel, occurred in 14 right-hemisphere lesioned animals, which was still present at the time of sacrifice 30 days postoperatively. NE depletion to approximately 65% of preoperative values in both ipsilateral and contralateral locus coeruleus and cerebral cortex was demonstrable by HPLC with electrochemical detection. By contrast, 16 left hemisphere operated animals showed neither behavioral nor biochemical differences from sham-operated controls. There were no significant differences in food or water intake between groups. Comparability of right and left-sided hemispheric lesions was demonstrated by histological examination of stained frozen or paraffin-embedded sections as well as by lesion volume assessment on a stereomicroscope equipped with horizontal and vertical vernier scales.

199.6 BRAIN ASYMMETRIES IN 2-DEOXY-D-GLUCOSE UPTAKE DURING POSTNATAL DEVELOPMENT OF THE RAT. D.A. Ross, S.D. Glick and R.C. Meibach., Dept. Pharmacol., Mt. Sinai Sch. of Med. of CUNY, New York, N.Y. 10029

A modification (Meibach et al., Brain Res., in press) of the 2-deoxy-D-glucose (DG) technique (Sokoloff, L. et al., J. Neurochem., 28:897-916, 1977) was used to investigate the postnatal ontogeny of functional brain development in the Sprague-Dawley rat. Different asymmetries in [^{3}H]-DG uptake in various micro-dissected brain regions were present at different ages and are listed in table 1. All asymmetries were significant at $p < .05$ using the Chi-Square test.

Days	Structure	Asymmetry
0-5	Hippocampus	R > L
	Brain Stem (Medulla/Pons)	L > R
0-15	Diencephalon	R > L
6-30	Cortex	L > R
31-60	Brain Stem	L > R
	Caudate Nucleus	R > L

In addition to these left-right asymmetries, we also looked for changes in [^{14}C]-DG activity within specific subanatomical fields of the hippocampus using radioautography. At birth the dentate gyrus had the highest activity. By day 5 the dentate activity decreased and that of CA 1 increased to render it the most active hippocampal subfield. Although the caudate asymmetry was associated with changes in circling behavior, the precise relationships between most of these findings and behavior remain elusive. However, the data do suggest that the two sides of the brain develop at differential rates.
(Supported by NS 14812 & DA 70082).

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199.7 ASYMMETRICAL DOSE-RESPONSE CURVES FOLLOWING UNILATERAL INTRACORTICAL 6-HYDROXYDOPAMINE INJECTIONS R.G. Robinson, T.G. Stitt*. Dept. of Psychiatry and Behavioral Science, Johns Hopkins University School of Medicine. Baltimore, Maryland 21205.

Following surgical ligation of the right middle cerebral artery in the rat, spontaneous hyperactivity occurs during the first 20 postoperative days and norepinephrine (NE) concentrations in the non-injured as well as injured areas of the cortex and in the locus coeruleus are depleted by about 30% to 50%. (Brain Res. 181:202, 1980). Left middle cerebral artery ligation, on the other hand, does not lead to an alteration in either the animals spontaneous activity or brain catecholamine concentrations (Science 205:707, 1979; Brain Res. 188:63, 1980).

Microinjections of the neurotoxin 6-hydroxydopamine (6-HDA) were made into either the right or left cerebral hemispheres of rats to determine what role injury to catecholamine containing terminals might play in the behavioral and biochemical asymmetries previously reported. 6-HDA (2µg/ul plus ascorbic acid 1mg/ml) was injected 1mm below the surface of the brain at a rate of 0.5µl/min in the area adjacent to the middle cerebral artery about where ligations were made. The dose-response curves for the right and left hemispheres are markedly different ($F., 55=126, p < .001$) with doses as small as 1µg (N=8) or as high as 6µg (N=12) producing hyperactivity which continued throughout the 30 day postoperative period. Left hemispheric injections did not produce any hyperactivity until the dose reached 6µg (N=7) when the animals mean activity was about 130% of control values. Analysis of norepinephrine concentrations using the high pressure liquid chromatography with electrochemical detection revealed that 6-HDA depleted NE concentrations in both the ipsilateral cortex and ipsilateral and contralateral locus coeruleus by about 30% to 50%. The 6µg dose of 6-HDA which caused hyperactivity when injected into the left hemisphere was the only dose which caused contralateral (right) hemispheric depletion of NE. The hyperactivity resulting from left hemispheric injection thus may be the result of right hemispheric mechanisms.

Since injections into either hemisphere cause ipsilateral depletions of NE but only right hemispheric injections cause hyperactivity, these data suggest that the asymmetrical neural pathways which lead to hyperactivity are postsynaptic to the cortical noradrenergic terminals.

199.8 THE DEVELOPMENT OF CIRCLING BEHAVIOR AND ROTATORY BIASES IN SHAKER-1 MUTANT MICE. John M. Cooke, Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605.

Shaker-1 mutant mice have been shown to exhibit stable patterns of circling behavior as adults (Cooke and Wolf, Soc. Neurosci. Abstr. 5, 1979). Both the rate of circling and bias for a particular direction of rotation were very reproducible in repeated tests of the same animal. In the present study, thirty-eight shaker-1 mice (15 females, 23 males) were observed for three minute test periods, three times a week, from the third to the ninth postnatal week in order to determine the time of onset of circling behavior as well as the time when rotatory biases were expressed.

Circling behavior was arbitrarily considered to be established when a particular mouse circled at a rate of at least 10 rotations per minute during two consecutive test periods. The transition from not circling to circling was typically quite abrupt and usually occurred within a period of one week. Among 15 females, nine had begun to circle by postnatal day 28, and all 15 were circling by day 43. In contrast, only seven of 23 males had begun to circle by day 28, and five were still not circling as late as day 50. The ability of each mouse to hear and to swim was also tested, and, in general, there was no clear correlation between the time when a particular mouse began to circle and the time when the same mouse showed behavioral evidence of inner ear disease.

Observation of numerous adult shaker-1 mice had previously indicated that the rotatory biases of adults were maintained, in most cases, over the lifetime of the individual. Twenty-five of the 38 mice in the initial part of this developmental study were also tested as adults. Seven of the 25 adults showed a bias for circling in a preferred direction that was greater than 3:1, that is, the ratio of the total number of circles in the preferred direction to the total number of circles in the non-preferred direction was greater than 3:1. Even as early as the first week of testing after the initiation of circling activity, five of these seven had displayed biases that were similar in magnitude and in the same direction as their adult rotatory biases. Likewise, shaker-1 mice which showed weak rotatory biases as adults tended to show little or no bias during initial periods of circling activity. These preliminary observations suggest that the mechanism underlying the behavioral asymmetry of shaker-1 mice may be established quite early, perhaps even before circling behavior is actually expressed.
Supported by NIH Grant RR 05712.

200.1 A LONG-LASTING BEHAVIORAL CHANGE IN THE NUDIBRANCH MOLLUSC AEOLIDIA PAPILLOSA. M.B. Boyle*, L.B. Cohen, L.G. Harris* and K.P. Irons*. Dept. of Physiology, Yale University School of Medicine, New Haven, CT, 06510, and Dept. of Zoology, University of New Hampshire, Durham, N.H., 03824.

We have attempted to repeat in the nudibranch, A. papillosa, a behavioral paradigm previously used in the prosobranch Urosalpinx cinerea (Wood, Malacologia 6, 267, 1968) and in the pulmonate, Achatina fulica (Croll and Chase, Behav. Biol. 19, 261, 1977). This paradigm, which has been called injestive conditioning, consists of feeding the animal only one food and testing for changes in orientation to food orders in an olfactometer. Aeolidia were collected from the coast of Massachusetts and New Hampshire where they prey on the anemone, Metridium senile. The Aeolidia were divided into two groups, one fed only Metridium and the other only Anthopleura elegantissima (clonal), an anemone obtained from the California coast and never before eaten by the Aeolidia. The two groups were then compared using a simple olfactometer consisting of a rectangular box 31 cm x 6 cm with two inlets at one end, and a 18 cm long, vertical partition dividing the box at the inlet end. The sea water coming into one inlet had passed through a container of Metridium; that coming through the other inlet had passed through a container of Anthopleura. In several trials with 8 to 12 animals in each group, animals which were fed Anthopleura showed a significant ($p < 0.02$, one-tailed t test) preference for the side of the olfactometer receiving Anthopleura odor when compared with animals which were fed Metridium. This effect was observed 24 hours after the last previous meal. There was, however, variability of unknown origin; in other trials the differences between groups were not significant although the direction of the change in preference was always the same.

Compared to the two snails, the Aeolidia nervous system may be more amenable to investigation of the physiological basis of such behavior changes. We hope that our experiment can be improved to the point that the change in behavior can be demonstrated in each animal.

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200.2 OPERANT CONDITIONING OF CLAW POSTURE IN THE CRAYFISH. W. Stern-Tomlinson, S.B. Ellis*, and G.L. Gerstein. Depts. of Physiology and Biology, U. of Pa., Philadelphia, PA 19104

Procambarus clarkii were given an electric shock train to the tail in order to reinforce the maintenance of the dactyl within a selected position range. This study differs in method from a previous one (Stafstrom and Gerstein, Br. Res., 134: 185, 1977), because the shock is now removed from the body part being conditioned; therefore, behavioral changes cannot be due to antidromic stimulation of the claw neuromuscular system. The present experiments were done to determine the feasibility and design of future neurophysiological investigations of conditioned claw behavior.

Measurements of dactyl position were made for both claws, although only one (E) was effective in controlling the shock. In principle, the noneffective claw (N) could serve as a naive control, and neurophysiological data pertaining to its behavior could then be used as a point of departure in assessing the determinants of conditioned behavior in the E claw. Our findings were as follows:

1. The E claw spends a significantly greater proportion of time in the position zone affording safety from shock than it did before training.
2. The conditioned behavior is usually maintained for a minimum of 30 minutes to an hour.
3. Animals can learn to open the claw from a more closed posture, or to close the claw from a more open posture, indicating the plasticity of the behavior. Therefore, the behavior is unlikely to be a reflex response to shock which, in such a case, would drive ascending central neurons directly or through tail receptors. Such behavior would be more stereotyped.
4. The behavior of the N claw is not random; it is consistently related to the behavior of the E claw. Therefore, it cannot be used as a naive control.

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200.3 First- and Second-Order Associative Learning in the Terrestial Mollusc, Limax maximus. Chris Sahley, Alan Gelperin & Jerry W. Rudy*. Departments of Biology and Psychology, Princeton Univ., Princeton, New Jersey, 08544.

Olfaction plays an important role in the localization and selection of food in many molluscs, including Limax. Potato (P) and carrot (C) odors normally signal the availability of preferred food sources and slugs readily locomote toward these odors. We present evidence that the signal value of these odors can be modified through first- and second-order conditioning such that slugs no longer approach the formerly highly attractive food odors.

In a two-phased Pavlovian conditioning paradigm, experimental slugs, Group SOC, were exposed first to pairings of carrot odor and quinidine sulfate, a bitter tasting plant extract (Phase 1) and in Phase 2 were exposed to paired presentations of carrot and potato odors. Slugs in two additional groups controlled for the Phase 1 and Phase 2 pairing operations. Slugs in Group U/P received unpaired (U) presentations of carrot and quinidine in Phase 1 and paired (P) presentations of carrot and potato in Phase 2. Slugs in Group P/U received paired presentations of carrot and quinidine in Phase 1 but unpaired presentations of carrot and potato odor in Phase 2.

First-order conditioning was demonstrated by the result that the slugs in the two groups that received Phase 1 carrot-quinidine pairings (SOC and P/U) displayed a reduced preference for carrot odor in comparison to slugs in Group U/P that experienced unpaired presentations of carrot and quinidine. Second-order conditioning was demonstrated by the result that slugs in Group SOC (that received both Phase 1 C-Q and Phase 2 P-C pairings) displayed a reduced potato-odor preference in comparison to slugs in Groups P/U and U/P. These observations not only suggest that the slug is capable of associative learning, they also suggest that associative learning processes may play an important role in regulating the food selection of slugs in their natural environment. Thus with behavioral procedures that allow a clear definition of the variables that influence associative learning by the intact slug, and with an in vitro preparation that is amenable to cellular analysis, we hope to make progress in understanding the synaptic interactions that underly associative learning.

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200.4 REWARD LEARNING IN DROSOPHILA MELANOGASTER. B.L. Tempel* and W.G. Quinn*. (SPON: R.W. Cholewiak). Dept. of Biology, Princeton University, Princeton, N.J. 08544.

The fruit fly, Drosophila melanogaster, can learn to avoid specific odors, colors, or body postures if they are presented with aversive reinforcers such as electric shock, mechanical vibration, or bitter quinine powder. We report here a new training procedure, using positive reinforcement, in which hungry flies learn to choose a specific odorant that was previously presented in association with a sucrose solution. After being starved 18-20 hrs, a population of hungry flies are run alternately towards two odorants, 3-octanol and 4-methyl-cyclohexanol, in 17 x 100 mm test tubes. In one of the odorant tubes they are given the opportunity to feed on a localized solution of 1.0 M sucrose. The flies' odor preference is then tested in a T-maze, with each of the two chemical odorants in one arm. Usually about 2/3 of the flies run toward the sucrose-associated odor, while 1/3 of the population runs toward the control odorant. This learning performance found with sucrose reward is comparable to that found with electric shock reinforcement. However, memory persists much longer after reward training.

The positive nature of the sugar reinforcements is seen most clearly when the flies encounter only one odor during training and choose between that odor and air at testing. The odorant alone is slightly aversive; however, after pairing the odor with sucrose, trained flies will choose that odor over air. Exposure to sucrose does not itself change the flies' natural aversion to an odorant unless it is presented simultanously with the odor.

For reward learning to occur, the reward solution must be delicious but not necessarily nutritious: flies will choose odorants associated with fucose, which is tasty but not metabolizable by the flies.

Three single gene mutants selected on the basis of their failure to learn negatively reinforced tasks have been tested in this sucrose reward paradigm. We find that one mutant (cabbage) fails both testing procedures while two others (dunce and rutabaga) show some learning on the sucrose reward task.

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200.5 PAVLOVIAN CONDITIONING IN PLEUROBRANCHAEA: DISCRIMINATIVE RESPONSES AND ELECTROPHYSIOLOGICAL CORRELATES. G. J. Mpitsos, C. S. Cohan*and A. D. McClellan*. Dept. of Anatomy, Case Western Reserve Univ., Cleveland, OH 44106.

Previous work on Pleurobranchaea has dealt primarily with operant food aversion training in which a squid stimulus (Ssq) was paired with electrical shocks (ES) (Mpitsos, Collins and McClellan, Science, 199: 497, 1978). Using a Pavlovian paradigm, we have trained these marine molluscs to cease feeding and to withdraw from another palatable stimulus prepared by boiling a commonly available beer to 0.1 volume and then diluting it to various concentrations (Sbr) as described for Ssq. In "discriminative test" experiments, animals were tested for feeding and withdrawal thresholds to Ssq and Sbr before and after training but were given only the Sbr and ES during training. Experimental animals (E's) were given only 5 trials spaced 2 hrs. apart of 60 sec. of Sbr with an overlapping ES during the last 50 sec.; concurrently run control animals (C's) were given the same amount of stimulation and handling but Sbr was unpaired from ES by 1 hr. Before training, all animals exhibited equal feeding thresholds and did not withdraw from the Ssq or Sbr, which, by definition, indicated that they did not discriminate between Ssq and Sbr. After training, the E's withdrew from and had greatly suppressed feeding behavior to Sbr but not to Ssq; C's were little changed from pretraining and were significantly different from E's. Similar associational response differences that persisted for several weeks after training were obtained from animals that were given the above E and C procedures together in a discriminative conditioning paradigm. Moreover, suspended and semi-intact preparations that had been dissected and attached with electrodes to muscles and nerves exhibited conditioned discriminations similar to those obtained from them before dissection. About 30% of the preparations that had ingested the CS^- (Ssq) not only withdrew and ceased feeding when the CS^+ (Sbr) was coapplied, but they also regurgitated or rejected the ingested CS^-. Despite the fact that obviously different behavioral responses were observed, the underlying motor programs recorded electrically were indistinguishable, except for the active phase of regurgitation as reported for untrained animals (A. D. McClellan, Soc. for Neurosci., 1978 and 1979). Along with feeding, regurgitation and rejection, the mouth, jaws and radula of Pleurobranchaea are also used cyclically in at least two other behaviors: defensive bite and self and inter-animal gill grooming. Thus, unless there are criteria for one behavior to distinguish it from all other motor programs produced by the same appendages, it is presently impossible to establish unambiguously in Pleurobranchaea, or other animals, the neurophysiology of conditioned or unconditioned responses without using preparations in which a vigil can be kept on the behavior itself. (NSF BNS 76-8123)

200.6 DEFENSIVE CLASSICAL CONDITIONING IN APLYSIA: EVIDENCE FOR CONDITIONED FEAR IN AN INVERTEBRATE. E.T. Walters,* T.J. Carew and E.R.Kandel. (SPON: R. Ambron). Div. of Neurobiol. & Behavior, Depts. Physiol. & Psychiat., P & S, Columbia Univ., N.Y. State Psychiatric Inst., New York, N.Y. 10032.

Aversive associative learning has recently been demonstrated in a number of simple invertebrates. In mammals aversive conditioning is thought to produce conditioned fear (McAllister and McAllister, 1971). This raises the question: to what degree do the effects of aversive conditioning in invertebrates resemble conditioned fear in higher animals? We have begun to examine this question in Aplysia californica, a gastropod mollusc. We have previously shown that Aplysia can form a classically conditioned association between a chemosensory conditioned stimulus (CS, shrimp extract) and an aversive unconditioned stimulus (US, electric shock to the head). The associative learning is expressed as a powerful facilitation of escape locomotion by the CS (Walters et al., 1979, PNAS, 76). One prediction of a conditioned fear hypothesis is that the fear-inducing CS should modulate additional behaviors in a motivationally consistent pattern: defensive behaviors should be enhanced while appetitive behaviors should be suppressed or unaffected (Konorski, 1967). To test this prediction we have examined whether the CS, after conditioning, can enhance four different defensive responses varying in complexity: escape locomotion (a complex fixed action pattern), inking (an all-or-none fixed act), and siphon and head withdrawal (two graded reflex acts).

In each experiment two groups of animals (8 to 15 animals per group) were trained: one with the CS and US temporally paired, the other with the CS and US explicitly unpaired. Twenty-four hours after training each group was tested using a blind procedure. Siphon withdrawal and escape locomotion were triggered by tail shock and were examined simultaneously in the same animals. Inking was triggered by noxious shock to the body wall. Head withdrawal was elicited by application of the CS to the head in a "split-foot" preparation (Hening et al., 1979, Brain Res: 179). We found that, in the presence of the CS, each of the four defensive responses was significantly facilitated in paired compared to unpaired animals ($p < .005$ in each case). We are presently examining whether the CS can inhibit appetitive responses such as feeding, in addition to facilitating defensive responses.

Our results show that, after aversive classical conditioning, the CS acquires the ability to facilitate four distinct defensive responses ranging from simple withdrawal reflexes to complex motor programs. The fact that several response systems are modulated by the CS in a motivationally consistent pattern supports the hypothesis that some invertebrates, such as Aplysia, can learn a form of conditioned fear functionally similar to that exhibited by vertebrates.

200.7 DEFENSIVE CLASSICAL CONDITIONING IN APLYSIA: FUNCTIONALLY DISTINCT MOTOR SYSTEMS SHOW DIFFERENT NEUROPHYSIOLOGICAL CORRELATES. T.J. Carew, E.T. Walters,* and E.R. Kandel. Div. of Neurobiol. & Behavior, Depts. Physiol. & Psychiat., P & S, Columbia Univ., and N.Y. State Psychiatric Inst., New York, N.Y. 10032.

Aplysia can learn to associate a chemical conditioned stimulus (CS, shrimp extract) with an aversive unconditioned stimulus (US, electrical shock to the head). After pairing with the US, the CS becomes capable of facilitating a number of defensive behaviors: escape locomotion, inking, siphon withdrawal and head withdrawal. These findings have led us to propose that Aplysia may exhibit a form of conditioned fear (Walters et al., 1979, PNAS, 76; Neurosci. Abstr., 1980).

As a first step towards a cellular analysis of associative learning we have searched for neuronal correlates of conditioning. All animals first received either paired or specifically unpaired (control) training. We then examined (blind) three separate motor systems: escape locomotion, inking, and siphon withdrawal, using a modified "split-foot" preparation (Hening et al., 1979, Brain Res: 179) for recording intracellularly from motor neurons during behavioral testing. To study escape locomotion we applied the CS to the head and then shocked the tail. In the presence of the CS, paired animals (N=10) showed significantly greater numbers of steps and significantly more action potential bursts in pedal motor neurons than unpaired animals (N=10; $p < .01$ and $p < .025$, respectively). To study inking and siphon withdrawal we first applied a test stimulus to the tail. The CS was then applied to the head and a second test stimulus was delivered. In the presence of the CS paired animals (N=8) showed significant facilitation of the amplitude and duration of complex EPSPs elicited by the test stimuli in both ink and siphon motor neurons compared to unpaired animals (N=9; $p < .005$ in each case).

These results demonstrate that neuronal correlates of associative learning are different in functionally distinct motor systems in Aplysia. In the locomotor system, where the learned behavior is expressed as an increase in a recurrent motor sequence, the CS enhances the output of the central program, leading to an increase in the number of spike bursts in the motor neurons. By contrast, in the inking and siphon withdrawal systems, where the learning is expressed as a decrease in threshold or an increase in response amplitude, the CS enhances afferent synaptic input directly onto the motor neurons.

Examining the effects of learning in the identified motor cells of several different behavioral systems is useful for two reasons: first, it may allow us to select from a number of different behaviors the simplest monosynaptic test system for examining the mechanisms by which a motor response is modulated by associative learning. Second, it may simplify the search for neurons that are causally related to the associative learning.

200.8 SUPPRESSION OF THE GILL WITHDRAWAL REFLEX OF APLYSIA BY ARGININE VASOTOCIN. J.A. Thornhill, Ken Lukowiak*, K.E. Cooper and W.L. Veale. Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

Over the last year we found that arginine vasopressin (AVP), in picomole concentrations, caused a marked reduction in the amplitude of the gill withdrawal reflex (GWR) in Aplysia evoked by tactile stimulation of the siphon. In addition, AVP caused the rate of habituation of the reflex to be significantly increased. Finally, AVP caused a reduction in the excitatory input to central gill motor neurons (CGMN's) evoked by siphon stimulation. Recently, our laboratory has shown (Fed. Proc. 39; 1743, 1980) that a related neurohypophyseal peptide, arginine vasotocin (AVT), is present in the nervous system of Aplysia, being most concentrated in the pleural ganglia. Thus, the present experiments were designed to determine if exogenously applied AVT affected the GWR and the neuronal activity evoked by siphon stimulation in a similar manner as AVP.

The preparation consisted of the siphon, mantle, gill and abdominal ganglion (PVG) of Aplysia californica. The branchial, ctenidial and siphon nerves were left intact, all other nerves were severed. The PVG was isolated in a leak-proof chamber from the rest of the preparation so that AVT could be infused directly over the PVG. The GWR was evoked by tactile stimulation (1g) of the siphon with the 'Tapper'. An infusion of AVT (10^{-12}M) for 15 min over the PVG caused a marked and significant decrease in the amplitude of the GWR and increased the rate of reflex habituation. In addition, AVT caused a decrease in the number of action potentials evoked in the CGMN's by the siphon stimulus. The suppressive effects of AVT were completely reversible following washout with artificial seawater and a 3 h rest. AVT did not affect the passive membrane properties of the CGMN's. It is thought that AVT brings about its effects by increasing the activity of neurons which pre-synaptically 'gate' the input from sensory neurons to the CGMN's and which also results in increased CNS suppressive output to the PNS in the gill which ultimately mediates gill reflex behaviours.

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200.9 THE EFFECTS OF VASOTOCIN ON ABDOMINAL GANGLION NEURONS IN APLYSIA ARE NOT MEDIATED BY THE NEUROSECRETORY BAG CELLS. J.P. Edstrom*, K. Lukowiak*, J.A. Thornhill (SPON: K.E. Cooper).
Division of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

Previous reports in the literature have described how bag cell activity and exogenously applied bag cell extract are able to inhibit the pacemaker cells L_3-L_6 in the abdominal ganglion of Aplysia for long periods of time while the same procedures increase the activity of another pacemaker, R_{15} (Mayeri et al., 1979, Sci., 204, 417).

Several recent series of experiments in this laboratory have investigated the effects of the peptides vasotocin and vasopressin on Aplysia neurons. These experiments have demonstrated the presence of significant concentrations of vasotocin in the Aplysia central nervous system and that the peptides exert a prolonged suppressive influence on the gill withdrawal reflex and inhibit pacemakers L_3-L_6 while exciting R_{15} (1980, in press). Because the hormone synthesized and released by the bag cells has been identified (Chiu et al., 1979, PNAS, 76, 6656) and is known to be neither vasotocin nor vasopressin, the similarity of the actions of vasotocin and bag cell activity on the identified pacemaker cells suggested the possibility that the effects of vasotocin and vasopressin on the pacemaker cells was an indirect one caused by activation of the bag cells. This was tested by simultaneously observing the activity of identified abdominal ganglion pacemaker and bag cells before, during and after bathing the ganglion in artificial sea water containing between 10^{-6} and 10^{-12}M/ℓ of either vasopressin or vasotocin. Standard intracellular techniques were used to record from the cells L_3-L_6, R_{15} and bag cells. The artificial sea water was maintained between 16 and 18 degrees Celsius and had a pH between 7.7 and 7.9. At no time was either vasopressin or vasotocin observed to activate the normally quiescent bag cell population. Subsequent stimulation of the right connective nerve could usually activate the bag cells. This resulted in a prolonged inhibition of the L pacemakers and activation of R_{15} and showed that the lack of response of the bag cells to either vasopressin or vasotocin was not due to an inability of the bag cells to fire. At the same time vasopressin and vasotocin had their normal effects on cells L_3-L_6 and R_{15}. Although a direct action of vasotocin and vasopressin on the pacemaker cells has not yet been proved, these results do indicate that the effects of vasotocin and vasopressin are not mediated by bag cells.

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200.10 BIOCHEMICAL CORRELATES OF A LONG-TERM ASSOCIATIVE BEHAVIORAL MODIFICATION IN HERMISSENDA: CHANGES IN THE LEVELS OF SPECIFIC PHOSPHOPROTEIN BANDS FOLLOWING A CONDITIONING PROCEDURE. J.T. Neary, T. Crow and D.L. Alkon. Lab. of Biophysics, NINCDS, NIH, Marine Biological Laboratory, Woods Hole, MA 02543.

A conditioning procedure consisting of the temporal association of light and rotation produces a long-term modification of photopositive behavior in the nudibranch mollusk, Hermissenda crassicornis (Crow, T. and Alkon, D., 1978, Science 201:1239). Cellular neurophysiological changes in B photoreceptors (persistent depolarization and increased input resistance) following training were correlated with the modification of photopositive behavior (Crow, T., and Alkon, D., 1980, Science, in press). In an effort to investigate biochemical correlates of the behavioral modification, we have examined protein phosphorylation in the eyes of animals from trained, unpaired, random, and normal control groups. Following three days of behavioral training, the circumesophageal nervous systems were dissected from trained animals and control groups and incubated separately in artificial sea water containing 0.625 mCi ^{32}Pi/ml for 2 hr at 15°C. Eyes were then dissected from the nervous systems on a cooling plate and dissolved in lysis solution. Total ^{32}Pi incorporation into proteins was determined by TCA precipitation. No significant differences were detected in total protein phosphorylation levels (paired $\bar{X}$=10,500 $\pm$ 4910 cpm/eye; random $\bar{X}$=10,900 $\pm$ 5410 cpm/eye; unpaired $\bar{X}$=9560 $\pm$ 3320 cpm/eye). Aliquots of the eyes were subjected to one-dimensional SDS-polyacrylamide gel electrophoresis, and autoradiograms of the gels were analyzed by densitometry. Seven phosphoprotein bands were detected in the eyes from all animals. In paired vs. control groups, there were no significant differences in five of the phosphoprotein bands. However, significant overall differences between paired and control groups ($p<.01$) were found for bands with approx. MW of 23,000 and 20,000 daltons. Post-hoc comparisons revealed that the paired group was significantly different from both the random and unpaired control groups ($p<0.01$) while the controls were not different from each other. One phosphoprotein band (approx. MW=23,000) was increased 49% in paired as compared to random controls. The second phosphoprotein band (approx. MW=20,000) was increased 56% in paired as compared to random controls. The changes in the two phosphoprotein bands, as mediated perhaps by protein kinases and/or phosphatases, may be related to the increase in input resistance in type B photoreceptors observed in trained animals and to the increase in the long-lasting depolarization in type B photoreceptors observed in isolated nervous systems following pairing. In summary, the data presented here indicate that the phosphorylation of specific proteins in the eyes is correlated with the acquisition of an associative behavioral modification in Hermissenda.

201.1 SPINAL MODULATION OF THE ACOUSTIC STARTLE RESPONSE: THE ROLE OF NOREPINEPHRINE, SEROTONIN, AND DOPAMINE. D.I. Astrachan* and M. Davis. Dept. of Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06508

The acoustic startle reflex is being used increasingly as a model system to study how different neurotransmitters modulate reactivity to sensory stimuli. Systemic administration of serotonin (5-HT), norepinephrine (NE), or dopamine (DA) agonists increase acoustic startle amplitude. Acoustic startle involves a brainstem-spinal cord reflex arc. Anatomical studies indicate that spinal motor neurons receive dense 5-HT and NE inputs. Single unit recording studies indicate that 5-HT and NE facilitates excitation of motor neurons produced by afferent stimulation. Behavioral studies indicate that 5-HT or NE facilitates spinal reflexes in spinal or decerebrate animals. It is thus possible that excitatory effects on startle produced by systemic administration of 5-HT or NE agonists result from a direct action in the spinal cord.

To test this rats were implanted with catheters in the lumbar enlargement of the spinal cord (Yaksh & Rudy, Physiol. Behav., 1976, 17, 1031-1036). After recovery they were presented with startle-eliciting noise bursts before and after infusion of different compounds onto the spinal cord (intrathecally).

Intrathecal administration of 5-HT increased acoustic startle. This effect was potentiated and prolonged by intraperitoneal (ip.) administration of pargyline. The 5-HT agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) also markedly increased startle. Effects of 5-HT or 5-MeODMT were blocked by ip. administration of the 5-HT antagonists cinanserin or cyproheptadine but not the NE antagonist WB-4101. Intrathecal administration of NE or the α_1-agonist phenylephrine also increased startle amplitude. These effects were completely blocked by the α_1-antagonist WB-4101 given ip., but not by cyproheptadine. In contrast, intrathecal infusion of a wide range of doses of the β-agonist isoproterenol, or the DA agonist apomorphine did not alter startle. Intrathecal infusion of d-amphetamine markedly increased acoustic startle. This effect was completely blocked by ip. administration of WB-4101, but not by cyproheptadine, propranolol, or haloperidol.

The results indicate that excitatory effects of systemically administered 5-HT and NE agonists on startle may be mediated partially or even entirely by the spinal cord. On the other hand, excitatory DA agonist effects must involve supraspinal systems. More generally, this study demonstrates the importance of the spinal cord as a potential target for mediating drug-effects on behavior since, in fact, most behaviors used in psychopharmacology ultimately involve spinal output.

201.2 EFFECTS OF LOCUS COERULEUS LESIONS ON SELF-STIMULATION BEHAVIOR IN RATS-REVERSIBILITY BY MORPHINE ADMINISTRATION. A. Tempel, S.S. Steiner, D. Ocheret*, C. Pavlides* and S. Berman*. Behavioral Physiology Lab., Dept. Psych., The City College of New York, New York, N.Y. 10031.

It has been shown that an important relationship exists between central nervous system mechanisms of reward and drugs of abuse, the nature of which were explored in the following study.

Nine rats were stereotaxically implanted with 2 pairs of bipolar stainless steel electrodes aimed at one of the following combinations: fields of forel (ff) and crus cerebri (cc); medial forebrain bundle (mfb) and ff; mfb and cc. Stimulation consisted of biphasic rectangular pulse-pairs. Each bipolar electrode was used in a monopolar fashion. Animals received 7 days saline (0.9%), 1 day naloxone(1mg/kg), 7 days saline, 7 days morphine (2.5mg/kg), 1 day morphine + naloxone and 6 days post-drug saline. Subjects were then given d- and l- isomers of amphetamine alternated in an ABBA series, followed by 7 days saline. All doses were 1mg/kg, injected s.c. 40 min. before the beginning of the ICSS session. Acute locus coeruleus lesions (LC) were then made unilaterally in all animals. Following the lesion, animals were run for 21 days of saline to allow for complete fiber degeneration and neuroheumoral depletion. ICSS drug paradigms post-lesion were identical to the pre-lesion paradigm.

Prior to lesion, animals showing a large facilitation in ICSS response rates during morphine administration, also showed a large increase in ICSS rates to the d- but not the l- isomer of amphetamine. Animals showing no response to morphine administration, had ICSS rates that were equally enhanced by both isomers of amphetamine.

Post-lesion saline rates were depressed in animals where the lesion included areas of the LC proper, ventral LC and/or subcoeruleus area. These animals maintained their response facilitations under morphine administration, which were naloxone reversible.

Animals, in which the LC was spared, showed no alterations in saline ICSS rates post-lesion and maintained their response facilitation under morphine administration.

These data indicate that lesions in the area of the LC produce marked decreases in ICSS behavior which are reversible by morphine administration.

201.3 ENDOGENOUS OPIOIDS AND MOTIVATION: AVERSIVE PROPERTIES OF NALOXONE IN DRUG-NAIVE RATS. D. van der Kooy, R.F. Mucha* and M. O'Shaughnessy*. Depts. of Anatomy and Pharmacology, Univ. of Toronto, and Addiction Research Foundation of Ontario, Toronto, Canada M5S 1A8.

We have previously reported that rats prefer environments in which they receive intravenous or intracranial morphine compared to environments in which they receive corresponding saline treatment (Soc. Neurosci. Abstr. 1979, 5, 657). In the course of investigating the pharmacological blockade of this place preference effect, it was found that naloxone alone (in drug-naive rats) produced a place aversion.

A detailed analysis of naloxone-induced place aversion is now reported. Male Wistar rats were placed in one of two separate environments: a black box with a black plexiglas floor or a similar white box with a sawdust floor. They received naloxone injections on daily 30 min trials for 4 days in one box. Similar saline training trials were also given each day in the other box. On day 5 rats were tested by providing simultaneous access to both training environments for 15 min and measuring the amount of time spent in each environment. Naloxone produced aversions, for the environment in which it was administered, over a wide range of intravenous doses (0.1, 0.5, 2.0 and 45.0 mg/kg).

At the lower doses naloxone injections during training did not produce any gross behavioral changes except for a possible small decrease in locomotor activity, but the 45.0 mg/kg dose produced behavioural convulsions during training and an especially strong aversion on the test day. The naloxone aversion was stereospecific, as 0.5 mg/kg (+)-naloxone failed to produce place conditioning. In morphine dependent rats (subcutaneous 75 mg morphine base pellet 3 days prior to training), potent naloxone aversions were seen at .004 and .025 mg/kg doses. This suggests that morphine dependence may simply be an exaggeration of a process present in drug naive animals.

In an attempt to see if the naloxone aversion could be mediated by receptors in the brain, naloxone training was carried out with 10 µg injected into the lateral ventricle. No aversion was seen with ventricular injections, however a total of 10 µg of naloxone injected bilaterally into the central nucleus of the amygdala did produce a clear aversion. This site has recently been shown to be particularly sensitive to naloxone induction of withdrawal in morphine dependent rats (Lagowska et al., Neurosci. Letter 1978, 8, 241). Bilateral morphine injections (10 µg total) into the central nucleus of the amygdala produced only small, relatively insensitive place preferences. These results suggest that decreasing activity in endogenous opioid systems may induce changes in reward, and further that other effects of naloxone (eg. in feeding) may be secondary to the motivational consequences.

201.4 SEROTONERGIC AND DOPAMINERGIC MEDIATION OF THE DISCRIMINATIVE STIMULUS PROPERTIES OF LISURIDE. Francis J. White and James B. Appel. Behavioral Pharmacology Lab., Dept. Psych., University of South Carolina, Columbia, S.C., 29208, USA.

Lisuride hydrogen maleate (LHM) is a semi-synthetic ergot derivative, structurally similar to LSD, which has many of the biochemical, physiological and behavioral effects of this potent hallucinogen. However, LHM is reportedly not hallucinogenic in man (Somerville and Herrmann, Headache, 18, 75-79, 1978) and is used clinically in the treatment of migraine as well as endocrine disorders. This study investigates the discriminative stimulus properties of LHM in order to elucidate its mechanism of action in vivo and to compare this mechanism with that of LSD in identical behavioral situations.

Male albino rats were trained to discriminate LHM (.08 mg/kg) from saline in a two-lever, water-reinforced drug discrimination task. Following acquisition of this discrimination, the rats were divided into three groups (n=13/group) which were then exposed to a 'fading' procedure in which the training dose of LHM was either increased gradually, decreased gradually or remained the same. By using this procedure, the three groups were trained to discriminate a relatively wide range of doses of LHM (.02 mg/kg, .08 mg/kg, .32 mg/kg) at high levels of accuracy (>95%). Substitution tests with various novel compounds, conducted under extinction conditions, revealed that LSD (.01-.64 mg/kg), and the dopamine (DA) agonist, apomorphine, (.25-4.0 mg/kg), produced significant LHM-lever responding which depended on both the dose of the test drug and the dose of the training drug (LHM). The serotonin (5-HT) agonists quipazine and 5-methoxydimethyltryptamine produced intermediate results. Both the DA antagonist, haloperidol, and the 5-HT antagonist, methiothepin, were capable of reducing (blocking) the LHM cue; the amount of reduction depended on both the dose of the antagonist and the training dose of LHM.

These results indicate that the LHM cue, unlike the LSD cue (Kuhn et al. Neuropharmacology, 17, 257-263, 1978), is mediated by interactions with both DA and 5-HT systems. They also support the hypothesis that transfer and antagonism results in drug discrimination studies depend, to some extent, on the dose used to establish the discrimination.

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201.5 MULTIPLE DA RECEPTORS AND HETEROGENEITY OF APOMORPHINE RESPONSES DURING SIX MONTHS NEUROLEPTIC ADMINISTRATION. John L. Waddington and Stephen J. Gamble. (SPON: A. Taylor). Div. of Psychiatry, MRC Clinical Research Centre, Harrow, Middlesex HA1 3UJ, U.K.

The concepts that the prolonged antipsychotic action of neuroleptic drugs might be mediated via cerebral DA receptor blockade and that neuroleptic-induced DA receptor supersensitivity may be a pathophysiological substrate of tardive dyskinesia show some superficial inconsistencies. We describe here behavioural sequelae of six months administration of phenothiazine neuroleptics.

Rats were treated with fluphenazine or trifluoperazine (FPZ and TPZ, 1.7 and 3.8 mg/kg/day in drinking water). After six months responses to low (0.15 mg/kg) and high (1.0 mg/kg) doses of apomorphine (APOM) were assessed while neuroleptic administration continued and compared with controls.

Low intensity features induced by 0.15 mg/kg APOM were attenuated after either neuroleptic. The more intense stereotypy induced by 1.0 mg/kg APOM was modified in a complex manner. After FPZ continuous sniffing was attenuated while locomotion was greatly enhanced. After TPZ continuous sniffing was attenuated and occurred with some locomotion, while 60% of TPZ animals also showed gnawing. Thus some aspects of APOM stereotypy were enduringly antagonised while others were facilitated during protracted phenothiazine administration.

Chronic neuroleptic treatment may differentially influence heterogeneous classes of DA receptor that mediate distinct DA-dependent behaviours.

We are grateful to E.R. Squibb Ltd., U.K., for facilitating this study.

201.6 EFFECTS OF SEROTONIN ANTAGONISTS ON PUNISHED AND UNPUNISHED RESPONDING IN RATS. Paul C. Mele* and Marjorie A. Caplan. Dept. of Psychol., Adelphi Univ., Garden City, N.Y. 11530

Drugs that deplete or antagonize serotonin (5HT) increase rates of schedule-controlled operant behavior that have been suppressed by electric-shock punishment. Many of these drugs have been shown to increase unpunished responding as well. It is difficult to determine whether these drugs selectively increase punished or unpunished responding, however, since in most studies punished and unpunished responding were maintained by different schedules of reinforcement and at different control rates of responding; these factors are determinants of drug effects on schedule-controlled behavior (McMillan, Fed. Proc., 1975, 34:1870). The present study therefore, sought to determine whether or not some anti-5HT agents selectively increase punishment-suppressed responding when these factors are controlled for.

The structurally distinct 5HT antagonists methysergide (ME) and cinanserin (CN) were tested in rats responding on a multiple schedule of milk reinforcement. The multiple schedule was composed of two fixed-interval 2-minute components; each component was signalled by a distinctive exteroceptive stimulus. Responding in one component was suppressed by foot-shock delivered after every fifth response (a fixed-ratio 5 punishment schedule). The sympathomimetic amine d-amphetamine (AM) and the benzodiazepine chlordiazepoxide (CD) were administered as reference drugs as their differential effects on punished and unpunished responding are well documented. ME (3-17mg/kg) and CD (5-20mg/kg) increased overall rates of punished responding while producing smaller increases in unpunished responding. CN increased punished responding at 32 or 64mg/kg but not at 8 or 16mg/kg; unpunished responding was not consistently altered by any dose of CN. AM (0.5-1.5mg/kg) produced dose-dependent decreases in both punished and unpunished responding. Since overall rates of punished responding ranged from one-half to one-fifth of unpunished rates, comparisons were made between drug-induced alterations in punished and unpunished rates from within the fixed-intervals, i.e. local rate comparisons. When local rates of punished and unpunished responding were similar, ME and CN generally increased these rates to comparable degrees. CD frequently increased punished responding more than comparable local rates of unpunished responding. AM primarily decreased local rates of punished responding while unpunished responding was increased or decreased less than punished responding. Thus, CD and AM selectively altered punished vs unpunished responding while ME and CN did not. These results do not support the notion of a serotonergic inhibitory system which specifically mediates punishment-induced response suppression. They do suggest a more general behavioral inhibitory system mediated by serotonin.

201.7 BEHAVIORAL EFFECTS OF PARAQUAT. W. Brock and J. Dougherty (R. Yokel), University of Kentucky and Veterans Administration Medical Center, Lexington, Ky.

There is evidence to suggest that the accumulation of paraquat in the body can become a toxicological problem. Because the symptoms associated with paraquat poisoning in humans include CNS changes, the effects of oral paraquat on two types of behavior were studied in two groups of Wistar rats. Paraquat dichloride was administered for 7 days in the drinking water in the home cages via graduated drinking tubes at concentrations of .07, .19, .38 and .77 mg/ml. The rats were exposed to each paraquat concentration 23 hr per day for seven days. The concentrations were administered in ascending order, with 3-10 days of water only access between 7-day exposure periods. Two behavioral procedures were used to evaluate the compound: non-cued single spatial alternation (SSA) and a fixed-ratio (FR-60) schedule. Both procedures were conducted in daily 1-hr sessions in operant chambers, required a lever press response, and utilized 45 mg sucrose pellets as reinforcers. In the SSA task, two retractable levers were presented to the rat on a discrete trial basis. Reinforcers were delivered only when alternation of responses between levers occurred on successive trials. Each daily session consisted of 100 trials, and each trial was terminated after a response, or 30 sec, whichever came first. A 10-sec inter-trial interval was programmed to occur between trials. Accuracy and speed of responding (latency) were measured. It was found that in 5 of 6 rats an average of 10-30 mg/kg/day paraquat over 7 days decreased accuracy 5-25% and increased latency 140-700% over pre-drug baseline performance levels. Significant behavioral effects persisted for 3-7 days following removal of the paraquat source. In the free operant FR 60 study, mean doses of 15-30 mg/kg/day over the 7-day exposure period in three rats resulted in a response rate decrease of 10-45%. Response rates gradually returned to baseline levels within 4-8 days after removal of paraquat from the water. Results from these studies indicate that paraquat reversibly decreases the speed of responding in discrete trial and free operant situations, and impairs the accuracy of choice behavior necessary for successfully meeting environmental contingencies.

203.1 CERVICAL SPINAL CORD POTENTIALS ELICITED BY NERVE STIMULATION. A.M. Sherwood, E.M. Sedgwick* and W.B. McKay*. Dept. of Clinical Neurophysiology, The Institute for Rehabilitation and Research, Houston, TX 77030.

A cervical somatosensory evoked potential can be recorded in man from surface electrodes placed over the 7th cervical vertebra and the suprasternal notch following median nerve stimulation at the wrist, at motor threshold. Epidural electrodes placed over the posterior midline of the cervical spinal cord were also successful in recording responses to median nerve stimulation, although with more complex morphology. (The electrodes were temporarily implanted, with distal leads externalized, as a part of the evaluation of the effectiveness of spinal cord stimulation on the patient's impaired motor control.) Responses were differentially amplified 25,000 times using a Teca AA6 MKIII amplifier, and averaged over 128 stimulus repetitions using a HP 1000 minicomputer system with a Preston 500 KHz analog to digital converter.

The responses appeared with a latency of 8 ms, a duration of 15 ms, and a peak amplitude of 10 to 30 microvolts. The early portion of the response consisted of multispike activity with a short refractory period superimposed on a slow wave background, while the latter portion consisted of only a longer duration, monophasic potential, with a longer refractory period. This waveform was recorded in three patients with electrodes at high cervical levels, above the 6th vertebra radiologically. In these patients, we were unable to elicit recordable potentials in the epidural electrodes in response to bilateral tibial nerve stimulation at the popliteal fossae.

However, recording from epidural electrodes at T1-T3 in another patient, stimulation of the tibial nerve at an amplitude sufficient to induce an H-reflex in the triceps surae muscle resulted in a response which consisted of a mono- or biphasic pulse of 1 microvolt amplitude, with a latency of 18 ms, and a duration of 3 ms. With the electrodes in this position, however, we were unable to record responses to median nerve stimulation.

These responses were compared to those made from surface electrodes, and from epidural records over the lumbar spine. The comparison showed the dependence of the large potentials upon the localized anatomy. These responses were found to be of nerve root and spinal cord origin. In contrast, the tibial responses recorded over the high thoracic spinal cord were of ascending fiber origin.

203.2 EFFECTS OF COMPRESSION AND ISCHEMIA ON ASCENDING AND DESCENDING SPINAL CORD CONDUCTION M.H.Bennett, Department of Neurological Surgery, University of Pittsburgh Medical School, Pittsburgh, PA 15261

Somatosensory evoked potentials (SEP's) have been used to monitor spinal cord function during various neurosurgical and orthopaedic surgical procedures. Since SEP's provide no information on descending long tract function, their limitations as an operative monitoring technique must be critically evaluated.

In cats ascending and descending spinal cord activity was recorded above and below the lesion sites in response to sciatic nerve and motor cortex stimulation. Potentials were measureed during the application of graded dorsal or ventral compression or following multilevel bilateral radicular artery coagulation until a conduction block occurred. SEP's and ascending activity were also measured during the production of the conduction block.

Data will be presented to show the relative decrements in ascending and descending long tract conduction produced by each type of lesion during the establishment and recovery of the conduction block. The degree of decrement of ascending and decending activity was highly dependent upon the site of compression. However, in no case was only one system affected by the compression. Ischemia equally affected both systems. SEP amplitude was approximately proportional to ascending activity amplitude. From these observations it was concluded that, although possible, it is unlikely that acute cord ischemia or compression would produce descending system deficits without detectable SEP changes. It was concluded that SEP monitoring has considerable merit as an operative monitor of spinal cord function.

203.3 A PRELIMINARY REPORT ON THE EFFECTS OF BARBITURATE COMA ON MULTIMODALITY EVOKED POTENTIALS (MEPS) IN PATIENTS FOLLOWING SEVERE HEAD INJURY. P. G. Newlon*, R. P. Greenberg and D. P. Becker. Div. of Neurosurgery, Med. Coll. of Va., Richmond, VA., 23298.

Pentobarbital (PB) has been advocated as a means of controlling intracranial pressure in patients with severe head injury. But therapeutic PB coma prevents adequate evaluation of brain function since information about a patient's neurological condition cannot be easily ascertained. We report preliminary results assessing brain function by means of multimodality evoked potentials (MEPs), i.e. visual (VER) and somatosensory (SER) cortical and somatosensory (SBSR) and auditory (ABSR) brainstem responses recorded from 13 PB treated (mean serum level 20.5 ug/ml) and 6 control severe head injury patients otherwise receiving equivalent treatments. MEP studies were obtained in treated patients and controls in the acute period following head injury (mean day 2.3) and after PB therapy was discontinued (mean day 14.9). Comparisons of evoked potential waveforms were made between treatment and nontreatment time periods in both patient groups.

All PB treated patients demonstrated an increased latency (1-3 msec) in all components of the SER and SBSR, seen as early as the Erbs Point potential - but returned to normal latency when PB was discontinued. No such change in latency was exhibited by controls. The amplitude of the P15-N20 complex in PB treated patients was not affected although SER later waves were markedly reduced in amplitude compared to controls. There were no significant amplitude or latency differences in ABSR's of PB treated patients or in the control group. The amplitudes of VER's in the PB treated group were markedly reduced and returned to control level after PB was withdrawn, but latency variability in these patients was similar to that seen in the control group.

The latency increases in the SER and SBSR may well be due to the effects of PB coma on body temperature and hence peripheral conduction velocity rather than as a result of a direct effect of PB. Mean core temperatures for the PB and control groups were 96.8 and 100.4 F. respectively. The reduced amplitude seen in cortical responses is due to depressant effects of the drug. These results demonstrate that while PB coma alters evoked potentials recorded in head injury patients, it is still possible to monitor brain function with them. Cortical activity is present, though diminished in amplitude and brainstem responses show little change except for slight shifts in latency which may be attributable to slowed peripheral conduction. Hence, as long as these effects are known and considered, assessment of neuroelectric activity in PB treated head injury patients remains a valuable technique with which to evaluate brain function. Supported by NINCDS Grant 5-20627 and T.I. Award 5K07NS00346-02.

203.4 NEURAL GENERATORS OF BRAINSTEM AUDITORY EVOKED RESPONSES. PART I: LESION STUDIES. R.H. Britt and G.T. Rossi. Div. Neurosurg. R155, Stanford Med School, Stanford, CA 94305 and Palo Alto Veterans Hospital, Palo Alto, CA 94304.

In a series of 70 pentobarbital anesthetized cats, the neural generators of click brainstem auditory evoked responses (BAERs) were studied by discrete lesions (44 animals) and gross electrode recordings (32 animals) in the nuclei and tracts of the ascending auditory pathway. Ten per second clicks were presented monaurally, filtered (150Hz-3 or 8kHz) and averaged from differential recordings between vertex and mastoid electrodes. Cochlear microphonic, N1 and N2 responses were recorded bilaterally from round window electrodes. Lesions were made either by visual aspiration, transection using a scalpel, or by stereotaxic radiofrequency thermocoagulation and verified histologically.

Five waves (I-V) were routinely recorded in this preparation. Wave I matched the latency of N1 and remained after complete aspiration of the cochlear nucleus (CN). The configuration of wave I elongated with complete CN lesions. If the CN were surgically isolated all but waves I and II disappeared. Wave II assumed an elongated configuration. Wave III was most affected by large lesions of either superior olivary complex (SOC). If the lesion was contralateral to the stimulus, the residual activity of wave III shifted to a shorter latency and merged with the latter part of wave II. Wave IV was still present but diminished in amplitude. If the lesion was in the ipsilateral SOC, all except waves I and II disappeared. Wave IV had its most significant reduction in amplitude with large bilateral lesions of the lateral lemnisci (LL) near the dorsal nucleus. Wave V could be significantly reduced by complete aspiration of the contralateral inferior colliculus (IC). A similar effect on wave V was seen with a sagittal midline transection of the brainstem extending from the commissure of the IC to the caudal medulla. The residual wave V was eliminated by a complete lesion of the ipsilateral IC. The effects of midline lesions on waves III and IV were similar to those seen with large lesions of the contralateral SOC.

These lesion studies suggest that wave I of the BAER arises at a site proximal to the cochlear nucleus, most likely activity in the eighth nerve. Wave II remained with CN isolation and disappeared with CN aspiration, suggesting that the ipsilateral CN is the principal neural generator of wave II. Waves III and IV have bilateral generators. Lesions of either SOC complex effected the greatest reduction of wave III. Wave IV was the most difficult to selectively effect with lesions, but the greatest reduction in amplitude occurred with large lesions of the rostral LL bilaterally. The studies suggest that wave V arises primarily in the contralateral IC. (Studies supported by VA RAG/Merit Review and NIH (NS 15860) Grants and the Neurosurgery Research Fund)

203.5 NEURAL GENERATORS OF BRAINSTEM AUDITORY EVOKED RESPONSES. PART II: ELECTRODE RECORDING STUDIES. G.T. Rossi and R.H. Britt. Div. of Neurosurgery R155, Stanford Med. Sch., Stanford, CA 94305 and Palo Alto Veterans Hospital, Palo Alto, CA 94304.

In a series of 32 pentobarbital anesthetized cats, gross electrode recordings to click stimuli were made in the nuclei and tracts of the ascending auditory pathway. With bipolar electrodes a biphasic waveform was recorded when the electrode was centered within a neural generator. On either side of the neural generator, a monophasic peak of the same latency was recorded, however, a reversal of polarity occurred when the electrode crossed from a site proximal to the generator to a site distal. Unipolar recordings were referenced to the same vertex electrode used to record the BAER and had a positive polarity with amplitudes best correlated with the biphasic responses recorded with the bipolar electrodes. The details of stimulus presentation and BAER recordings are given in the companion abstract. Electrode tracts and positions were histologically verified.

Recordings from cochlear nucleus (CN) showed activity comparable with wave II. The dorsal CN contributed little when compared with the high amplitude of activity recorded from the ventral CN. Recordings from the midline trapezoid body (TB) showed activity that extended from the latter part of wave II through wave III. Recordings from either superior olivary complex (SOC) demonstrated high amplitudes of activity corresponding to wave III. Wave IV activity was maximally seen in the rostral lateral lemnisci (LL) bilaterally, but was also seen caudally down to the SOC and rostrally to the inferior colliculus (IC). Activity comparable with wave III, although maximally present in the SOC, was also seen in TB recordings and rostrally in the LL and, finally, to a minor extent, in the superficial aspect of the inferior IC. Activity with a latency of wave V was first recorded at the level of the inferior colliculus; with the majority of activity arising from the IC contralateral to the stimulus.

These studies support the concept of multiple neural generators for most of the component waves of the click evoked BAER. We have divided the neural generators into "primary" and "secondary" based on their relative contribution to each component wave: Wave I: primary - 8th nerve; Wave II: primary - CN, secondary - TB; Wave III: primary - SOC bilaterally and TB, secondary - LL and IC; Wave IV: primary - LL bilaterally, secondary - SOC and IC bilaterally and Wave V: primary - contralateral IC, secondary - ipsilateral IC.

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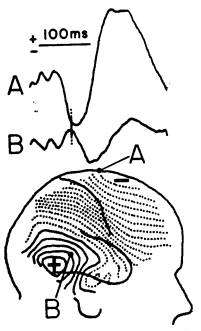
203.6 SCALP DISTRIBUTION OF HUMAN AUDITORY EVOKED POTENTIALS: A REASSESSMENT. C.C. Wood* and J.R. Wolpaw (SPON: T. Allison). VA Medical Center, West Haven, CT 06516 and Armed Forces Radiobiol. Res. Inst., Bethesda, MD 20014

Inferences about the origin of human auditory evoked potentials (AEPs) have depended heavily on their scalp distribution. Vaughan and Ritter (EEG J., 28:360, 1970) reported that the auditory vertex potential (VP), a prominent negative-positive sequence with latencies of 80-100 and 160-200 msec, reversed in polarity across the Sylvian fissure. They concluded that this distribution was consistent with a dipole source for the entire VP located in the supratemporal plane in or near primary auditory cortex. Kooi et al. (EEG J., 31:166, 1971) suggested that the apparent polarity reversal was an artifact of VP activity at the nose reference used by Vaughan and Ritter, since no reversal occurred with a chest reference. Later work has supported the Kooi et al. interpretation using even more distant noncephalic references (Picton et al., Multidisci. Perspect. in ERP Research, 1978). Wolpaw and Penry (EEG J., 39:609, 1975) reported that additional potentials occur at temporal scalp locations during the latency range of the VP.

AEPs to a monaural or binaural click (50 dB SL, 3.2 sec isi) were recorded simultaneously from 20-channel arrays over the right hemisphere in 11 adults (ages 15-49). Other electrodes were located on the nose, ear, mastoid process, knee, ankle, and at 3-cm intervals down the neck. All were referred to a balanced thorax electrode; AEPs based on other references were derived by computer subtraction. The results indicate: (1) VP-like activity is present in recordings between the nose and noncephalic locations in some but not all subjects. (2) With a noncephalic reference, polarity reversals between temporal and fronto-central scalp occur at a number of latencies, including the region of the VP negativity (see fig., which is based on grand average AEPs from all 11 subjects). However, the reversal during the VP is better characterized as the summation of potentials from several sources than as a reversal due to a single dipole source. (Note the focal temporal distribution of the 80-msec positive peak.) (3) The ear and the mastoid process, both widely used as references in AEP recordings, are significantly active relative to noncephalic references.



203.7 FAST AND SLOW COMPONENTS OF THE HUMAN AUDITORY BRAINSTEM RESPONSES. O. Özdamar* (SPON: Y. Geinisman). Siegel Institute, Michael Reese Hospital, Chicago, Illinois 60616.

Human auditory brainstem response (ABR) consists of a series of fairly regular peaks and valleys (Jewett waves) superimposed upon a broad response. These two responses, designated fast and slow components, have energy concentrations in the high and the low frequencies respectively. Recent studies (Davis and Hirsh, 1980; Huang, 1980) suggest that these components originate from different anatomical structures with different electrophysiological mechanisms. The objective of this study is to explore the characteristics of these components in normal subjects and in neurological patients.

A method of differentiating fast and slow components of the ABR is to utilize different filter bandwidths. In this study wide-band ABRs, which include both the fast and the slow components, were recorded with filter settings at 3-2000 Hz. Fast ABRs were recorded using 100-2000 Hz filter bandwidths. The slow component was isolated by subtracting the fast ABR from the wide-band ABR. To expedite recording time, simultaneous two channel recordings with different bandwidths were used in most recordings. Click stimuli were used in all the recordings.

In normals, slow responses are characterized by a vertex positive wave, occasionally followed by a negative wave of smaller amplitude. The vertex positive wave begins 4-5 msec post-stimulus onset and has a peak latency of 5-7 msec. Latencies of fast responses conform to previously published norms.

This study shows that slow and fast responses may be independently affected in neurological patients. In demyelinating diseases such as multiple sclerosis, the later components of the fast ABR can be small and fused. However, the slow component often is not, or less, affected by the disease. Generally the slow response is prolonged, diminished in amplitude or absent only at advanced stages of the disease. Data from neurological patients support the hypothesis that fast and slow components of the ABR are generated by different physiological mechanisms.

203.8 MAGNETIC AUDITORY EVOKED FIELDS: INTERHEMISPHERIC ASYMMETRY. M. Reite, J.T. Zimmerman* and J.E. Zimmerman*. Univ. of Colo. Med. Center, Denver, CO 80262 and Nat. Bureau of Standards, Boulder, CO 80302.

We recorded magnetic auditory evoked fields (AEF) from 10 normal adult human subjects using binaural and monaural stimulation. The magnetic sensor was a 2" diameter 2-D first derivative SQUID gradiometer. Subjects were recorded in an aluminum shielded room. The magnetic sensor was placed about 1 cm from the scalp centered over a point thought to approximately overlie the primary auditory cortex (approximately 1/3 of the distance from T_3 to C_3 or T_4 to C_4).

Stimuli were irregularly spaced 0.1 msec clicks with a modal inter-stimulus interval of 700 msec (range 500-1500). Sound was conducted to the ears by a system of plastic tubing. The magnetoencephalogram (MEG) was amplified, filtered with a 0.1-30 Hz bandpass, and averaged for 500 msec following 128 stimuli.

AEFs exhibited considerable interindividual variability. There was additionally some evidence of intraindividual phase reversals across time. Within an individual, recording from the same location and during a single recording session, responses were very similar. These responses were used for amplitude analysis. The amplitude of the largest magnetic responses occuring within the first 400 msec were measured for bilateral, contralateral, and ipsilateral stimulation.

Recording from the right hemisphere, 8 of 10 subjects exhibited larger amplitude AEFs to contralateral stimulation, with contralateral responses being 134$\pm$ 37% of ipsilateral responses. Using all data (several subjects were recorded more than once), mean contralateral response amplitudes from the right hemisphere were 176$\pm$ 39 femptotesla (fT) peak-to-peak; mean ipsilateral response amplitude was 134 $\pm$ 33 fT. Recording from the left hemisphere, 7 of 7 subjects had higher amplitude contralateral AEF amplitudes, with contralateral responses being 147$\pm$ 52% of ipsilateral response amplitudes. Using all data, mean left hemisphere contralateral response amplitude was 187$\pm$ 47 fT; mean ipsilateral response amplitude 129$\pm$ 43 fT. Contralateral and bilateral responses were similar in amplitude, with contralateral being of slightly greater magnitude over the left hemisphere.

These findings suggest that cortical current dipoles produced by contralateral auditory stimulation are of greater magnitude than those resulting from ipsilateral stimulation. They are compatible with the anatomical evidence that a majority of centripetal auditory input is crossed. The similar magnitude AEFs to bilateral and contralateral stimulation suggests the relationship is not simply linear. (Supported by Office of Naval Research Contract No. N00014-79-C-0383).

203.9 LUMINANCE-DEPENDENT PATTERN VEP DELAY IN HUMAN DEMYELINATING DISEASE. J. Camisa, I. Bodis-Wollner, and L. Mylin*. Department of Neurology, Mt. Sinai School of Medicine, New York, N.Y. 10029

VEP measurements are widely used in the diagnosis of MS. In humans, increased latency is commonly attributed to impaired conduction due to demyelinization in the optic nerve. However, VEP latency is not an absolute measure: parameters of the visual stimulus do affect whether or not an abnormality is found. The existence of spatial frequency and orientationally selective abnormalities in MS have been studied by psychophysical (Regan, et al., 1980) and electrophysiological (Camisa, et al., 1979) methods. Now we report the effects of pattern luminance on VEP in MS.

Visual evoked potential latencies were measured for each eye of 42 patients. Observers viewed a 2.3 c/d checkerboard pattern (4^{o} visual angle) reversing at a rate of 1 Hz. For each patient, latencies were recorded at two mean luminance levels--1.7 log ft lamberts and .7 log ft lambert--at the identical pattern contrast (90%). The patients' latencies at each luminance level were compared to the normal (15 control observers) bivariate distribution of right eye/left eye latencies. Of the 42 patients, 23 (55%) were categorized as abnormal in the high luminance condition, and 31 (74%) of the patients were classified as abnormal for the low luminance condition. This difference was significant ($p < .05$). Comparing the amplitude and the "width" of the P-100 "component" revealed no systematic differences between the two luminance conditions, either for patients who had abnormal latencies at both or only at the low luminance level.

We conclude that luminance is an important parameter in studying conduction velocity with VEP methods. Since an enhanced abnormality at low luminance does not appear to be accompanied by either VEP amplitude reduction or waveshape broadening, the data do not so far suggest either 1) less synchrony in multiple pathways or 2) a reduction in the number of pathways responding as explanations of the luminance-specific conduction defect in MS.

Supported by grant no. EY01708 of the National Eye Institute, N.I.H.; grant no. NS11631-5 of the Clinical Center for Parkinson's Disease & Allied Disorders; Core Center Grant no. EY01867 of the N.I.H.; and grant no. RR-00071 of the Division of Research Resources, General Clinical Research Center Branch, N.I.H.

203.10 SPATIO-TEMPORAL ANALYSIS OF THE HUMAN VISUAL EVOKED RESPONSE. E. Sutter. Smith-Kettlewell Inst. of Visual Sciences, San Francisco, California 94115.

Some of the factors which can cause variations of the visual evoked response with retinal stimulus position are known. They are: gradients in retinal receptor populations, gradients in receptive field properties, cortical magnification factor and gross cortical anatomy. A spatio-temporal analysis thus provides important information on these anatomical and physiological factors. Some of the data presented here were recorded with very high spatial resolution. Surprisingly, they contain features which cannot be explained on the basis of these factors alone.

A technique using spatio-temporal white noise stimuli has been employed for a simultaneous local investigation of extended retinal areas. Results from two sets of experiments are presented:

1. large area (10°), low resolution (50').
2. small area (1.5°), high resolution (3').

In both experiments, remarkable differences were found between luminance and edge response, the edge response showing a much less uniform spatial distribution. The high resolution recording showed extremely localized features extending less than 10' of arc. To determine their physiological significance, it will be necessary to study their dependence on stimulus orientation and electrode position.

204.1 TRANSIENT CATECHOLAMINERGIC CELLS IN MOUSE EMBRYONIC PANCREAS COEXIST WITH CELLS PRODUCING GLUCAGON. G. Teitelman, T.H. Joh and D.J. Reis, Laboratory of Neurobiology, Dept. of Neurology, Cornell Univ. Med. College, New York, NY 10021.

In the embryonic rat, cells containing catecholamine (CA) biosynthetic enzymes tyrosine hydroxylase (TH) and dopamine-B-hydroxylase (DBH) appear in several sites which in the adult do not contain adrenergic neuronal perikarya. Such transient CA cells are found, among other locations, in the gut of rat embryos before midgestation (Teitelman et al., PNAS 76:509, 1979). The fate of this transient population of cells is not known: they may transform into cells which synthesize other substances, possibly peptides. To test this hypothesis, we sought to determine: (a) whether a transient population of catecholaminergic (CA) cells can be detected in the embryonic pancreas; and, if so, (b) whether the presence of the CA cells overlap in time with those containing glucagon; and (c) if the CA cells are localized within the glucagon islets.

Mouse embryos at different gestational ages were processed for immunocytochemical localization of TH, L-dopa decarboxylase (DDC), DBH, and glucagon according to the PAP technique.

Cells containing TH were found in the dorsal pancreatic anlage only between days 11 and 12 of development (E11-E12). Between 12 to 25 stained cells were present in each section throughout the pancreatic primordia. These cells lack neurite-like processes and are found either dispersed or in clumps. The intensity of the PAP reaction was typically lower than that of sympathetic ganglia cells present in the same section at different sites. While cells containing immunoreactive DBH were also found in the gut, no DBH was detected in cells of the embryonic pancreas although some of them did contain DDC. Therefore, these cells are probably dopaminergic. Sections were also stained for glucagon to determine whether cells containing pancreatic peptides were localized in the same regions as the TH cells. Cells containing glucagon were found in the pancreas at E12 and their distribution and number were similar to that of the TH cells. However, while cells containing TH disappear at E12, those containing glucagon persisted throughout life. When sections were sequentially incubated with both antibodies, cells containing TH were found within the glucagon islets.

We conclude: (a) that the embryonic mouse pancreas contains a population of cells transiently expressing TH and DDC but not DBH and (b) that the TH cells coexist in time and space with glucagon producing cells. These findings suggest an intimate developmental and functional interrelationship between neural and pancreatic tissue.

(Supported by NIH grant HL 18974).

204.2 DEVELOPMENTAL ASPECTS OF SEROTONIN N-ACETYLTRANSFERASE STIMULATION AND TRYPTOPHAN METABOLISM IN PINEAL MONOLAYER CULTURE. V. Rowe, J. Parr*, and V. Steinberg*. University of Kansas Medical Center, Kansas City, Kansas 66103, and Veterans Administration Medical Center, Kansas City, Missouri 64128.

We have previously shown that pineal cells derived from neonatal rat pineal glands can be maintained in monolayer culture for extended periods. Further studies on the adrenergic pharmacology of these cultures show that stimulation of serotonin N-acetyltransferase (NAT, E.C. 2.3.1.5.) activity is mediated by receptors having both α-and β-adrenergic properties. In addition, concentration response curves for NAT stimulation suggest that α-adrenergic properties increase as the cells age and develop in culture. Furthermore, L-propranolol, a β-adrenergic antagonist, exhibits partial agonist characteristics in these cultures. Finally, rat serum was found to stimulate synthesis of metatonin, 5-methoxyindole acetic acid, 5-methoxytryptophol, N-acetylserotonin, and 5-hydroxytryptophol.

204.3 FACTORS AFFECTING THE DEVELOPMENT OF PINEAL β-ADRENERGIC RECEPTORS IN CULTURE. M. Blair Clark* and Benjamin Weiss, Dept. of Pharmacol. Medical College of Pennsylvania, Philadelphia, Penna. 19129.

Previous studies have shown a marked developmental increase in the density of β-adrenergic receptors in rat pineal gland. The present experiments were designed to determine if β-adrenergic receptors continue to develop in an in vitro preparation and whether exogenously administered catecholamines can influence their development. Timed pregnant Sprague-Dawley rats were kept under an alternating 12 hr light-dark cycle for at least one week prior to parturition. Dispersed cell cultures of pinealocytes were prepared by a modification of the method of Buda and Klein (Endocrinol. 103:1483, 1978). Cells were placed in plastic 60 mm culture dishes at a density of 2-3 x 10^6 cells/dish in a supplemented Dulbeccos Modified Eagles Medium. Whole pineal glands were cultured on stainless steel grids in plastic organ culture dishes in a modified BGJ_b Fitton-Jackson medium. The β-adrenergic antagonist ^{3}H-dihydroalprenolol (DHA) was used to quantitate β-receptors in sonicates prepared from cells or whole glands. The density of β-adrenergic receptors (Bmax) of dispersed pinealocytes prepared from 2-day-old rats increased about 2-fold after 24 hr in culture. There was no appreciable change in the dissociation constant for DHA binding (Kd). After 16 days in culture, these cells exhibited a 10-fold increase in both the Kd and Bmax for DHA binding, suggesting that long term culture causes qualitative as well as quantitative changes in DHA binding sites. Cultured whole pineal glands removed from 2-day-old rats also showed an increase in DHA binding following 24 hr or 48 hr in culture. In fact, the increase in DHA binding was quantitatively greater in culture than that seen developmentally in vivo. Whole pineal glands removed and cultured from adult rats also exhibited an increase in DHA binding. These results suggest a type of adrenergic denervation supersensitivity of β-receptors caused by the removal of catecholamine input. To examine this possibility we exposed whole pineal glands to l-isoproterenol (10 μM)during a 48 hr culture period. This treatment completely blocked the rise in DHA binding. In summary, these experiments show for the first time an increase in pineal β-adrenergic receptors in vitro. This increase apparently is genetically predetermined but can be modified by the degree of sympathetic input. This in vitro system may provide a useful model for studying further the neural and hormonal factors that modulate the development of β-adrenergic receptors. Supported by USPHS Grants NS13768 and NS16242 and NS07061.

204.4 LONG TERM CHANGES IN DOPAMINE REGULATION OF PROLACTIN SECRETION IN OFFSPRING FOLLOWING MATERNAL TREATMENT WITH HALOPERIDOL. N.R. Plach, G. Jawahir,* L.J. Grota, J.A. Seggie and M.P. Rathbone. Neuroscience Dept., McMaster Univ., Hamilton, Ontario, L8N 3Z5.

Dopamine (DA) neurons of the tuberoinfundibular system exert a tonic inhibitory influence on pituitary prolactin (PRL) secretion. This inhibitory influence can be blocked by DA antagonists such as haloperidol. We investigated whether administration of haloperidol during the prenatal period when tuberoinfundibular neurons are first developing would lead to long term changes in DA regulation of PRL secretion in offspring. Pregnant Wistar rats received daily injections of haloperidol (1.2 mg/kg SC) during the last trimester. At birth, both control (CON) and haloperidol (HAL) litters were fostered to untreated dams. Except for weighing and visual inspection once per week, the pups were left undisturbed until weaning on day 21 when they were sexed and housed in groups of 6. On postnatal day 25, half of the HAL and half of the CON offspring received an acute injection of haloperidol (0.3 mg/kg SC). The other half of each group received a control injection of vehicle. One hour later, trunk blood was collected. The results showed that serum PRL levels in response to vehicle injections were the same in both the prenatal HAL and CON groups. The rise in serum PRL levels of animals receiving the haloperidol challenge, however, was significantly less in the case of prenatal HAL offspring as compared to CON offspring.

One interpretation of these results is that the pituitaries of offspring who received prenatal haloperidol treatment developed supersensitivity to DA inhibition. In order to test this possibility, we investigated the direct effect of DA on PRL secretion in isolated pituitary incubation experiments. The anterior pituitaries from 25 day old animals, treated prenatally as in the previous experiment, were bisected and preincubated for 1 hour in aerated Medium 199 at 37°C. One of each pair of hemipituitaries was then transferred into fresh medium containing DA, the other into control medium (no DA). Following a 3-hour incubation period, the amount of PRL released in each sample was assayed and dose-response curves for HAL and CON pituitary PRL secretion were compared. The results of these experiments indicated that the pituitaries of offspring treated prenatally with HAL were more sensitive to inhibition by DA than that of CONs. From these data we conclude that maternal treatment with haloperidol can produce long term supersensitivity to DA inhibitory effects on PRL secretion in the pituitaries of offspring.

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204.5 DEVELOPMENT OF SEX DIFFERENCES IN STRIATAL DOPAMINE RELEASE in vitro. Jill B. Becker and V.D. Ramirez. Neural and Behavioral Biology Program and Dept. of Physiology and Biophysics, Univ. of Ill., Urbana, Ill. 61801.

In earlier experiments, we have reported sex differences in the amphetamine (AMPH)-stimulated release of dopamine (DA) from rat striatal tissue in vitro. In striatal tissue from adult females, the release of DA by AMPH stimulation is altered by the in vivo presence or absence of gonadal steroids. In contrast, the AMPH-stimulated DA release from striatal tissue obtained from adult males is not affected by hormonal manipulations.

The present experiments were conducted to investigate the development of these sex differences. Employing an in vitro perifusion system, we examined the effect of AMPH on DA release from striatal tissue of male and female rats: 1) pre-pubertally; 2) in adults which had received hormonal treatments neonatally that are known to alter the pattern of gonadotropin release; and 3) in the adult rat, following post-pubertal ovariectomy (OVX) or castration (CAST).

There were no sex differences in DA release at 4, 19 or 31 days of age. AMPH stimulated significant DA release from striatal tissue at all ages, regardless of the sex of the donor. Striatal tissue from feminized males (CAST at day 2, tested as adults) and control males (CAST at day 15, tested as adults) showed significant AMPH-stimulated DA release. Striatal tissue obtained from both androgenized females (500 µg testosterone propionate day 2, OVX day 15, tested as adults) and control females (OVX day 15, tested as adults) also showed significant AMPH-stimulated DA release. In contrast, with striatal tissue from females OVX as adults, there was a dramatic decremant in the release of DA in response to AMPH stimulation. In adult OVX females, there was attenuation of AMPH-stimulated DA release at 4, 11, or 45 days following OVX. However, the effect of CAST in adult males did not differ from its effect in young male rats. 45 days after CAST, there is significant AMPH-stimulated release of DA from striatal tissue.

These experiments confirm our previous report of a sex difference in striatal DA release in response to AMPH stimulation. We conclude that this sex difference in AMPH-stimulated DA release from the striatum is not found pre-pubertally and is not a function of the organizational effects of neonatal androgen exposure. Our results suggest that there are specific ovarian events which occur later in development, perhaps in association with puberty, which directly or indirectly induce changes in the striatal DA response to AMPH stimulation in female rats.
(RIAS GRANT NSF-SER 76-18255)

204.6 CHLORPROMAZINE ELEVATED PROLACTIN SECRETION IN PREPUBERTAL MALE RATS. C. Hoops* (SPON: S. Rosner). Dept. of Anatomy, Thomas Jefferson University, Phila., Penna. 19107.

Since hypothalamic dopamine acting on the pituitary prolactin cell is viewed as the principle regulatory inhibitor of prolactin secretion and chlorpromazine (CPZ) is thought a dopamine receptor blocker, administration of CPZ to rats normally causes increased prolactin secretion indicated by elevated serum prolactin concentrations. The purpose of this study was to determine whether such a response to chlorpromazine would occur in prepubertal male rats thus indicating prolactin cell secretory capacity and the presence at this stage of a dopaminergic inhibitory apparatus.

Prepubertal male Long Evans rats each received a total of 5 mg/200 g body weight in equally divided doses given in daily intraperitoneal injections over a period of time and were sacrificed on the last day of injection, at 16 or 25 days of age. (Control group animals received injections of PBS.) Serum prolactin concentrations in the chlorpromazine treated rats were significantly ($P < 0.005$-0.010) higher (22 to 55 ng/ml) than those in the control rats (≤2.5 to 16 ng/ml). Examination by electron microscopy of pituitary prolactin (mammotrophic) cells from 25 day old animals suggested morphological development (i.e. increased granule formation, cell hypertrophy) of these cells in the CPZ treated animals, presumably a result of increased secretory activity in these cells during the CPZ treatment period.

It is inferred from this work that a neural dopaminergic mechanism effecting inhibition of prolactin secretion is present at prepuberty, that a dopaminergic receptor is normally operative at this developmental stage on pituitary prolactin cells, and that the prolactin cell is capable of increased secretory activity. It remains to be determined to what extent the dopamine inhibition has matured, when the onset of neural regulation of prolactin secretion begins, and what events of neuron maturation attend the maturation of dopaminergic inhibition of prolactin secretion during the perinatal and prepubertal periods.

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204.7 CERVICALLY-STIMULATED PROLACTIN RELEASE IN THE FEMALE RAT: MODULATION BY NEONATAL STEROID TREATMENTS. L.C. Krey* and F. Kamel*, (SPON: L.-M. Kow). The Rockefeller University, New York NY 10021.

In the female rat, cervical stimulation induces a pattern of prolactin release characterized by nocturnal and diurnal surges. These surges are necessary for pregnancy. In the present study, we report that neonatal exposure to testosterone or estrogen blocks cervically-stimulated prolactin release in the mature rat.

Neonatal female rats were implanted with Silastic capsules filled with 1,4,6-androstatriene-3,17-dione (ATD, day 2-10); testosterone (T, day 3-10); 20% testosterone (LoT; day 3-10: T + ATD; or LoT + ATD. Additional females were injected with 10µg estradiol benzoate on day 3. At 60-70 days of age, the rats were castrated. At 1800 the following evening, they were subjected to cervical stimulation with a glass rod. Groups of rats were rapidly decapitated at 3-hour intervals beginning at 30 hours following stimulation. Serum prolactin and corticosterone levels were measured by radioimmunoassay.

Rats treated with ATD or LoT + ATD had ovaries filled with corpora lutea. In response to cervical stimulation, they displayed nocturnal (0300) and diurnal (1800) prolactin surges. In contrast, rats in the other treatment groups had polyfollicular ovaries and displayed a constant pattern of prolactin secretion with no evidence of nocturnal or diurnal surges. However, the diurnal pattern of corticosterone secretion was comparable in all treatment groups.

These results indicate that neonatal exposure to testosterone will influence the organization of the brain circuits mediating cervically-stimulated prolactin release as well as those regulating preovulatory gonadotropin secretion. The findings that estrogen mimics testosterone action while the aromatase inhibitor ATD blocks testosterone action suggest that the organizational influences of androgens on these neuroendocrine circuits depend on the intraneuronal conversion of androgen to estrogen and the subsequent uptake of this steroid by estrogen receptor systems.
(Supported by grant RF 70095 from the Rockefeller Foundation)

204.8 ONTOGENY OF PITUITARY THYROTROPIN-RELEASING HORMONE RECEPTOR IN THE RAT. C. Prasad and A. Banerji* (SPON: D.G. Kline). Section of Endocrinology, Department of Medicine, LSU Medical Center, New Orleans, LA. 70112

Pituitary thyrotropin (TSH) responsiveness to exogenous thyrotropin-releasing hormone (TRH) is significantly greater in neonatal than in adult rats (Prog. Endocr. Soc. Ann. Mtg. Abs. #179, 1976). This exaggerated TSH response to TRH has been explained partly by a marked inability of L-triiodothyronine (T_3) to suppress this response in young, but not adult rats. However, since in the adult there is a close correlation between the TRH-receptor density and TSH response to TRH, we postulated that the exaggerated response in the young may be due to an increase in the number of pituitary TRH-receptors.

The present study was undertaken to determine if there is any change in pituitary TRH-receptor concentration during the first two weeks of postnatal development in male and female Sprague-Dawley rats and to compare this to adult animals. The measurement of TRH-receptor number was performed as described elsewhere (Endocrinology 100: 1496, 1977) except that the homogenization buffer also contained 250 mM sucrose. The binding of ^{3}H-TRH to pituitary membranes was very low in one day old male rats (< 10 fmole TRH bound/mg protein) but increased linearly with time to a maximum at day 4 (168 fmole TRH bound/mg protein). It then declined steadily to an undetectable level by day 12. The female rats followed a similar pattern except that the maximum binding was observed at day 7 (186 fmole TRH bound/mg protein) and the decline in binding was much slower. At almost all ages studied, females possessed higher number of TRH receptors than males. The following table shows for the first time that the neonatal pituitary has significantly higher numbers of TRH-receptor than the adult.

Age	fmole TRH bound/mg protein	
	Male	Female
4-7 Days	115.0 ± 25.5(4)	164.7 ± 14.1(4)
Adult	37.0 ± 5.8(10)	58.9 ± 7.7(10)

We conclude that the availability of TRH-receptors could be an additional regulatory step in the action of TRH on the pituitary gland and the exaggerated TSH response to TRH in the neonatal pituitary may be a function of an age dependent increase in TRH-receptor density.

205.1 SELECTIVE ISCHEMIC DAMAGE TO STRIATAL GABAergic NEURONS
A.J. Francis* and William Pulsinelli* (Spon. T.E. Duffy)
Dept. Neurol., Cornell Univ. Med. Coll., New York, NY 10021

Sensitivity to ischemic damage differs markedly among neuronal populations. There are no accepted chemical or physiological explanations for this phenomenon. We reported striatal GAD (glutamic acid decarboxylase) activity was depleted (54±6% of control) at 5-8 days following 40 min of ischemia in rats, while striatal CAT (choline acetyltransferase) was stable. This suggested selective damage to striatal GABAergic neurons (Neurol 30 431). The present study determined if damage to striatal GABAergic neurons affects striatal GABA binding or GAD activity of substantia nigra (SN). Striatal GABA binding should increase in response to GABAergic denervation and GAD activity in SN should be depleted by damage to striatonigral GABAergic neurons. Transient forebrain ischemia was induced for 30 or 40 min in Wistar rats by reversible occlusion of the common carotid arteries 24 hr after cauterization of the vertebral arteries (Stroke 10 267). In this model, blood flow to striatum is 1% of control, but brainstem is partially perfused (27-35%). Neuronal damage to striatum is severe and reproducible, while brainstem neurons including those of SN are histologically normal. GAD activity was estimated by trapping CO_2 enzymatically released from L-(1-^{14}C)-glutamic acid. ^{3}H-GABA binding was modified from Campochiaro et al. In preliminary studies, GAD activity in SN was measured at 2-6 days following 30 (N=2) or 40 (N=8) min of ischemia. Activity was 78.7±2.5% and 67.4±6.4% of control respectively. This suggests depletion of GAD from SN occurs by striatonigral denervation, since the SN neurons were histologically normal. Striatal GABA binding was studied (at 5 nM) following 40 min of ischemia:

Post-isch. survival	^{3}H-GABA bound(%control±SEM)	N
6 hrs	133 ± 8	4
1 day	175 ± 27	4
3 days	427 ± 24	5

Kinetic studies of ^{3}H-GABA binding are in progress. Increased GABA binding to striatum after ischemia may represent denervation supersensitivity, similar to that reported in kainic acid lesions which also deplete striatal GAD (Campochiaro et al. Brain Res 136 501). Both depletion of GAD activity from SN and enhanced striatal GABA binding after ischemia are consistent with selective ischemic damage to striatal GABAergic neurons.

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205.2 IRON DEFICIENCY AND THE DOPAMINE RECEPTOR IN THE CAUDATE IN THE RAT. R. Ashkenazi, D. Ben Shachar* and M.B.H. Youdim*.
Department of Pharmacology, Technion, Haifa, Israel.

Behavioral and biochemical studies have demonstrated that iron deficiency in rats inhibits both 5-HT and DA mediated behaviors, but has no effect on either presynaptic synthesis of monoamines or monoamine catebolizing enzyme activity (Youdim et al., Neuropharmacol., 19:259, 1980). 21 days old rats were made iron deficient by feeding them on semi-synthetic diet low in iron, while their control received the same diet with iron supplement. After one week on the diet it was found that the behavioral response to apomorphine (2 mg/kg i.p) was decreased. The iron deficient group was anemic as was judged from the hemoglobin as serum iron values. At the same time, ^{3}H-haloperidol binding in the caudate of the iron deficient group was about 50% of that of control. When iron deficient rats (4 weeks on iron deficient diet) were put on control diet for 2 weeks it was found that only hemoglobin levels returned to normal values. The behavioral response to apomorphine, the serum iron, the non-hem iron in the brain and the ^{3}H-haloperidol binding in the caudate remained low. The decrease in the DA receptor caused by iron deficiency may explain the inhibition of the DA mediated behavior. Our results also indicate that the iron deficiency and not anemia per se may play an important role on receptor function and the response to drug treatment.

205.3 CHANGES IN CENTRAL EPINEPHRINE AND β-ADRENERGIC RECEPTORS ELICITED BY ANTIDEPRESSANTS AND α_2-ADRENERGIC BLOCKERS.
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The findings that the PNMT inhibitor SKF 64139 is a potent α_2-adrenergic blocker (M. Goldstein, M. Saito, J. Y. Lew, P. Hieble and R. G. Pendelton, Eur. J. of Pharmacol., in press) prompted us to investigate its effects alone or in combination with desmethylimipramine (DMI) on central epinephrine (E) levels and on the binding characteristics of β-adrenergic receptors. Sprague-Dawley rats were treated with SKF 64139, DMI, or with a combination of both drugs. Sixteen hours after termination of treatment the catecholamine levels were determined in the hypothalmus, and the specific binding of ^{125}I-Iodohydroxybenzylpindolol (IHYP) was measured in cerebral cortical membranes. Treatment with SKF 64139 for three days (once a day 25 mg/Kg, i.p.) resulted in a small reduction of E (10-15% and in a small reduction of the specific binding of IHYP (5-10%). Treatment with DMI (10 mg/Kg, i.p. once a day) resulted in a small reduction of E (10-15%) and in a small reduction of the specific binding of IHYP (10-15%). The combined treatment of SKF 64139 with DMI resulted in a greater decrease of E levels (35-40%) and in a greater decrease of the specific binding of IHYP (25-30%) than the treatment with each drug separately. The combined treatment with SKF 64139 and DMI decreases the B_{max} to a much greater extent than treatment with DMI alone. (B_{max} controls: 50, DMI treated: 42, SKF 64139 + DMI; 35, f moles/mg protein). These results suggest that decrease in central E levels may contribute in some brain regions to the subsensitivity of β-adrenergic receptors elicited by antidepressants (AD). Thus, central E may be involved in the pathology of affective disorders. Furthermore, treatment with α_2-adrenergic antagonists in combination with AD may be of therapeutic value in affective disorders.

205.4 HYPERSENSITIVITY OF RATS WITH CHRONIC CEREBELLAR LESIONS TO ABNORMAL BEHAVIORS INDUCED BY APOMORPHINE. Vernice Jackson, Judy Glassgold*, Carl Miller*, and Stuart R. Snider.*
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Radiographic, surgical and pathologic evidence for anterior cerebellar atrophy in approximately 40% of patients with schizophrenia was recently presented by Weinberger et al. and Heath et al. A possible link between the cerebellum and schizophrenia is provided by reports from other investigators of a pathway from cerebellum to ventral tegmental dopamine (DA) neurons; abnormal function of DA systems is postulated in the "dopamine hypothesis" of schizophrenia. The present study was initiated to determine whether limited cerebellar lesions would result in abnormalities of DA-mediated behavior.

Groups of control rats (n=16) or rats with anterior cerebellar lesions (n=32) were tested with intraperitoneal apomorphine in open trials at 1, 2, 3 and 8 weeks after surgery. Motor behavior was recorded on a 0 to +4 scale. At most testing intervals, abnormal oral behaviors (stereotyped licking, biting, carrying or eating feces) were more severe and frequent in the lesioned than the control animals. Aggressiveness, irritability and locomotor hyperactivity were increased at all intervals compared to controls while apomorphine-induced grooming was less frequent. Stereotyped limb movements and sympathetic signs were equally frequent.

These results indicate that chronic anterior cerebellar lesions result in abnormal behavioral sensitivity to the DA agonist, apomorphine. They support the possibility of a role of the cerebellum in DA-influenced abnormal behavior in human psychosis.

205.5 DDT-INDUCED MYOCLONUS AND CEREBELLAR BENZODIAZEPINE RECEPTOR BINDING. Eunyong Chung Hwang and Melvin H. Van Woert*. Depts. of Neurology and Pharmacology, Mt. Sinai Sch. of Med., New York, N.Y. 10029.

Certain types of stimulus-sensitive arrhythmic myoclonus in patients are particularly responsive to therapy with benzodiazepine derivatives as well as serotonin precursors such as L-5-hydroxytryptophan. Intragastric administration of the insecticide p,p'-DDT (200-600 mg/kg) to rodents produces an animal model of stimulus sensitive myoclonus which also can be reduced by treatment with benzodiazepines and serotonin agonists.

Paul and Skolnick (Science 202:892, 1978) reported that pentylenetetrazol- and electroshock-induced seizures produced an increase in benzodiazepine receptor binding in rat cerebral cortex. Since myoclonus may be a type of seizure, the effect of p,p'-DDT (600 mg/kg) on specific (^{3}H)-flunitrazepam binding to washed membranes from rat brain cerebellum, cortex and remaining areas of the brain was examined. (^{3}H)-flunitrazepam binding was increased only in the cerebellum of DDT-treated rats. The increase in cerebellar (^{3}H) flunitrazepam binding is gradual, being significantly higher 2 hours after DDT administration (when mild myoclonus starts), reaching a maximum elevation at 3.5 hours and remaining elevated at 5 hours (during maximum intensity of myoclonus). Scatchard plot analysis revealed that 3.5 hours after DDT, the total number of cerebellar binding sites (B max) was 140 pmoles/g tissue weight which is a 13% increase from controls (124 pmoles/g). The K_D was not affected by DDT treatment (4.00 and 3.85 nM for control and DDT, respectively).

Although the mechanisms by which DDT rapidly increases the number of cerebellar benzodiazepine binding sites is not known, the close correlation of these biochemical changes with the myoclonic movements suggests that the major pathophysiological changes in DDT-myoclonus and the antimyoclonic action of clonazepam may be localized in the cerebellum. Furthermore, the site of antimyoclonic action of benzodiazepines may be distinct from their anticonvulsant action. (Supported by USPHS grants NS 12341, NS 05802 and NS 71631).

205.6 EFFECTS OF PURKINJE CELL DEGENERATION ON THE NORADRENERGIC PROJECTION TO MOUSE CEREBELLAR CORTEX. S. Roffler-Tarlov and M. J. Zigmond. Dept. of Neuroscience, Children's Hosp. Med. Ctr., Boston, MA 02115 & Dept. of Biol. Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Purkinje Cell Degeneration (pcd) is a mouse mutant in which all cerebellar Purkinje cells die between 20 and 40 days of age (Mullen et al., P.N.A.S. 73, 1976, 208-212). Purkinje cells are thought to be the principal targets for the plexus of noradrenergic axons within cerebellar cortex which originate in locus coeruleus. Thus, pcd offers the opportunity to examine the impact of loss of target cells on noradrenergic neurons. We report here the results of measurements of transmitter stores and of activity of the rate limiting biosynthetic enzyme, tyrosine hydroxylose (TH), in 9 pairs of pcd mutant mice and control littermates aged 2 to 6 months. Samples were homogenized in 50mM Tris-HCl buffer, pH 6.0. One aliquot was taken for analysis of NE content using a radioenzymatic assay involving methylation of NE by ^{3}H-S-adenysl methionine and COMT (Saller & Zigmond, Life Sci. 23, 1978, 1117). A second aliquot was used to determine the activity of TH by the hydroxylation of 75μM ^{14}C-carboxyl labelled tyrosine in the presence of 3mM 6MPH$_4$, followed by conversion of the product to $^{14}Co_2$ in the presence of excess dopa decarboxylase (Kapatos & Zigmond, Brain Res. 170, 1979, 299).

We observed a significant and permanent increase in NE content of mutant cerebellar cortex, an area which was reduced to 52% of control weight. The NE content had increased significantly whether expressed per gram tissue (314±25% control) or per cerebellar cortex (164±11% control). In contrast, TH activity increased slightly on a per weight basis (128±7 percent but was reduced when expressed per area (68±5% control) resulting in a large decrease in the ratio of TH activity to NE content (42±3% control). The NE content of hippocampus (a region believed innervated by the same locus coeruleus cells but with no known cell loss) was increased in some but not all cases: the average value was 131±14% control whereas TH activity was unaltered.

These results show that the noradrenergic projection to cerebellar cortex is maintained despite the loss of its principal target cells. They also indicate that compensatory adjustments take place leading to a decrease of TH activity. Such changes are not seen in hippocampus, another terminal field of the same cell group. It is possible that in the absence of the principal targets, NE afferents are less active resulting in a selective accumulation of transmitter stores and a decline in the availability of biosynthetic enzyme in these terminals.

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205.7 DECREASED STRIATAL AND NIGRAL SUBSTANCE P LEVELS IN HUNTINGTON'S DISEASE. S.H. Buck*, T.F. Burks, H.I. Yamamura, E.D. Bird*, M. Rossor*, and M.R. Brown. (SPON: R.P. Gruener). Dept. Pharmacol., UAHSC, Tucson, AZ 85724; MRC Neurochem. Pharmacol. Unit, Cambridge, England CB2 2QD; Peptide Biology Lab, Salk Institute, La Jolla, CA 92037.

The undecapeptide, substance P (SP), is found in discrete regions and nerve tracts in the brains of laboratory animals. The substantia nigra contains the highest levels of SP in brain and lesion studies indicate that most of the nigral SP originates from a striatonigral neuronal pathway. SP is also found in human brains with a regional distribution similar to that in animals (Duffy et al., Neuropharmacol. 14, 615, 1975). It has been reported that in brains from humans with Huntington's disease (HD), in which there is marked atrophy of basal ganglia, there is moderate reduction of SP content that is limited to globus pallidus and nigra (Gale et al., J. Neurochem. 30, 633, 1978).

We have re-investigated alterations in SP levels in HD using a radioimmunoassay for SP. Rabbit anti-SP serum was used which showed cross-reactivity of 1% with physalaemin and 0.01% with eledoisin. Using ^{125}I-tyr^8-SP as tracer, sensitivity was about 10 fmol SP. Tissues were extracted 2X with cold 2M acetic acid; extraction efficiency was 90.3 ± 1.1% (n=6) as determined using labeled tracer. Assay of varying dilutions of samples (control and HD) produced curves that were parallel to the standard curve.

SP Immunoreactivity - pmol/g wet weight ± s.e.m. (n)

	Controls	HD	↓	P
Cortex (BA 32)	5.74 ± 0.60(10)	5.70 ± 0.92(10)	0.0%	NS
Caudate	1.43 ± 0.42(10)	0.83 ± 0.31(4)	42.0%	NS
Putamen	32.38 ± 5.02(10)	9.78 ± 2.88(10)	69.8%	<.0025
Globus pallidus	115.17 ± 19.87(10)	20.57 ± 5.64(10)	82.1%	<.0005
S. nigra -				
p. compacta	267.16 ± 48.07(5)	83.91 ± 20.64(5)	68.6%	<.005
p. reticulata	866.34 ± 100.82(5)	66.12 ± 9.33(5)	92.4%	<.0005
Thalamus	1.42 ± 0.31(8)	1.86 ± 1.21(7)	+31.0%	NS
Hypothalamus	64.15 ± 12.04(10)	60.69 ± 12.29(10)	5.4%	NS

Our results confirm the relative distribution of SP in normal human brain. Highest levels are in basal ganglia and nigral p. reticulata contains 3 - 4 times more than p. compacta. In HD, there are reductions in SP that are limited to basal ganglia. In caudate, SP was undetectable in 6/10 HD and 0/10 control brains. These data indicate that in HD there is depletion of SP throughout basal ganglia and that in some areas this depletion is nearly total. This is the first time that evidence has been presented that the reduction in SP levels in HD also occurs in the neostriatum.

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205.8 DOPAMINE RECEPTORS IN SCHIZOPHRENIC HUMAN BRAINS: DIFFERENTIAL CHANGES. Tyrone Lee and Philip Seeman. Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

Abnormal dopamine receptors have been demonstrated in schizophrenic human brains using ^{3}H-haloperidol or ^{3}H-spiperone to label the post-synaptic receptors (Lee et al., Nature 274:897, 1978 and Am. J. Psychiatry 137:191, 1980). Such abnormalities were indicated by elevation of ^{3}H-neuroleptic binding in dopamine-rich regions of the schizophrenic brain: caudate nucleus, putamen and nucleus accumbens. In order to determine whether the enhancement in binding was due to a change in receptor affinity (K_D in nanomoles/liter) or receptor density (B_{max} in femtomoles/mg protein), Scatchard analysis was carried out in postmortem normal and schizophrenic brains. Post-synaptic dopamine receptors (D-2 receptor sites) in the caudate and putamen were labeled by ^{3}H-spiperone while the high-affinity presynaptic dopamine receptors (D-3 receptor sites) were labeled by ^{3}H-dopamine. N represents the number of brains assayed. The results are as follows:

^{3}H-SPIPERONE BINDING (D-2 RECEPTORS)				
	NORMAL		SCHIZOPHRENIC	
	CAUDATE	PUTAMEN	CAUDATE	PUTAMEN
B_{max}	115±14	128±13	198±24**	191±23*
K_D	0.39±.06	0.31±.03	0.39±.05	0.51±.09
N	15	17	16	13

*$P < 0.05$; **$P < 0.001$.

^{3}H-DOPAMINE BINDING (D-3 RECEPTORS)				
	NORMAL		SCHIZOPHRENIC	
	CAUDATE	PUTAMEN	CAUDATE	PUTAMEN
B_{max}	46.9±5.4	53.9±3.9	38.4±6.7	50.5±4.2
K_D	1.16±.22	2.04±.51	0.62±.08	1.23±.13
N	8	11	5	6

Dopaminergic hyperactivity in schizophrenia could result from too few pre-synaptic dopamine receptors or too many post-synaptic dopamine receptors. The present results indicate that the density of the presynaptic D-3 sites is normal, but that the density of the post-synaptic D-2 receptors is increased.

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206.1 QUANTAL ASPECTS OF SYNAPTIC TRANSMISSION IN THE LAMPREY SPINAL CORD. M. E. Selzer. Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

In giant interneurons of adult sea lamprey (Petromyzon marinus) spontaneously occurring depolarizing potentials with amplitudes in the 0.1-1 mV range persisted in tetrodotoxin (TTX), which blocks all known action potentials in the lamprey. Interval histograms of TTX resistant potentials deviated slightly from the negative exponential density function expected for a random process because of under-representation of very short intervals. Amplitude histograms showed a skewed distribution with under-representation of small potentials. Both of these findings are most likely secondary to bias in signal detection due to noise. The mean amplitude of TTX resistant potentials (approx. 0.5 mV) increased during intracellular passage of hyperpolarizing current and decreased with depolarizing current. Therefore they are probably "miniature" EPSPs. Spontaneous hyperpolarizing potentials were also observed, but the vast majority were eliminated in TTX.

Attempts were made to estimate mean quantal content (m) and quantal voltage (q) at synapses between presynaptic Muller axons and postsynaptic lateral cells. At six synapses, activated at 2-5 second intervals, mean steady state EPSP amplitude ($\bar{V}$) was 1.90 mV (range 0.81-3.15). m was calculated from long series of EPSPs using a correction for noise by the formula $m = \frac{\bar{V}^2}{SD^2 - RMS^2}$ where SD^2 = variance and RMS = root mean square of noise (generally .05-.10 mV). m averaged 43 quanta (range 11-114) and q averaged 75 μV (range 18-210). These calculations assume a Poisson behavior of quanta, including a low probability of release. There was no consistent change in q when $\bar{V}$ was decreased by reducing Ca^{++}. For example at one synapse $\bar{V}$ was 1.2, 2.3 nd 3.1 mV at bath (Ca^{++}) of 0.5, 1.0 and 2.6 mM respectively. Corresponding qs were 47, 46 and 49 μV. When pairs of impulses were initiated in the presynaptic axon, facilitation was seen at interstimulus intervals shorter than 50-75 msec, while synaptic depression was seen at longer intervals. Thus a large number of $\bar{V}$s was obtained by varying interstimulus intervals in different series of paired EPSPs. Once again, q was constant, suggesting a low probability of release.

The data presented above suggest that true mEPSPs occur in the lamprey spinal cord and that the statistical behavior of EPSPs at an identified synapse is Poisson in nature, suggesting a quantal mode of transmission.

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206.2 "SPONTANEOUS" QUANTAL RELEASE OF TRANSMITTER ABSENT IN VIVO. A.R.Blight and W.Precht*. Max-Planck Institute for Brain Research, Frankfurt/M 71, Federal Republic of Germany.

Spontaneous release of transmitter at rates up to several quanta per second is generally accepted as a normal property of synaptic terminals though the few recordings of such release in intact animals do not exclude the possibility that it was promoted by mechanical damage. Recording intracellularly from lumbar motoneurons of the in situ spinal cord of curarised frogs (R.temporaria) we noticed that spontaneous activity, previously shown to be partly quantal release in isolated cord (J.Physiol.Lond.168,389), was usually absent, of very low frequency, or clearly generated by action potentials in presynaptic neurons. Cutting the thoracic spinal cord (a procedure intrinsic to the isolated preparation) increased the incidence of spontaneous potentials.

Further analysis was made with the neuromuscular junction of m.extensor digitorum longus IV of intact, anaesthetised or spinalised fogs. First, random microelectrode penetrations were made over the middle third of the muscle length (exposed under mineral oil), each cell held 1-3 min. The peroneal nerve was cut in one leg of each frog 1-24 hr before recording and the presence of miniature potentials (MEPPs) compared in the muscle with intact and that with cut innervation. Of 257 penetrations in 8 lesioned muscles MEPPs could be recorded in 52, compared with 6 in 257 penetrations from intact muscles. 5 of 8 intact muscles showed no MEPPs (in 158 penetrations). Secondly, recordings were made from muscles with innervation vitally stained by methylene blue. 57 penetrations close to stained innervation in 10 intact muscles produced one fibre with MEPPs; 83 penetrations in 12 muscles with cut peroneal nerve showed 31 with MEPPs.

Finally, the presence of effectively "silent" endplates in phasic muscles fibres of the frog was confirmed by recording up to 10 min from muscle fibres with no spontaneous potentials, then superfusing with hypertonic saline, causing release of MEPPs. This was also done by cutting the peroneal nerve under TTX saline while recording from single muscle fibres, to demonstrate directly the effect of presynaptic lesions in producing "spontaneous" discharge. We conclude that most if not all cases of quantal release we have recorded in vivo were due to mechanical damage. It remains to be determined whether spontaneous release is totally absent at the normal junction or occurs at rates which are orders of magnitude less than those found in isolated preparations.

206.3 POST-TETANIC POTENTIATION OF EPSPS IN MOTONEURONS OF DIFFERENT SIZE: FURTHER EVIDENCE REGARDING PRESYNAPTIC MECHANISM. Elwood Henneman, Paul Ruenzel* and Hans-R. Lüscher. Dept. of Physiology, Harvard Medical School, Boston, MA 02115.

Stimulation of muscle nerves with single shocks that set up maximal volleys in their Ia fibers evokes aggregate EPSPs in their motoneurons, which are large in small cells and small in large cells. Recently we showed (Nature 282: 859, 1979) that a 500/sec conditioning tetanus applied to the nerve for 10 sec caused post-tetanic potentiation (PTP) of these aggregate EPSPs that was greatest in large cells and least in small cells. These results suggested 1) that Ia impulses normally invade the relatively simple terminal ramifications of fibers going to small motoneurons more completely than the extensive arborizations on large cells and 2) that hyperpolarization of Ia fibers during PTP, with consequent increases in the amplitude and duration of their impulses, presumably facilitates invasion of Ia terminals, leading to activation of more synaptic endings and that this effect is greater for large cells which normally have more uninvaded terminals.

To determine precisely how potentiation of EPSPs in large and small motoneurons depends on duration of tetani, 500/sec conditioning shocks were applied for 2, 4, 8, 16 and 32 sec. Percent potentiation increased with prolongation of tetani, but no ceiling in PTP was reached, even with 32 sec stimulation. When duration of tetanus was plotted against % potentiation on a semilogarithmic scale, a strikingly linear relationship was apparent. The slope of this relationship was greatest for large cells and least for small cells. Sensitivity to duration of tetanus was clearly greatest in the biggest motoneurons.

Since temperature is known to influence conduction at branch points, control and potentiated responses were studied at 34° to 41°C. Postsynaptic population potentials (PSPPs) elicited by electrical stimulation of single Ia fibers were recorded from ventral roots by the sucrose gap technique (J. Neurophysiol. 42: 1146, 1979). Amplitude of control PSPPs was linearly related to temperature ($p < 0.001$). There was a clear, but non-linear, correlation between early potentiation of PSPPs and temperature. The results are consistent with the view that lower temperature and PTP both facilitate conduction at presynaptic branch points where conduction failures occur.

These findings add further evidence that invasion of Ia terminals is generally more complete in the terminal arborizations on small cells because they have fewer branch points.

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206.4 TWO DIFFERENT MECHANISMS FOR CALCIUM CURRENT MODULATION UNDERLY HABITUATION AND SENSITIZATION IN APLYSIA. M. Klein* and E. R. Kandel, Div. of Neurobiology & Behavior, P & S, Columbia University, New York, N. Y. 10032.

The gill-withdrawal reflex of Aplysia undergoes habituation and sensitization. Each of these has been shown to result from alterations in transmitter release from sensory neuron terminals (Castellucci & Kandel, 1974, 1976), and both are associated with changes in the Ca^{++} influx of the presynaptic terminal (Klein and Kandel, 1978, 1980). To analyze the mechanisms underlying the modulation of the Ca^{++} current we voltage clamped the sensory neurons and caused them to release transmitter with brief depolarizing pulses. To unmask the Ca^{++} current, we blocked the Na^+ and K^+ currents. Repeated depolarization steps simulating habituation caused a parallel decline in the presynaptic Ca^{++} inward current and in the postsynaptic potential, while application of serotonin, the presumed facilitating transmitter, had no effect. In the presence of K^+ currents, however, serotonin reduced the outward currents and enhanced the inward currents. In another set of experiments, Cs^+ ions were substituted for intracellular K^+ using the antibiotic nystatin. Serotonin again had no effect on the membrane current as long as K^+ was absent. When K^+ was injected into the cell, however, serotonin reduced the partially restored K^+ current. These results imply that homosynaptic depression results from a direct action on the Ca^{++} channel, while presynaptic facilitation results from an indirect action on the Ca^{++} current due to an increase in duration of the action potential secondary to a decrease in the repolarizing K^+ current.

In order to determine whether the small increases in action potential duration seen in normal solutions after heterosynaptic stimulation were adequate to increase transmitter release, we studied the sensory neuron output as a function of the duration of voltage-clamp depolarizations. We found that small increases in step duration caused large increases in the amplitude of the postsynaptic potentials. Because it prolongs the Ca^{++} current, the increase in duration of the action potentials might in turn cause a change in the shape of the PSP. When the shape of the postsynaptic potentials was analyzed we found that the time-to-peak did not change during habituation, but was prolonged after sensitizing stimulation. This finding also supports the hypothesis that habituation affects the Ca^{++} channel directly and is independent of the K^+ currents which determine the configuration of the action potential, while sensitization modulates the Ca^{++} current indirectly by depressing the K^+ current and increasing the duration of the action potential. Thus, although both habituation and sensitization involve a common site of synaptic plasticity, the presynaptic Ca^{++} current, modulation is direct in habituation and indirect in sensitization.

206.5 SPECIFIC IONIC CURRENTS UNDERLYING POST-TETANIC POTENTIATION IN APLYSIA. Robert Kretz,* Eli Shapiro,* Eric R. Kandel (SPON: M. Jacob). P & S, Columbia University, New York, N. Y. 10032.

Four types of synaptic plasticity have been analyzed in Aplysia and shown to be due to a modulation of the calcium current of the presynaptic terminal: homosynaptic depression, presynaptic facilitation and inhibition, and membrane potential-dependent modulation of transmitter release (Klein and Kandel, 1978; Shapiro, Castellucci and Kandel, 1980a;b). We have now begun a voltage-clamp analysis of a fifth type of plasticity, post-tetanic potentiation (PTP) using the synapses made by cholinergic cell L10. Firing L10 at .2 Hz in an unclamped state elicits stable PSPs in the follower cell. After a tetanus of 100 spikes at 4 Hz, follower cell PSPs are potentiated about 200% for 5-10 min. During the tetanus the spike of L10 increases in duration, but returns to control levels in less than 2 min. after the tetanus. Tetanic stimulation also hyperpolarizes L10 by 5-10 mV. This lasts for 5-10 min, and correlates well with the duration of PTP.

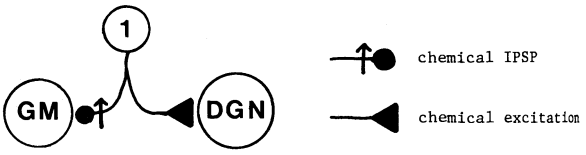
We next voltage clamped cell L10 in TTX solutions. Depolarizing test pulses (50 msec) from -40 to 0 mV at .2 Hz elicit stable PSPs. A tetanus of 100 test pulses at 4 Hz caused PTP of about 200% lasting 5-10 min. Three changes in ionic currents were observed: 1) Inactivation of the delayed voltage-dependent K^+ current. This current rapidly returns to control levels after the tetanus and correlates well with the time-course of spike-broadening. 2) Partial inactivation of the transient Ca^{++} current (measured during pharmacological blockage of the K^+ currents with TEA^+ and 4-AP). This current also returns rapidly to control levels after tetanus. 3) Activation of a slow non-voltage dependent outward current. This persists for 5-10 min after the tetanus and parallels the potentiation of the PSP and hyperpolarization of cell L10 in unclamped cells. This outward current was blocked by Co^{++} and by replacement of Ca^{++} by Ba^{++}, suggesting that the current is a Ca^{++}-dependent K^+ current. In other molluscan cells the amplitude of the Ca^{++}-dependent K^+ current has been shown to be proportional to the intracellular concentration of Ca^{++} (Eckert and Tillotson, 1978).

Our data suggest that repeated activation of L10 during the tetanus increases the intracellular Ca^{++} concentration. This in turn activates a Ca^{++}-dependent K^+ current and also causes increased transmitter release. These experiments support the idea (Rahamimoff, 1968) that a residual Ca^{++} mechanism contributes to PTP and indicate that intracellular Ca^{++} metabolism can affect synaptic transmission in Aplysia. Since L10 also undergoes modulations of the Ca^{++} current in response to presynaptic inhibition and membrane potential-dependent control of transmitter release, both Ca^{++} current modulation and changes in intracellular Ca^{++} concentration seem capable of contributing to different types of synaptic modifiability in the same set of synapses.

206.6 GRADED TRANSMISSION AND PULSATILE TRANSMISSION FROM ONE NEURON TO TWO DIFFERENT POSTSYNAPTIC NEURONS. B. Mulloney and D.H. Edwards, Jr. Zoology, University of California, Davis, CA 95616.

One neuron that has overshooting action potentials causes discrete, classical IPSPs in some of its follower cells, yet causes smoothly graded chemical excitation of another follower in the same ganglion. This chemical excitation shows no evidence of pulsatile secretion of transmitter synchronous with impulses in the presynaptic neuron.

The presynaptic neuron is Interneuron 1, in the stomatogastric ganglion of Panulirus (Selverston and Mulloney, 1974). This neuron is a spontaneous pacer that fires regularly at 5-10 Hz when the neural circuit of which it is part is not generating periodic bursts of impulses. Interneuron 1 makes seven synapses in the ganglion: six are inhibitory and one excitatory. We have studied transmission at two of these synapses:



Impulses in Interneuron 1 cause discrete, constant-latency IPSPs in GM. These IPSPs facilitate as the impulse frequency of Interneuron 1 increases. Interneuron 1 excites DGN, but no discrete EPSPs that follow presynaptic impulses can be recorded either from the soma or from the integrative segment of DGN. At its average membrane potential (-60 mv ± 5 mv), Int 1 excites DGN continuously. Small hyperpolarizations of Int 1, that slow its spontaneous firing, cause graded hyperpolarizations of DGN. Longer hyperpolarizations of Interneuron 1 cause no further response in DGN.

Both synapses are blocked by elevated extracellular Mg^{++} concentrations and augmented by elevated extracellular Ca^{++} concentrations. We have looked for evidence of an electrical synapse between DGN and Interneuron 1 by passing current into each neuron while recording the membrane potentials of both neurons; we have found no evidence of electrical synapses between these neurons.

The evidence in hand shows that different synapses made by the same neuron can have postsynaptic effects with very different temporal characteristics. Whether these differences arise from differences in the presynaptic release mechanisms or from differences in the postsynaptic membranes is under investigation.

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206.7 EXTRACELLULAR POTASSIUM ACTIVITY AND AXON EXCITABILITY IN PERIPHERAL NERVES OF CRAYFISH AND RAT. D.O. Smith. Department of Physiology, University of Wisconsin, Madison, WI 53706.

Using K^+-selective microelectrodes, changes in extracellular levels of K^+, Δa_K, were measured during repetitive stimulation of the excitor axon of the opener muscle of the crayfish walking leg and the phrenic nerve innervating the rat diaphragm muscle. Double-barreled electrodes were used with which action potentials and Δa_K were recorded; the distance of the tip from the nerve was measured with a reticle in the microscope eyepiece.

In crayfish, levels of Δa_K in the periaxonal space rose within 5s to about 0.86 mM during stimulation at 50 impulses/s; this maximum was maintained at a steady value in most preparations although it declined in some during continual stimulation. Conduction failure occurred in several preparations after at least 90s of stimulation; levels of Δa_K were not abnormally high in these cases, suggesting that increased $[K^+]$ extracellularly may not have been directly responsible for the blockage. The diffusion coefficient for K^+ through the extracellular space was estimated to be 2×10^{-6} cm^2/s; treatment of the preparation with 0.1% collagenase increased this to 2×10^{-5} cm^2/s, which is quite close to the value observed in aqueous solution. Also, the magnitude of Δa_K was lowered significantly after treatment with collagenase. Soaking the preparation for 30 min in 1 mM ouabain resulted in maximum values of Δa_K which increased by a factor of 1.34; ouabain also eliminated any decay in Δa_K during continued axon stimulation.

In a related series of experiments, Δa_K was measured in the end-plate region of the phrenic nerve-diaphragm neuromuscular junction of young (9 mos) and aged (26 mos) rats. The results were qualitatively similar to those observed in crayfish. At 50 impulses/s, Δa_K rose within 5s to 0.5 mM; the diffusion coefficient was 6×10^{-6} cm^2/s. In aged rats, these values were 0.6 mM and 1×10^{-6} cm^2/s, respectively. After soaking the tissue for 1 hr in 0.1% collagenase, the diffusion coefficient rose to 3×10^{-5} cm^2/s in the young animals and 4×10^{-5} cm^2/s in the old rats.

It is concluded that K^+ accumulates extracellularly during axon stimulation due mainly to restricted diffusion through the collagenous components of the extracellular space. In crayfish, the magnitude of this accumulation is limited by reduced flux of K^+ per action potential and continuous activity by the Na^+-K^+ pump. It is also concluded that conduction failure in these preparations may not be a direct consequence of extracellular K^+ accumulation but that it may be the result of indirect intracellular mechanisms related to altered ionic activities. Supported by NIH grants NS13600, AG01572, and NS00380 (R.C.D.A.) and the Alfred P. Sloan Foundation.

206.8 THE IMPORTANCE OF Ca^{++} IN THE POTASSIUM STIMULATED NEURONAL RELEASE OF ^{14}C-SEROTONIN FROM THE CEREBRAL GANGLIA OF MYTILUS EDULIS: PHARMACOLOGICAL EFFECTS OF Ba^{++}, Mg^{++}, Co^{++} and La^{+++}. J. Brogan*, G.B. Stefano, E.J. Catapane. Dept. Natural Science. Medgar Evers College, C.U.N.Y., Brooklyn, New York 11225.

The potassium stimulated neuronal release of ^{14}C serotonin in the cerebral ganglion of the bivalve mollusc Mytilus edulis was examined to determine the influence of various ions on this process. The normal artificial sea water (ASW) used in these experiments contained 423mM Na^+, 9.0mM K^+, 9.3mM Ca^{++}, 48.4mM Mg^{++}, 26.0mM SO_4^{--} and 496mM Cl^-. When Ca^{++} free ASW was used isomolarity was maintained by substituting sorbital for Ca^{++}. When Mg^{++}, Co^{++}, Ba^{++}, La^{+++} or additional Ca^{++} were added as chloride, NaCl was lowered accordingly. The cerebral ganglia were placed in a 2 ml chamber with a constant superfusion of 0.2 ml/min. Aliquots (0.1 ml) of the superfusion solution were collected and counted for radioactivity every 5 min. The ganglia prior to being placed in the perfusion chamber were incubated in ASW containing ^{14}C-serotonin (1×10^{-6}M, specific activity 57 mCi/mM) for 30 min. The study demonstrated the following; (1) calcium was required for serotonin release, (2) neuronal release occured in tissue placed immediately in Ca^{++} free medium but not in tissue which was maintained in a Ca^{++} free medium for one hour, (3) high concentrations of Ca^{++} (27.9mM) prevented release, (4) the chelator, EDTA (400 uM) prevented release. Addition of Ba^{++} restored release in a Ca^{++} free medium. Mg^{++} (100mM), Co^{++} (60mM) and La^{+++} (5mM) when added to ASW inhibited serotonin release. This work was supported in part by Grant 1 S06 RR 08171-01 from DRR and N.I.M.H. and by Grant 1-T32GM07641 from the MARC Program of NIGMS.

206.9 PRESYNAPTIC EFFECTS OF ANTICHOLINESTERASES. David F. Wilson, Department of Zoology, Oxford, Ohio 45056

The influence of inhibiting acetylcholinesterase on neuromuscular transmission was reexamined in the cut-muscle preparation of the rat diaphragm (Wilson, Am J. Physiol. 237:C31-C37, 1979). Intracellular recording techniques were used to monitor end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs). The preparations were examined in control saline and in the presence of an acetylcholinesterase inhibitor (neostigmine or eserine). In the presence of neostigmine ($5x10^{-8}$ to $1x10^{-6}$ g/ml) quantal release was unaffected by low frequency stimulation (2/sec). At a frequency of 10/sec or higher, a gradual buildup of endplate depolarization occurred and the EPP amplitudes declined. The decrease in EPP amplitude can only be partially explained by the maintained decrease in membrane voltage (2-8 mV). Desensitization appears unlikely since MEPPs can be observed between EPPs during maintained stimulation and desensitization has a relatively slow time course (several seconds) while the EPP decline occurs rapidly. Saturation of the receptors cannot explain the EPP decline since the EPP depolarization is not close to the reversal potential. The conclusion that quantal release is inhibited during moderate and high frequencies in the presence of neostigmine or eserine is supported by the observation that the acetylcholine output of this preparation (Straughan, Brit. J. Pharmacol. 15:417-424, 1960) closely parallels the quantal release data. The magnitude of quantal release inhibition by the anticholinesterases is dosage dependent.

It is postulated that the presynaptic acetylcholine receptors may serve to limit the amount of transmitter released by the motoneuron and function in a negative feed-back pathway. Under in vivo conditions, nerve impulses in a myasthenic patient may reach frequencies up to 50/sec. The present results can explain why high doses of anticholinesterase treatment can have deleterious effects on neuromuscular transmission. In moderate or high doses the net result will be small EPP's during a tetanus; failure to maintain tension in a muscle. The critical level of anticholinesterase treatment that is beneficial to myasthenic patients may be the dosage that prolongs the time course of the EPP while permitting removal of the acetylcholine before onset of the next motor nerve impulse.

206.10 RELEASE AND TURNOVER OF ACETYLCHOLINESTERASE IN RAT DIAPHRAGM S. Brimijoin and J. Carter*. Dept. of Pharmacology, Mayo Medical School, Rochester MN 55901.

Our recent work suggests that a significant fraction of the acetylcholinesterase (AChE) in nerve-muscle preparations is subject to spontaneous release. In a systematic study of this process, phrenic nerve-hemidiaphragms were removed from rats exsanguinated by perfusion with 0.9% NaCl and were incubated in vitro at 37° in 1.5 ml of oxygenated HEPES buffered salt solution containing glucose and albumin. After 3 h, baths were collected and tissues were homogenized in detergent-containing buffer for assay of AChE. Normal hemidiaphragms released enzyme activity at the rate of 30 U (μmol ester hydrolyzed/h) per h; over a 24 h period this would correspond to 12% of the total AChE activity of the tissue. The rate of release was not affected by supplementing the bathing medium with insulin and leucine or with pepstatin (10^{-5} M); release was increased, however, in muscles that had been denervated by transection of the phrenic nerve 1 to 7 days earlier. Ultracentrifugation of samples on 5-20% sucrose density gradients showed that released AChE was mainly of the 10S form although the muscles contained significant amounts of 16S and 4S enzyme in addition. The rate of spontaneous release was much greater than that of arrival of enzyme at the neuromuscular junction by rapid axonal transport along the phrenic nerve (estimated at 3.1 U/h from the rate of accumulation of AChE activity against a ligature applied for 24h in vivo). We concluded that most of the released enzyme originated in the muscle. In order to compare the rate of release with that of turnover in the muscle, rats were treated with the protein synthesis inhibitor cycloheximide, 20 mg/kg, sc, and the fall of AChE activity in the diaphragms was measured. The half-life derived from this experiment was 32 h and the daily turnover was calculated as 2200 U per hemidiaphragm. Approximately 1/3 of this turnover can thus be accounted for by spontaneous release. However, further sucrose density gradient experiments showed that only the 4S form of AChE was lost to a significant extent after cycloheximide treatment (estimated halflife 6h), whereas the turnover of 10S AChE was too slow to measure (half-life > 72h). If released enzyme is derived from 10S AChE in the muscle, therefore, then release may represent the sole fate of this slowly turning-over form. (Supported by Mayo Foundation and by NIH grant NS11855).

207.1 CODING OF SPECIES-SPECIFIC SIGNALS IN MORMYRID ELECTRIC FISH: I. FREQUENCY CHARACTERISTICS. A.H. Bass and C.D. Hopkins, Dept. of Ecology and Behavioral Biology, University of Minnesota, Minneapolis, Minnesota 55455.

During a recent trip to the Ivindo River district of Gabon, West Africa, we examined some response characteristics of Knollenorgans - the putative social communication receptors of mormyrids. Realizing the wide diversity in waveform and spectra of the Electric Organ Discharge (EOD) of these fishes (Hopkins, Behav. Ecol. Sociobiol., '80) we hypothesized a comparable divergence in the frequency response characteristics of their Knollenorgans.

Detailed information was gathered for five local fishes with distinct EOD's: Brienomyrus brachystius triphasic (BBTP; peak power of EOD spectrum -PPW- at 0.7 kHz), Brienomyrus brachystius long biphasic (BBLB; PPW at 0.5 kHz), Marcusenius paucisquamatus (MP; PPW at 2.5 kHz), Petrocephalus sp. (PE; PPW at 4.0 kHz), and Stomatorhinus corneti (SC; PPW at 9.0 kHz). We recorded the spike activity of individual Knollenorgans; stimuli consisted of 100 msec tone bursts of sine waves. Three response paradigms characterized receptors: conventional tuning curves, response profiles of frequency vs. spike activity, and best frequencies (BF's) determined by visual and auditory inspection of spike activity. The positions of all receptors were mapped and together with the BF data, determined topographic variation in BF's of receptors. BF's range from 0.3-3.5 kHz for BBTP, 0.4-2.4 kHz, 1.1-5.7 kHz for MP, 2.0-6.5 kHz for PE, and 0.5-17.2 kHz for SC. BBTP, BBLB, and MP display bimodal distributions of receptor types: a low frequency population (median at 0.6, 0.8, and 1.5 kHz respective BBTP, BBLB, and MP) localized at opercular levels, and a high frequency population (median at 1.3, 1.8, and 4.0 kHz respective BBTP, BBLB, and MP) with a widespread rostral-caudal extent. PE has a unimodal distribution with the median at 4.0 kHz. SC has a trimodal distribution with medians at 1.0, 2.5 and 6.0 kHz; with no systematic topography.

In conclusion, there is: (1) wide overlap in the BF range for both sympatric and allopatric species, and (2) no strict correlation between BF range and the PPW for each species examined. We believe these filter characteristics are a "communication window". A more discrete mechanism must govern the recognition of species-typical EOD's.

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207.2 CODING OF SPECIES-SPECIFIC SIGNALS IN MORMYRID ELECTRIC FISH: II. TEMPORAL CHARACTERISTICS. C.D. Hopkins & A.H. Bass. Dept. Ecol. & Behav. Biol.; Univ. Minnesota; Minneapolis, Minn. 55455.

1. African electric fish (Mormyridae) produce trains of electric pulses characterized by: the waveform (or the Electric Organ Discharge -- EOD) and by the repetition rate or pulse rhythm (RHY). EODs are usually species-specific stereotyped "signatures" while RHYs vary greatly with context and show broad overlap between species.

2. One species with sexually different EODs (Brienomyrus brachyistius TP.) has an electric calling song made from bursts of EODs called "rasps". Rasps are used to attract females during the breeding season. We quantified rasps produced by males during field playback of EODs in Gabon (W. Africa).

3. Males sing when presented with computer-synthesized tapes of their own female's EOD combined with their own female's RHYs. They tend to ignore tapes of male's EODs or EODs of other species -even if driven by the female's RHY. They sing in response to the female's EOD even when paired with another species' RHY or with a scrambled RHY. Thus EOD, not RHY is used by males for female recognition.

4. Males sometimes are confused and sing in response to the EODs of the wrong species, but only if that species is allopatric.

5. Males can discriminate EODs whose phase spectra differ but whose power spectra do not. Time-reversed EODs of females elicit half the number of rasps that normal EODs will; phase-shifting the EOD by 90^{0} renders it ineffective. These results suggests that EODs are recognized using phase- or temporal-cues.

6. Natural EODs are presented to Knollenorgan electroreceptors on the skin of the fish. Spike responses recorded external to the skin are phase-locked to the stimulus. Each different EOD presented evokes a unique temporal pattern of spikes; spike number varies from 2 to 5 per EOD. The EOD of female B. brachyistius TP is coded by two spikes separated by 0.4 msec.

7. A square wave stimulus, 0.4 msec in duration imitates the neural code of the female's EODs yet the stimulus waveforms are quite different.

8. Field playback of 0.4 msec square waves to males evokes singing.

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207.3 NEURAL CORRELATES OF A NON-JAMMABLE ELECTROLOCATION SYSTEM. Joanne A. Matsubara. Scripps Institution of Oceanography, UCSD, La Jolla, Calif. 92093.

Weakly-electric fish perceive objects in their immediate surroundings by emitting electric signals and evaluating small distortions in these signals as they are bent around objects. The electrosensory system is sensitive to all such electrical signals: not only their self-imposed Electric Organ Discharges (EOD) but also the EODs emitted by other nearby fish of the same species. An individual's electrolocation system is therefore subject to jamming by the discharges emitted by its neighbors, and various behavioral modifications have evolved to minimize this interference. One of these behavioral strategies, the Jamming Avoidance Response (JAR) is possessed by all but one genus of wave-type electric fish. Sternopygus unlike the others has no JAR, yet can electrolocate even in the presence of electrical jamming signals.

Since Sternopygus differs so drastically from other fish, we predicted that these differences would be reflected in the way the central nervous system processes information regarding electrolocation. A comparative study between Sternopygus and Eigenmannia, a closely related gymnotiform with a JAR, was undertaken. Extracellular, single unit recordings from the posterior lateral line lobe (pLLL) will be discussed. The pLLL is the first central processing area for electroreceptive inputs and is organized in a somatotopic fashion. Some pLLL cells have electroreceptive fields on the body surface which can be mapped using a localized amplitude-modulated signal. Simple receptive fields are either excited (Class 1) or inhibited (Class 2) by an increase in EOD amplitude. Class 3 cells have more complex receptive fields, consisting of both an excitatory and an inhibitory area. Results show that Class 3 cells, so far found only in Sternopygus, are unique in that they are responsive to EOD amplitude modulation only if it occurs over a limited portion on the cell's receptive field. Hence, Class 3 cells respond to moving objects since moving objects produce localized distortions in the EOD. Class 3 cells, however, do not respond to jamming signals because they produce large-field amplitude modulations which stimulate entire receptive fields. This mechanism reminds us of certain visual receptive fields in the vertebrate retina which respond to local contrast but not to overall changes in illumination.

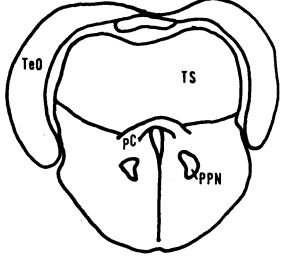
Hence, Sternopygus protects electrolocation not via a behavioral strategy but via central processing mechanisms which can be seen already at the first stage of central processing. This new mode of protection against jamming of the electrolocation system is similar to the lateral inhibitory mechanisms for contrast enhancement in the retina.

207.4 MISSING LINKS IN THE NEURONAL HARDWARE OF THE JAMMING AVOIDANCE RESPONSE (JAR) IN THE ELECTRIC FISH, EIGENMANIA. W. Heiligenberg, T. Finger, J. Matsubara, and C. Carr. Scripps Inst. of Oceanography, UCSD, San Diego, CA. and Univ. Colo. Med. Ctr.,Denver, CO.

The gymnotid fish, Eigenmania, emits a stable high frequency electric signal which the animal uses for electrolocation as well as social interactions. Eigenmania is able to shift the frequency of its electric organ discharge (EOD) in order to avoid interference from the EOD of a neighboring fish with a similar frequency. This jamming avoidance response (JAR) requires that the animal first sense a mixture of its own and its neighbor's EOD's, and then direct an appropriate shift in the frequency of discharge of the electric organ pacemaker nucleus in the medulla. An essential part of the sensory processing necessary for the JAR is performed in the second order electrosensory nucleus, the torus semicircularis (TS). We are studying pathways through which the TS or higher order electrosensory nuclei ultimately control the activity in the pacemaker nucleus.

Using either pressure or iontophoretic delivery methods, we injected small amounts (<20 nl) of HRP into the medullary pacemaker nucleus in six fish. After a 2-4 day survival period, the animals were perfused with 4% glutaraldehyde and processed for HRP by means of the Hanker, et al. method.

In 1 case, the HRP injection was entirely confined to the medullary pacemaker nucleus. In this case, retrograde label occurs only in a cluster of neurons, the prepacemaker nucleus (PPN) lying at the meso-diencephalic junction (see Figure). The PPN lies 300 µm lateral of the midline and about 500 µm ventral to the posterior commissure (PC). Axons leave the prepacemaker nucleus in a caudo-ventral direction and come to lie along the ventral margin of the brain in the rostral rhombencephalon. Just rostral to the level of the pacemaker nucleus, the fibers turn dorsally and medially to enter the pacemaker nucleus along the raphe.



In an attempt to further delineate the pathways involved in the JAR, we are now attempting to inject HRP into the area of the prepacemaker nucleus. We hope to be able to trace the pathway by which lateral line sensory input can influence the frequency of EOD.

207.5 2-DEOXYGLUCOSE IDENTIFICATION OF ELECTRORECEPTIVE PATHWAYS IN THE SOUTH AMERICAN WEAKLY ELECTRIC FISH, EIGENMANNIA. *Curtis L. Baker, Jr. and Catherine E. Carr.* Neurobiology Unit, Scripps Inst. Oceanography, U.C.S.D., La Jolla, CA 92093.

Electrolocation and the Jamming Avoidance Response (JAR) in weakly electric fish have been studied neurophysiologically as far as midbrain levels, while diencephalic and telencephalic electroreceptive pathways have only recently been explored.

The 2-deoxyglucose technique was used to map central electroreceptive areas in the gymnotiform, Eigenmannia virescens. Fish were curarized and injected intraperitoneally with 1 uCi ^{14}C-2-DG. A stimulus of two sine waves of slightly differing frequency, known to activate the JAR and affect electrolocation (Heiligenberg et al, 1978), were presented to the fish for an hour in a quiet, dark room.

Previous experiments demonstrated that the resolution of the Sokoloff et al(1977) 2-DG method was inadequate for studies on a brain of this size (3 mm length). We therefore adapted procedures used by T. Sejnowski (pers. comm.). The fish was perfused with saline and the brain placed overnight in cold anhydrous acetone (4°C). Xylene was substituted for the acetone, and the tissue processed for paraffin histology, modified to avoid contact with water. Sections were cut at 15 u and dry-mounted on albumin-coated slides. The slides were dewaxed, air-dried, and dipped in liquid emulsion (Kodak NTB-2). Slides were developed after 2-5 days.

Every region previously known to be electroreceptive was labelled: posterior lateral line lobe, lobus caudalis, eminentia granularis, n. praeeminentialis and torus semicircularis. Certain areas believed not to be electroreceptive were only slightly above background: torus longitudinalis, parts of the cerebellum and the reticular formation. In addition a number of unidentified diencephalic and telencephalic areas were also labelled.

We thank T. Sejnowski for his invaluable suggestions for alternative 2-DG procedures, and W. Heiligenberg for sponsorship. (Supported by NIMH grant PHSMH-2614904 and NSF grant BNS76-20761 to W.H.).

207.6 PRELIMINARY OBSERVATIONS ON THE NEURAL CONTROL OF SOUND PRODUCTION IN THE ATLANTIC CROAKER, Micropogon undulatus. L.S. Demski, E.J. Williams*, and J.G. Dulka*. School of Biological Sciences, Univ. of Kentucky, Lexington, KY 40506 and Gulf Coast Research Laboratory, Ocean Springs, MS 39564.

Croaking has been evoked by electrical stimulation of the brain in seven anesthetized croakers ranging in size from 11½ to 13 cm standard length. The fish were caught in a trawl in the Mississippi sound in July 1979. The techniques used were very similar to those used with toadfish (see Demski and Gerald, Anim. Behav. 20: 507). Due to an equipment failure, single pulses rather than biphasic square-wave pulse pairs were used. The anesthetic was a solution of 1 to 40,000 MS-222 in seawater. Frequencies of 10-100 Hz were used with currents up to 150 μA at a constant duration of 1 msec. *Thresholds ranged from 80 to 150* μA. Evoked responses occurred both during and at the termination of the stimulation (after-responses). Most responses sounded normal as compared to croaking evoked by gently squeezing anesthetized, unoperated fish. A total of 43 electrode tracts were run in a dorsoventral direction. Fourteen of 28 positive sites were marked and identified histologically. These sites were located on 13 tracts which were completely reconstructed. Five sites were in the area of the midbrain equivalent to the sonic midbrain area (SMA) of toadfish (see Demski and Gerald, Brain Behav. & Evol. 9: 41). Three sites were in the basal midbrain and their position suggests that a functional system may extend in ventral direction from the caudal part of the SMA into the reticular formation of the basal tegmentum. Six other sites were found in the ventrolateral medulla in positions analogous to many positive points in toadfish. These data compare quite well with the results of the toadfish studies and suggest that similar sonic mechanisms may exist in the two types of fishes studied. A group of croakers also received a series of brain transections in an attempt to determine grossly if the midbrain region from which sounds were evoked was critical for normal croaking. The animals were anesthetized in solutions of either 1 to 30,000 or 1 to 40,000 MS-222 and seawater and then tested for croaking in response to gentle squeezing. Fish that responded underwent brain transections (a sharpened spatula was used). Following this they were retested for croaking. Although the brains have not yet been examined histologically, it seemed apparent at the time of surgery that cuts through the rostral part of the midbrain had no striking effect on croaking, whereas cuts made in caudal midbrain near the cerebellum resulted in a loss of, or at least, a noticeable weakening in sound production. These preliminary observations are consistent with the idea of a SMA as defined from the stimulation studies.

207.7 THE MAUTHNER NEURON MEDIATES THE INITIAL STAGE OF A DIRECTIONAL AVOIDANCE RESPONSE IN GOLDFISH. Robert C. Eaton & William A. Lavender*. Dept. E.P.O. Biology, Univ. Colorado, Boulder, CO 80309

One of the most commonly observed behavior patterns in fish is the startle response to sudden and unexpected stimulation, as when an aquarium is bumped with the hand. Several lines of evidence support a hypothesis that this "fast-start" behavior is initiated by the Mauthner cells (M-cells), a single pair of large interneurons located in the hind-brain.

The typically observed fast-start pattern, the C-start, is biphasic. The initial stage is a brief, C-like, contraction of the body musculature on one side. The second stage is a highly variable, but strongly propulsive contraction on the side opposite the initial contraction. Because the first contraction lasts only 15-20 ms, it is usually apparent only after viewing high-speed cine films of the behavior. The major unresolved question is whether the M-cell initiates the first stage, the second, or possibly both.

To answer this, we filmed at 500 frames/sec, fast-start behavior in six goldfish, chronically implanted with single microelectrodes near one of the M-cells. This implantation procedure is based on that of Zottoli (J. Exp. Biol. 66:243, 1977), and the stimulus was a 74 gm ball dropped into the aquarium from above the fish. We found that the M-cell fires only once, 9.3 ms ±0.2 (±S.E., n=25) before the onset of stage 1 of the C-start. Neither M-cell appeared to directly activate the motoneurons initiating the second stage.

C-starts have been shown to occur in predator avoidance responses of goldfish, and an appealing hypothesis is that the M-cell system can mediate directional avoidance maneuvers that will carry the animal away from the predator. Our experiments confirm this for objects falling into the water and it is likely that the result can be extended to predatory strikes as well. We dropped the ball 18 times on the same side of the fish as the recorded M-cell soma, and in 15 trials (83%) the recorded M-cell fired, causing the fish to turn away from the side of the ball. The fish turned into the path of the ball in only 3 trials (17%).

The present experiments provide the first direct association between the M-cell action potential and a documented behavior pattern, occurring in freely-swimming animals. We demonstrate a precise time-locked correlation between the M-spike and the first stage of the C-start, and furthermore show that the M-cell system is activated so that the behavior is used for directional avoidance maneuvers.

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207.8 MÜLLER AXON FIRING DURING MOVEMENTS IN LAMPREY. R.D. Clark and W.O. Wickelgren. Dept. of Physiology, Univ. of Colorado Medical School, Denver, Colorado 80262.

To obtain information about the role of lamprey giant reticulospinal cells (Müller cells) in motor behavior in this animal, intracellular recordings were made from the giant spinal axons of these cells during spontaneous and evoked writhing/swimming movements. Larval and adult lamprey were anesthetized briefly with MS-222 and a small region of spinal cord was exposed in the trunk. The underlying notochord was pinned in a saline-filled chamber at 5-8°C; the tail and head of the animal were lightly restrained as needed. Müller axons were identified by their depth in the cord and their characteristic resting and action potentials; identification was confirmed in several cases by dye injection. Movements in this nearly intact preparation were evoked by cutaneous stimulation of the sucker mouth and were monitored by a piezoelectric device in contact with the trunk. An attempt was made to sample from as many different Müller axons as possible; however, it is likely that in any single experiment an axon may have been penetrated more than once. Further, the precise timing of firing with respect to the onset of movement was not determined.

Of 47 Müller axon penetrations in adults, 21 (53%) fired a single spike or a burst in response to tactile stimulation of the sucker mouth; of these, 10 (21%) fired during spontaneous or evoked movements. The remainder (47%) were unresponsive even to stimulation which caused strong movements. In larval lamprey, 51 presumptive Müller axons were impaled. 32 (63%) fired once or a burst in response to sucker mouth stimulation; of these, 16 (31%) fired continually during spontaneous or evoked movement. 19 of 51 (37%) did not fire even during evoked strong movements. Identification of the particular Müller axons which fire during movement has not been made, except for one particularly active axon in larvae which has been tentatively identified as belonging to the anterior medullary cell body group (either B_2 or B_3) (cf. Rovainen, Physiol. Rev. 59, 1008, 1979).

Müller cells receive polysensory inputs and send outputs to spinal interneurons and motoneurons; further, stimulation of single Müller axons can evoke weak trunk and fin movements (Rovainen, ibid.). Thus it is somewhat surprising that many Müller axons were inactive during the movements studied here. This suggests that many Müller cells may have specialized motor functions not revealed by the present experimental conditions.

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208.1 ANTAGONISM OF 5-HYDROXYTRYPTAMINE (5-HT) EXCITATORY EFFECTS ON SYMPATHETIC PREGANGLIONIC NEURONS (SPGN). K. Kadzielawa. University of Florida, College of Medicine, Dep. Pharmacol. Ther., Box J-267, Gainesville, Florida 32610.

The purpose of this study was to apply pharmacological analysis of the serotonin effects on SPGN , located in the thoracic segments of the spinal cord. These neurons are the main central sympathetic output station regulating heart rate and blood pressure.

Experiments were performed on cats anesthetized with a mixture of chloralose and urethane. SPGN (Th2-Th3) were identified with antidromic stimulation of the white rami of the stellate ganglion or the ramus to the Th3 intercostal nerve. The rate of the spontaneous firing of SPGN was analyzed. Serotonin (HCl-0.3 M, oxalate- 0.1 M, creat. sulf.-0.04 M; pH 3.5-4.5) and its antagonists: methysergide(maleate-0.01 M, pH 4.0) and cinnanserin (HCl -0.15 M, pH 4.5) were applied by means of microiontophoresis or with pressure ejection, from seven barrel micropipettes. 3-chloroimipramine (HCl-0.1 M, PH 4.0) was used as an inhibitor of 5-HT uptake.

The spontaneous activity of about 75 % of the 50 neurons tested was increased by 5-HT (40-80 nA) in proportion to the current applied and to the duration of ejection. Methysergide (60-80 nA) and cinnanserin (80-100 nA) attenuated or antagonized the excitatory effects of 5-HT, while chlorimipramine (40-60 nA) potentiated 5-HT action. When ejected for longer periods of time (5-10 min) 5-HT antagonists decreased the rate of firing of SPGN (inhibition of the endogenous 5-HT excitatory input ?).

These findings confirm the earlier report of DeGroat, W.C. and Ryall, R.W. (Exper. Brain Res., 1967, 3, 299-305) on the excitatory effects of 5-HT and indicate that this action of serotonin on SPGN can be blocked by specific 5-HT antagonists and that it is potentiated by an inhibitor of 5-HT uptake.

208.2 EVIDENCE FOR A TONIC GABAERGIC CONTROL OF SEROTONIN NEURONS IN THE MEDIAN RAPHE NUCLEUS OF RAT. C. M. Forchetti* and J. L. Meek* (SPON: E. Costa). Lab. Preclin. Pharmacol., NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

A method to determine which transmitters are secreted by neurons that innervate serotonin (5-HT) cell bodies is to inject into a raphe nucleus agonists and antagonists and then to measure 5-HT turnover in brain areas whose 5-HT innervation is by axons arising from these cell bodies. Iontophoretic studies of raphe nuclei have shown that GABA inhibits the activity of 5-HT neurons suggesting that 5-HT neurons contain GABA receptors on their cell bodies. We have examined whether the injection of GABA agonists and antagonists into the median raphe nucleus modifies the 5-HT turnover and/or the 5-hydroxyindoleacetic acid (5-HIAA) steady state level in the hippocampus of rats. This brain region receives its 5-HT innervation primarily from the median raphe nucleus. Since benzodiazepines amplify receptor responses to GABA we have studied the action of these drugs on hippocampal 5-HT turnover. The 5-HT turnover was estimated by measuring the rate of accumulation of 5-HIAA after probenecid (400 mg/kg i.p.); the 5-HIAA content was measured by High Pressure Liquid Chromatography (HPLC). The GABA agonist muscimol (100 ng) caused a 22% decrease in 5-HIAA content and reduced the 5-HIAA accumulation after probenecid by 30%. Subconvulsive doses of the GABA antagonists picrotoxin (2 µg) and bicuculline methiodide (4 µg) increased 5-HIAA steady state level by 47% and 33% respectively. Muscimol was able to almost completely block the effects of GABA antagonists and its action was potentiated by diazepam (5 mg/kg, i.p.). These results provide biochemical evidence of a tonic inhibition by GABA of 5-HT neuronal activity in the median raphe nucleus; this inhibitory effect is potentiated by a benzodiazepine.

208.3 EFFECTS OF IONTOPHORETICALLY APPLIED SEROTONIN ON MEMBRANE POTENTIAL, RESISTANCE, AND EXCITABILITY OF RAT FACIAL MOTONEURONS. C.P. VanderMaelen and G.K. Aghajanian. Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06508

Previous studies utilizing extracellular single unit recording techniques have found that microiontophoretic application of serotonin onto facial and spinal motoneurons results in a facilitation of excitatory inputs, but does not, by itself, cause these neurons to discharge (McCall & Aghajanian, Brain Res., 1979, 169, 11-27; White & Neuman, Brain Res., 1980, 188, 119-127). In an attempt to determine some of the underlying membrane mechanisms involved in this phenomenon we recorded intracellularly from rat facial motoneurons during extracellular microiontophoretic application of 5-HT, methysergide, or norepinephrine (NE).

Male albino rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic device. A hole was drilled in the skull to allow placement of the recording electrode assembly, and a cisternal drainage was performed. 5-barrel micropipettes were affixed with dental acrylic to intracellular glass microelectrodes. Recording electrodes were filled with 2 M KCl and had resistances of 15-25 MΩ. Iontophoretic barrels were filled with 5-HT creatine sulfate (0.04 M, pH 4.0), methysergide bimaleate (0.005 M, pH 4.0), l-norepinephrine bitartrate (0.1 M, pH 4.0), and NaCl (2 M or 4 M) for current balance. Sometimes the 5th iontophoretic barrel was filled with a solution of fast green for marking the recording location in the brain. Tip separations between recording and iontophoretic electrodes ranged from 25-60 µm. The facial nucleus was located by the presence of a characteristic antidromic field potential in response to stimulation of the zygomatic muscle branch of the facial nerve.

Iontophoretic application of 5-HT (25-60 nA for 30-120 sec) resulted in a slow depolarization of about 5 mV which remained subthreshold, accompanied by an increase in input resistance (measured by voltage displacement by a 1.0 nA hyperpolarizing pulse), and an increase in excitability (measured by responses to direct intracellular depolarizing pulses). NE also caused these same changes. Methysergide, a 5-HT receptor blocker, antagonized the effects of 5-HT but not NE, indicating that these actions of 5-HT are receptor mediated. We conclude that these membrane changes induced by 5-HT are responsible for the facilitation of excitatory inputs noted in previous extracellular studies, and underlie some of the major components of the excitatory behavioral "5-HT syndrome" seen in animals injected with 5-HT receptor agonists.

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208.4 SEROTONERGIC MODULATION OF TRIGEMINO-FACIAL REFLEX ACTIVITY IN THE RHESUS MONKEY. D. S. Charney*, M. Davis and G. R. Heninger, Dept. of Psychiatry, Yale Univ., Sch. Med., New Haven, CT 06508.

Anatomical studies indicate that the facial motor nucleus receives a dense serotonin (5-HT) input, and single unit studies in rats have demonstrated that excitation of the facial motor nucleus is facilitated by 5-HT. The functional significance of these findings was investigated by determining the effects of 5-HT antagonists on shock-elicited trigemino-facial reflex activity. Reflex activity was elicited in 4 unanesthetized rhesus monkeys by delivering 0.5 msec constant current shocks through needle electrodes implanted over the left supraorbital branch of the trigeminal nerve. Stimulus intensity varied between 2 and 15 mAmps. Reflex activity was recorded from two subcutaneous needle electrodes placed over the ipsilateral orbicularis oculi muscle just below the pupil. Drugs or placebo were given with rapid intermittent infusions through a catheter placed in the superficial saphenous vein to determine cumulative dose-response effects over the 2 hour recording session. Total cumulative doses of the 5-HT antagonists were: cyproheptadine (1.5 mg/kg), methysergide (3 mg/kg) and cinanserin (9 mg/kg). Parametric variations of stimulus intensity and stimulation rate produced effects on the reflex that have previously been demonstrated in humans. Two reflex components were evoked; an early response (R_1) with a latency of approximately 7 msec and a later, more prolonged response (R_2) with a latency of 15-25 msec. Paired stimuli with interstimulus intervals between 100 and 350 msec led to an increased R_1, but a decreased R_2. The three structurally dissimilar 5-HT antagonists markedly reduced both components of the reflex in a dose-related fashion, in the absence of gross changes in behavior. The decrease in R_2 appeared at a lower cumulative dose of the 5-HT antagonist than the decrease in R_1. The data indicate that the serotonergic system exerts a chronic facilitatory effect on the trigemino-facial reflex in the unanesthetized monkey, probably via facilitation of inputs to the facial motor nucleus as shown in single unit recording studies in the rat. Changes in the activity of trigemino-facial reflex may be a clinically useful method for the investigation of serotonergic effects on sensitization of sensory motor systems in humans.

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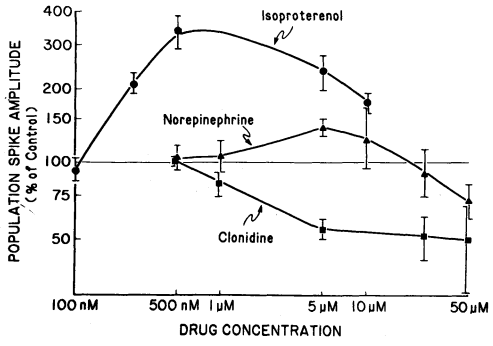
208.5 COMPARISON OF THE INTERACTION BETWEEN IONTOPHORETICALLY APPLIED GABA AND DIAZEPAM WITH SEROTONIN ON CEREBELLAR PURKINJE CELLS. Jean C. Strahlendorf, Howard K, Strahlendorf,† and Charles D. Barnes. Departments of Physiology and Pharmacology and Therapeutics,† Texas Tech University Health Sciences Center, Lubbock, Tx. 79430

Previous electrophysiologic studies from our laboratory have revealed that stimulation of the raphé nuclei (RN) inhibits spontaneous and evoked simple and complex spike activity of cerebellar Purkinje cells (PCs). Furthermore, RN conditioning stimulation, at current strengths subthreshold for inhibition of spontaneous PC activity, produced a marked augmentation of GABA mediated "off beam" inhibition. Preliminary findings have also revealed that iontophoretically applied serotonin (5-HT) at currents which are ineffective in changing ongoing PC activity potentiates the inhibition produced by concomitant iontophoretic pulses of GABA. Since recent neurochemical studies indicate an association between central nervous system GABA receptors and benzodiazepine receptors, we have compared the effects of 5-HT on GABA and diazepam (DIAZ) induced depression of spontaneous PC activity.

Experiments were performed on albino rats anesthetized with chloral hydrate or urethane. PCs located in the anterior and posterior vermis were recorded with conventional 5 or 7 barrel iontophoretic pipettes. Drug barrels were filled with 5-HT (0.05M, pH4.0), GABA (0.5M,pH4.5), DIAZ (0.05M,pH4.0), or sodium glutamate (0.2M,pH8,0). Recording and balance barrels contained 2M and 4M NACl respectively. Twenty to 30 second intermittent pulses of GABA or DIAZ were applied to PCs which resulted in a diminution of firing frequency during application. Following a control series, 5-HT was applied continuously at subthreshold currents while GABA or DIAZ continued to be pulsed. PCs showed a greater degree of inhibition to GABA and DIAZ in the presence of 5-HT; however, 5-HT potentiation of GABA actions (N=10 cells) exceeded that seen with DIAZ (N=8 cells). These studies support our previous contention that 5-HT may function as a neuromodulator or bias setter within cerebellar circuitry by influencing inhibitory processes. (Supported by the Tarbox Parkinson's Disease Institute of Texas Tech Univ. Health Sci. Ctr.)

208.6 NOREPINEPHRINE AND SYNAPTIC TRANSMISSION IN THE IN VITRO RAT HIPPOCAMPUS. Alan L. Mueller*, Barry J. Hoffer*, and Thomas V. Dunwiddie (SPON: Warren O. Wickelgren). Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

Adrenergic responses in the rat hippocampus were studied using evoked field potentials in CA1 of the in vitro hippocampal slice after stimulation of the Schaffer-commissural afferents. Superfusion of norepinephrine (NE) caused a weak and highly variable effect on population spike amplitude. This effect appeared to be dose-dependent, with lower doses generally producing weak excitations (approx. 30%) and higher doses more often producing inhibitions (0-40%); see Fig. 1. No effect on EPSP amplitude was seen. Application of isoproterenol (ISO), a β agonist, produced a much larger increase in population spike amplitude, up to 300%, with an EC_{50} of 270 nM. The β antagonists propranolol and timolol were able to completely block this effect of ISO. Clonidine, an α agonist, produced a dose-dependent inhibition of population spike amplitude, up to 50%, with an EC_{50} of approx. 2 uM. Phentolamine, an α antagonist, blocked this effect of clonidine while enhancing the effect of ISO. No change in EPSP amplitude was seen with either ISO or clonidine. Taken together, these results strongly suggest that NE acts at both α and β receptors to alter pyramidal cell excitability. We hypothesize that activation of β receptors leads to increased excitability while activation of α receptors leads to decreased excitability.



Supported by FS 02011 to B.J.H. and NS 05962-02 to T.V.D.

209.1 EFFECTS OF CONVULSANT BARBITURATES ON THE RELEASE OF ^{3}H-ACh FROM MOUSE HIPPOCAMPAL SLICES. J.R. Holtman, Jr.* and J.A. Richter, Depts. of Pharmacology and Psychiatry, Indiana Univ. School of Medicine, Indianapolis, IN 46223.

We have studied the actions of 5-(2-cyclohexylidene ethyl)-5-ethyl barbituric acid (CHEB) and (+) 1-methyl-5-phenyl-5-propyl barbituric acid (MPPB), both convulsant barbiturates, as well as (-) MPPB which possesses only depressant action in an effort to elucidate the mechanism of action of the convulsant barbiturates. The effects of these compounds on the release of preformed ^{3}H-ACH was studied in an in vitro superfusion system. In vivo studies were also performed for correlation with the in vitro results. The dose of CHEB causing convulsions in 50% of a population of mice was found to be 17 mg/kg. The brain level of CHEB at convulsion was then measured by radioimmunoassay in several brain regions of the same mice and did not differ significantly among regions (mean ± S.E.M., n=7):

Region	CHEB nmoles/gm
Cerebellum	14.8 ± 2.7
Cortex	14.0 ± 2.3
Midbrain	16.9 ± 2.7
Pons-Medulla	15.9 ± 2.6
Striatum	10.2 ± 1.0
Hippocampus	12.9 ± 2.3

CHEB brain levels in those mice not convulsing was lower in all regions. A concentration of 5.0×10^{-4}M CHEB was tested on the in vitro release of ^{3}H-ACh from mouse hippocampal slices. In preliminary exp eriments this concentration of CHEB was found to produce a near maximal drug-i nduced effect in mouse forebrain slices. Hippocampal slices were first loaded with ^{3}H-choline and then superfused at 0.25 ml/min with normal phosphate buffered medium for 30 min at which time a constant release of ^{3}H-ACh was observed. The slices were then superfused at the same rate for 60 min with medium containing 5.0×10^{-4}M CHEB or 50 mM K^+. This concentration of CHEB stimulated ^{3}H-ACh release to 166 ± 17% of resting release compared to 2213 ± 196% by the 50 mM K^+. The time courses for the releasing action of CHEB and K^+ were both similar with peak release occurring in the same fraction. After peak release, ^{3}H-ACh decreased in the remaining fractions collected to a point approximately the same as resting release. When (-) and (+) MPPB were tested in the same system neither isomer effected ^{3}H-ACh release. Further experiments will evaluate the dose dependency of the in vitro effects of these drugs as well as the Ca^{++} dependence and specificity of transmitter release by CHEB. (CHEB kindly supplied by Dr. H. Downes; MPPB isomers by Dr. J. Knabe).

209.2 ETHANOL POTENTIATES GABA-MEDIATED INHIBITION IN RAT HIPPOCAMPUS. J.N. Nestoros, K. Krnjević, R.J.Reiffenstein* & A.I. Shapovalov*. Anaesthesia Research and Physiology Depts., McGill University, Montreal, Canada H3G 1Y6.

Ethanol (ethyl alcohol) is the oldest antianxiety agent, and antianxiety drugs (benzodiazepines, barbiturates) are known to potentiate GABAergic neurotransmission. Furthermore, ethanol selectively augments only the GABA-evoked depolarization of primary afferent terminals in the amphibian spinal cord *in vitro* (Davidoff, Arch.Neurol.,28, 60, 1973) and the GABA-mediated inhibitions of feline cerebral cortical neurons *in vivo* (Nestoros, Science, in press). We have therefore studied in ketamine-anaesthetized rats the effects of intravenously administered ethanol on (1) the IPSPs and (2) the field potentials evoked in the CA1 and CA3 regions by stereotactic stimulation of the ipsilateral fimbria or entorhinal cortex.

Recording intracellularly with KCl electrodes we monitored continuously the neurons' membrane potential and input resistance at "rest" and during the IPSPs, by injecting with the help of a Linc 8 computer regularly repeated series of known current pulses through the recording electrode. Most often each pulse lasted 10 msec, it had a hyperpolarizing direction and 3 such sets of pulses were injected at intervals of 30 msec to measure the time course of the IPSPs. We found that ethanol consistently prolonged the time course of IPSP conductance increase independently of membrane potential changes. The prolongation of the IPSPs' time course occurred with doses as low as 8 mg/kg (the highest total dose used in these studies was 2,105 mg/kg). On the other hand, there was no clear change in the maximum of conductance increase at the peak of IPSPs.

With extracellular recording, the IPSPs evoked by low frequency (< 2 Hz) fimbrial stimulation generate a characteristic positive field response in the superficial pyramidal cell layer. Stimulation at higher frequencies rapidly leads to the appearance of single or multiple negative "population" spikes; and, if sufficiently prolonged, "seizure" activity is generated (desynchronized firing, after-discharges, followed by post-ictal depression). We found that intravenously administered ethanol (from 8 mg/kg) increased significantly the threshold (frequency of stimulation; time of stimulation) for the occurrence both of negative population spikes and seizure activity. Ethanol also antagonized the effects of picrotoxin (also given intravenously).

These findings further implicate GABAergic neurotransmission in the effects of ethanol on the CNS, and may have significance for both the etiology and treatment of alcoholism.

Supported by the Canadian Medical Research Council.

209.3 EFFECT OF SUBACUTE IN VIVO ETHANOL ADMINISTRATION ON ^{3}H-DIAZEPAM BINDING IN VITRO. R. L. Kochman, J. D. Hirsch, and G. A. Clay*. Dept. Biol. Res., G.D. Searle & Co., Chicago, IL 60680

The potentiation of the depressant effects of ethanol by benzodiazepines has been well documented clinically. The present study was designed to assess a possible interaction between ethanol and diazepam at the benzodiazepine receptor. Seven week-old male CD/CR rats (Charles River Breeding Laboratories, Wilmington, MA), weighing 185-230 grams and maintained on a Sustagen® (Mead Johnson Laboratories, Evansville, IN) liquid diet, were administered (p.o.) 2.5 grams/kg of 20% ethanol four times a day for 4.5 days. Control rats received 2.5 ml/kg of distilled water. Two hours after the last administration, all rats were decapitated. Crude mitochondrial pellets (P_2) from the whole brains with cervical spinal cords were prepared in 0.32M sucrose, osmotically shocked in deionized water, and frozen at -70°C. Saturation isotherms were determined using 0.5-40 nM ^{3}H-diazepam in the absence and presence of a 1000-fold greater concentration of unlabelled diazepam, 0.5-0.7 mg of homogenate protein from control or ethanol-treated rats, and 50mM tris·citrate (pH 7.4 at 0°C) in a total volume of 2 mls. for one hour at 0°C. The dissociation constants (K_D) for control (5.05±0.16 nM; n=8) and for ethanol-treated rats (5.30±0.27 nM; n=8) were not significantly different. However, ethanol treatment significantly lowered B_{max} values from 0.77±0.03 pmole/mg protein (control) to 0.68±0.02 pmole/mg protein (ethanol-treated)($p < .05$). These results suggest that the interaction between diazepam and ethanol may be mediated by a direct effect of ethanol at the benzodiazepine receptor.

209.4 DOSE-DEPENDENT EFFECTS OF ETHANOL ON THE EXCITABILITY OF IN VITRO CENTRAL MAMMALIAN NEURONS. P. Carlen, N. Gurevich, D. Durand, J.M. Wojtowicz and J.F. MacDonald. Playfair Neuroscience Unit, Addiction Research Foundation, University of Toronto, Toronto, Ontario, Canada, M5T 2S8.

The actions of ethanol, using conventional intracellular recording techniques, were examined on two preparations:
1) identified CA1 neurons of rat hippocampal slices (500 μm)
2) unidentified neurons of dissociated brain cultures derived from mouse foetuses and grown for a period of at least 4 weeks.

Ethanol was included in the perfusion medium of the hippocampal slices (20-200 mM) or applied by pressure microperfusion (10-200 mM) to single cultured neurons. Synaptic activity was assayed by stimulation of the stratum radiatum to evoke EPSPs and a subsequent long lasting IPSP (sometimes > 1 sec) in the case of slices, but was usually suppressed in cultures by elevating magnesium concentrations (10 mM $MgCl_2$).

Ethanol (20-50 mM) hyperpolarized and reduced the membrane resistance of CA1 neurons. Similarily cultured neurons were hyperpolarized by ethanol applications (10-100 mM) provided recording electrodes contained K Acetate (although most but not all cells, in either preparation, were responsive). Such responses were inverted to depolarizing responses when KCl electrodes were substituted (cultured neurons) and a correspondence of inversion potentials for ethanol and GABA responses indicated an increase in chloride permeability. Higher concentrations of ethanol (>150 mM) were significantly less effective in evoking this response in either preparation but were associated with a local anaesthetic action on spikes. After 10-15 minutes perfusion of ethanol (100 and 200 mM), CA1 cells occasionally demonstrated slow oscillations of membrane potential (3-8 sec period) and an increased frequency of presumed dendritic spikes. At all doses, an increase in the size of the stratum radiatum EPSP and IPSP were observed.
(Supported by MRC grants MA-7216, MA-6019 and NIH grant 1R01 NS16660-01 ALCB).

209.5 ETHANOL INDUCES A NEGATIVE SLOPE RESISTANCE REGION IN APLYSIA NEURONS. L. Silver*and S.N. Treistman (SPON: E. Thomas). Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010, and Worcester Foundation for Experimental Biology, Shrewsbury, MA. 01545.

Identified neurons from the buccal and abdominal ganglia of Aplysia californica were examined under voltage clamp to elucidate the effects of ethanol and other alcohols. As has been reported for a number of neural preparations, ethanol is capable of blocking the fast inward currents associated with the production of action potentials. However, at concentrations in which the action potential is only partially reduced, previously silent neurons were induced to generate "bursting pacemaker" discharges. This change in activity pattern was associated with a change in the quasi-steadystate current-voltage plot, characterized by the appearance of a negative slope resistance region (NSR). These events were very concentration-dependent, and in the silent cells studied, occurred at concentrations of 0.6M ethanol. At higher concentrations, the I-V plot became more linear, and membrane oscillations no longer occurred. The effects of ethanol were reversible with washing.

For comparison purposes, we examined the effects of 0.6M ethanol on cell R15, which exhibits a spontaneous bursting pattern and NSR. In this cell, 0.6M ethanol induced either extended bursts or beating activity, and reduced or abolished the NSR.

Ethanol is typically considered to be a membrane fluidizer. Our results suggest the possibility that the expression of NSR occurs in a relatively restricted fluidity range, and a normally bursting cell could be pushed out of this range by concentrations of ethanol which place silent cells into compliant range. The effects of ethanol were blocked by low temperature and decanol, which may act as membrane ordering agents.

Supported by NIH grant NS 15195-01

209.6 EFFECT OF ALCOHOL ON THE ELECTRICAL ACTIVITY OF CULTURED CNS NEURONS. D.L. Gruol. A.V. Davis Ctr. for Behav. Neurobiol., The Salk Inst., La Jolla, CA 92037.

The acute effects of ethyl alcohol (ETOH) on the activity of CNS neurons was tested using cultured fetal mouse spinal cord neurons as an in vitro model system and conventional electrophysiological techniques. ETOH was applied extracellularly by superfusion. Intracellular recordings were made from large (25 to 50 um) neurons which had been maintained in culture for 1 month or longer. Spontaneous depolarizing and/or hyperpolarizing potentials were observed in all spinal cord neurons studied (N=30). These potentials occurred singly or in burst discharges and were interpreted as presumptive excitatory and inhibitory synaptic potentials (EPSP's or IPSP's), respectively. Single or repetitive spikes were evoked by the spontaneous depolarizing potentials.

ETOH (30 to 500 mM) altered the pattern and frequency of the spontaneous activity of the cultured neurons. In neurons which showed a bursting discharge pattern, the lower ETOH concentrations (close to threshold for intoxicating blood levels) reduced the frequency of burst discharges and the number of synaptic potentials within each burst while the number of single potentials which occurred between burst discharges was increased. In neurons which did not show a bursting pattern, the frequency of the spontaneous potentials was reduced by ETOH treatment. These changes were not associated with a pronounced alteration in membrane potential, input resistance or depression of spike height in most neurons. At the higher ETOH concentrations tested, all spontaneous activity was suppressed and alterations in membrane potential and input resistance frequently occurred, the most consistent effect being an increase in membrane input resistance (10 to 30%). All ETOH effects were readily reversible.

In preliminary studies the response of the neurons to putative neurotransmitters was also evaluated in the presence and absence of ETOH in order to identify possible post synaptic effects of ETOH. ETOH (30 to 500 mM) reversibly potentiated the inhibitory GABA and glycine responses evoked by extracellular application of these amino acids via a glass micropipette. The excitatory glutamate response was unaltered or slightly depressed. The potentiation of the inhibitory responses could be partially explained by an increase in membrane input resistance produced by ETOH in these neurons.

These studies indicate that ETOH has multiple effects on the activity of cultured CNS neurons but the specificity and mechanisms underlying these effects remains to be elucidated. (Supported by Grant AA 03504.)

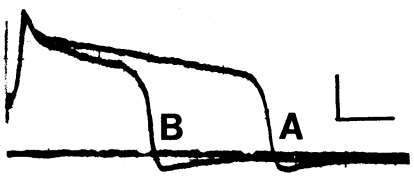
209.7 ALCOHOL DECREASES THE CALCIUM COMPONENT OF SENSORY NEURON ACTION POTENTIALS IN CULTURE. S. George Oakes* and R. S. Pozos (SPON: D. Forbes). Dept. of Physiology, Univ. of Minn.-Duluth, Sch. of Med., Duluth, MN 55812.

Ethanol has been reported to influence neurotransmitter levels (Hunt and Majchrowicz, J. Neurochem. 23:549, 1974) possibly by changes in release due to alterations of Ca^{2+} ion movement (Levett, Morini and Jack, Currents in Alcoholism, Vol. III, ed. Seixas, 1978). To investigate this possibility, electrophysiological measurements of sensory neurons exposed to ethanol were performed. Recently, Dunlap and Fischbach (Nature, 276(569), 837-839, 1978) have reported that increased extracellular Ca^{2+} accentuates the calcium component of DRG neurons producing an increased full width at half maximum amplitude (FWHM) action potential. The investigators feel that decreases in FWHM indicate decreased Ca^{2+} permeability in this model.

DRG were dissected from 17 day rat embryos, dissociated and maintained for 4-6 weeks. After 4 weeks recordings were made in a balanced salt solution containing 5.4 mM Ca^{2+} and 3.0 mM Ba^{2+}, which inactivates the K^+ conductance and passes through activated Ca channels. Ethanol was placed directly on the cell with a diffusion pipette. Control and post application recordings were recorded on an FM tape recorder and analyzed on a PDP-12 computer. Exposure to 0.2 gm% ethanol produced a marked decrease in the FWHM when compared to the control value (Fig. 1). The effect appears dose dependent since exposure to 0.5 gm% ethanol produced a much greater decrease in FWHM. The alteration was reversible within several minutes. Resting membrane potential and spike amplitude were unaffected by exposure to these levels of ethanol.

These data suggest ethanol in physiological dosages influences the calcium current in sensory neurons. Decreased calcium movement may account for changes in neurotransmitter release found with exposure to ethanol.

Figure 1 demonstrates (A) the FWHM before the application of ETOH and (B) the immediate effect on the FWHM of the application of .2 gm% ETOH (calibration: 20 mv and 10 ms).



Supported by a grant from the Minnesota Office of Alcohol and Other Drug Abuse Programming.

209.8 TOLERANCE TO ETHANOL-INDUCED CHANGES IN EVOKED POTENTIALS. Larry P. Gonzalez, Mitchell B. Friedman*and Boris Tabakoff. Department of Physiology and Biophysics, Univ. of Illinois Medical Center and the West Side V.A. Medical Center, Chicago, Il.

Acute administration of ethanol is known to alter the function of striatal dopaminergic neurons. While acute ethanol administration is believed to increase striatal dopamine release, such treatment produces an inhibition of acetylcholine in cortical and subcortical areas. In addition, an increase in hippocampal cholinergic receptors has been shown to accompany chronic ethanol administration. To further investigate the involvement of these neuronal systems in the mechanisms of action of ethanol, we have examined the acute effects of ethanol on evoked potentials in the caudate nucleus and in the hippocampus, both before and after chronic ethanol administration.

Averaged evoked potentials were recorded from the caudate nucleus in response to electrical stimulation of the substantia nigra and from the hippocampus in response to septal stimulation. Although the particular effects varied from subject to subject, the general effect of an acute injection of ethanol (1.5g/kg, 20% w/v, ip.) was a significant increase in the peak-to-peak amplitude of the early and late components of the evoked response of the caudate nucleus, and a decrease in these measures of the hippocampal evoked response. These results are consistent with the view that acute ethanol increases the activity of the nigrostriatal dopaminergic neurons but decreases the activity of cholinergic neurons terminating in the hippocampus.

Following this initial test of the acute effect of ethanol, the animals were maintained in an ethanol vapor chamber in which they were exposed to a low concentration of ethanol vapor (17.6 mg/l) for 12 hours per day following a total dose of 3.0g/kg. Evoked potentials were recorded before and after acute ethanol administration on every third day, 8 hours after the removal of ethanol vapor. The animals showed no signs of intoxication and ethanol was not detectable in the blood. After as little as 3-days of exposure to this regimen of ethanol administration, the effect of acute ip. ethanol on the evoked response was significantly reduced at both recording sites. At the same time, a reduction was observed in the effect of ethanol (total dose, 3.0 g/kg, ip.) on the righting reflex.

We interpret these results to indicate that alterations in the function of both dopaminergic nigrostriatal neurons and cholinergic septal-hippocampal neurons accompany the behavioral and physiological development of ethanol tolerance.

Supported by grants from PHS BRSG (79507), NIAAA (2696), NIDA (2024 and 1951), the state of Illinois DMH&DD (8083-13) and the Medical Research Service of the Veterans Administration.

209.9 EFFECT OF ETHANOL ON PROTEIN PHOSPHORYLATION IN HUMAN BLOOD PLATELET PROTEINS. Harish C. Pant* and Forrest F. Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, 12501 Washington Ave., Rockville MD 20852.

Incubation of intact human platelets with $^{32}PO_4$ reveals multiple phosphorylated proteins. Agents that induce the secretion of platelet granules (the platelet release reaction) cause an increase in the phosphorylation of specific platelet proteins. In the present study, we investigated in vitro, the effect of ethanol on protein phosphorylation in intact human platelets. Platelets were isolated by differential centrifugation from fresh EDTA-anticoagulated human blood and suspended in isotonic tris-buffered saline, pH 7.4. Aliquots of 100 µl of this suspension (approximately 10^8 platelets) were incubated with different concentrations of ethanol for 30 min. The phosphorylation reaction was initiated by adding 0.05 mCi of carrier free (^{32}P) P_i to each sample; the samples were incubated for 60 min at 37°C. The reaction was inhibited by adding 10% trichloroacetic acid. The phosphorylated proteins were analysed by sodium dodecylsulfate-polyacrylamide gel electrophoresis and liquid scintillation counting. The total amount of phosphorylated proteins was progressively decreased from 100% to 36% as the concentration of ethanol was increased from 0 to 500 mM. This decrease was found in all phosphorylated protein peaks. Thrombin induces the secretion of platelet granules. The application of thrombin (1 unit/ml) increased the phosphorylation of platelet proteins with molecular weights of 48,000-48,000 and 25,000-19,000 daltons. This increase in specific protein phosphorylation was not significantly affected by low concentrations of ethanol (5 to 250 mM). In addition, in thrombin treated platelets, these concentrations of ethanol did not significantly affect the other phosphorylated proteins. The results suggest that thrombin inhibits the effect of low concentrations of ethanol on protein phosphorylation in human platelets.

209.10 THE EFFECTS OF CHRONIC ALCOHOL CONSUMPTION ON THE LEVELS OF MONOAMINES IN DIFFERENT CNS REGIONS OF ALCOHOL-PREFERRING RATS. J.M. Murphy, W.J. McBride, L. Lumeng* and T.-K. Li*. Depts. of Psychaitry and Medicine, Inst. of Psychiatric Research, Indiana Univ. School of Medicine, Indianapolis, IN 46223.

The present study compared the regional levels of norepinephrine (NE), dopamine (DA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the CNS of rats selectively bred to prefer (P) or not prefer (NP) ethanol. The effects of chronic voluntary ethanol consumption on the CNS regional levels of these monoamines were also assessed in a group of P rats.

Two groups of adult males, one P (N=9) and one NP (N=9) group, were housed individually with ad lib food and water. A third group (N=9) of P rats (P-EtOH) was maintained in the same manner, except that free-choice ethanol (10% V/V) was available for 6-8 weeks. Rats in the three groups were killed by the near-freezing method (R. Takahashi & M.H. Aprison, J. Neurochem. 11: 887, 1964). The brains were dissected at -20°C into nine areas: cerebral cortex (CX), striatum (STR), septum (SEP), thalamus (TH), hypothalamus (HY), hippocampus (HIP), midbrain (MID), pons-medulla (PM), and cerebellum (CB). The tissue was frozen at -70°C until assayed by HPLC with electrochemical detection.

The most consistent finding was that the levels of 5-HT and 5-HIAA were 10-30% lower in the STR, TH and MID of the P animals than were found for the NP group. In addition, ethanol appeared to decrease the turnover of 5-HT in the SEP, HIP and HY regions as indicated by the 25-40% lower levels of 5-HIAA found in the P group relative to the P-EtOH animals. There were no significant differences in the levels of 5-HT or 5-HIAA in the CX, SEP, HY, PM or CB of the P compared to the NP group. CX NE levels of the P group (1.94 $\pm$ 0.13 nmol/g) were found to be higher than the NP group (1.46 $\pm$ 0.15 nmol/g), whereas NE content of the PM and CB was 15-20% lower for the P group ($P<0.05$). No significant differences were observed in DA levels for the P compared to the NP group or the P-EtOH compared to the P group.

The lower levels of 5-HT and 5-HIAA in several CNS regions of the alcohol preferring animals in comparison to the control NP group suggest a possible metabolic dysfunction or pathway deficiency in the serotonergic system of the P animals. The findings from the present study support the view of an involvement of serotonin in abnormal alcohol drinking behavior. (Supported in part by PHS Grant AA-03243 and MH 00203).

210.1 INTERACTIONS OF LIGANDS WITH MORPHINE AND ENKEPHALIN RECEPTORS ARE DIFFERENTIALLY AFFECTED BY SODIUM IONS AND GTP. E. Hazum*, P. Cuatrecasas*, A. Killian* and K.-J. Chang* (SPON: C.G. Lineberry). Dept. of Mol. Biol., Wellcome Research Laboratories, RTP, NC 27709.

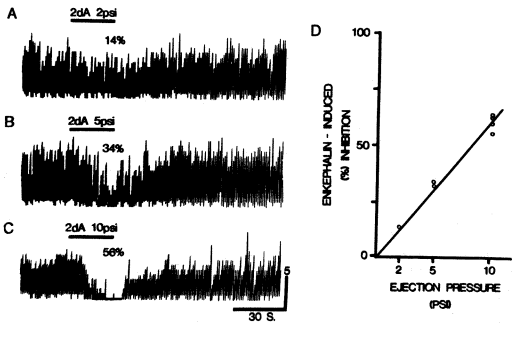
The effects of sodium ions (Na^+) and guanosine-5'-triphosphate (GTP) on morphine (μ) and enkephalin(δ) receptors were examined by using binding assays of [^{3}H]naloxone to rat brain membrane preparations and [^{3}H]diprenorphine to neuroblastoma cell membranes, respectively. The potencies of many opiate agonists and opioid peptides in competing with the binding of the labeled antagonist are reduced by Na^+ (100 mM) and GTP (0.1 mM). These effects are qualitatively similar for both subtypes of opiate receptors. However, quantitatively, the effects of Na^+ and GTP are much more profound for morphine receptor than for enkephalin receptors. Na^+ does not alter the affinity of opiate antagonists. GTP reduces the affinity of naloxone to morphine receptors by a factor of 2.5. Mg^{2+} (5 mM) increases the potencies of opiate agonists and enkephalins for both receptor sites. The combination of Na^+, GTP and Mg^{2+} further reduces the affinity of enkephalins and opiate agonists for enkephalin receptors and the affinity of Met- and Leu-enkephalin for morphine receptors. However, the combination of Na^+, GTP and Mg^{2+} partially restores the affinity of [D-Ala^2, Leu^5]- and [D-Ala^2, D-Leu^5]-enkephalin and morphine for the morphine receptors. These differential effects of cations and nucleotide further support the subtle differences between morphine and enkephalin receptors and indicate the complex interactions of cations and nucleotide with opiate receptors.

210.2 EVIDENCE FOR THE PRESENCE OF ENDOGENOUS FACTORS WHICH MODIFY NARCOTIC LIGAND BINDING CHARACTERISTICS. R.W. Barrett* and J.L. Vaught* (Spon: M.T. Spoerlein, Dept. Pharmacol., College of Pharmacy, Rutgers University) Piscataway, N.J. 08854.

We have recently reported the presence of non-opioid peptide factor(s) in the supernatents(S) of mouse brain homogenates which reduce the specific binding(SB) of ^{3}H-dihydromorphine (^{3}H-DHM) (The Pharmacologist, 1980). To further investigate the role of these factor(s) in drug-opioid receptor interactions, the ability of opiate ligands to inhibit the SB of ^{3}H-DHM was determined in the presence and absence of S. Membranes from pooled mouse brains, minus cerebellum(CB), were prepared by a single homogenization (unwashed membranes;UM) or by standard techniques (washed membranes;WM). In some experiments, S from the first centrifugation (49,000 xg;20 min) was used in place of fresh buffer to resuspend the final WM pellet. All binding assays were performed with 2.2nM ^{3}H-DHM±Levallorphan(37°C;20 min). Each experiment was repeated at least five times in independent membrane preps. The IC50's of naloxone (N) and morphine (M) in UM(4.0±0.1nM and 6.0±0.5nM; respec.)were significantly lower than those in WM(15.9±2.4nM and 12.2±1.3nM respec.). SB of ^{3}H-DHM,in the absence of any drugs, did not differ between UM and WM. When S was immediately removed from UM by centrifugation and the resulting pellet resuspended in fresh buffer, the N IC50 no longer differed from that of WM (11.6±1.1nM). Substitution of S for fresh buffer in the final resuspension of the WM pellet resulted in a significant reduction of the N IC50 to 6.1±1.9nM. Additionally, the SB of ^{3}H-DHM alone was reduced 54%. However, when S was dialyzed (12,000MW cut-off) before addition to WM, the N_2IC50 (10.4±1.8nM) no longer differed from WM, although the SB of ^{3}H-DHM alone was still reduced 42%. S similarly prepared from CB did not effect the N IC50 of WM (13.2±1.2nM) but decreased the SB of ^{3}H-DHM alone by 55%. These data indicate that in addition to factor(s) which reduce the SB of ^{3}H-DHM, S contains separate factor(s) which enhance the inhibitory potency of non-labelled opiates vs. ^{3}H-DHM. These results closely parallel those previously reported by Chau-Pham and Dewey (Life Sci.21: 1977). The fact that this new factor(s) is not present in S from CB suggests that it is not a preparative artifact and makes it more likely that this factor may play an important physiological role in drug-opioid receptor interactions (Supported in part by BRS Grant PHS RR 7058-14).

210.3 PHYSICAL AND PHYSIOLOGICAL CHARACTERISTICS OF MICROPRESSURE EJECTION OF DRUGS FROM MULTIBARRELED PIPETTES. B.J. Hoffer*, M.R. Palmer, S.M. Wuerthele. (SPON: S. Roper.) Department of Pharmacology, Univ. of Colorado, Denver, CO 80262.

The micropressure ejection technique was characterized in vivo as well as in vitro. Using a crystal clock controlled solenoid (Medical Systems Corp.) and fiber filled thinwall (1.5 mm O.D., 1.1 mm I.D.) multibarreled micropipettes, it could be demonstrated that volumes of radiolabelled drug released by this method increase linearly with increasing ejection pressure or time. This is reflected in vivo by graded inhibitions of cortical cell activity with increasing ejection pressures of enkephalin (2dA):



The linearity of this repsonse is independent of interejection time, suggesting that there is no warm-up phenomenon with the micropressure ejection technique. In vitro, interejection times do not affect the amount of 3H-sucrose released by micropressure ejection. The volume of drug released is highly reproducible. Likewise, responses of cerebellar Purkinje cells to repeated applications of norepinephrine or GABA are uniform over long periods of time. The variability in drug release between two barrels of a multibarreled pipette, or between two pipettes using this technique, is also considerably less than that observed with microiontophoresis. In addition to the linearity and reproducibility of micropressure ejection, these studies demonstrate that substances which are difficult to iontophorese, such as peptides, are readily applied by this method. Supported by USPHS Grants DA-07043 and ES-02011.

210.4 EFFECT OF INCREASING DOSES OF MICROIONTOPHORETICALLY INJECTED MORPHINE ON CAUDATE NEURONS IN NAIVE AND MORPHINE-DEPENDENT RATS. Avital Schurr, Benjamine M. Rigor*, Beng T. Ho and Nachum Dafny. Dept. Anesthesiol. and Dept. Neurobiol. and Anat., Univ. Texas Med. Sch., Houston, TX 77025

Experiments were performed on naive and morphine-dependent (using s.c. pellet implantation) Sprague-Dawley rats anesthetized with urethane (1.2g/kg). Unitary activity was recorded from caudate nucleus (CN) using glass multibarreled micropipettes. Barrels were filled with morphine (0.1M, pH 6.5), naloxone (0.1M, pH 6.5) and two with 4M NaCl for recording and current neutralization. Only those units which showed spontaneous activity and which remained stable over a period of at least 5 min were selected to be recorded and tested. Each unit was given 5 different doses of morphine (2,5,10,20,50 nA). Each injection lasted for 60 sec and was given simultaneously with the recording which continued for an additional 200 sec post-drug injection. A recovery period of 3 min was allowed between each morphine treatment. Naloxone was tested for its ability to reverse the effect induced by morphine, always after and at the same dose as the last morphine injection. Fifty units were recorded from naive animals in which 95% of these units changed their firing rate upon application of at least one of the morphine doses. The response of the CN units to morphine was found to be dose-dependent and could be divided into two classes: units which responded in a monophasic fashion (30%) either by increase or decrease in their discharge rate, and units whose response can be described as biphasic (70%) showing an increase in activity when low concentrations of morphine were injected and decreases in activity when high doses of the drug were applied, or vice versa. In morphine-dependent animals (n=20), two patterns of response were also observed, i.e. monophasic and biphasic. However, the percentage which responded to morphine in a monophasic manner increased from 30% to about 70%, while 30% of the units responded in a biphasic fashion. The neuronal population of the CN in morphine-dependent animals exhibited lower sensitivity to morphine (did not respond to the first two doses of the drug). In conclusion, the chronic morphine treatment changed the dose-response pattern in CN units, as compared to naive animals.

Supported in part by U.P.S.H.S. Grant NIDA00803.

210.5 ENKEPHALIN'S EXCITATORY ACTION ON HIPPOCAMPAL NEURONS CANNOT BE EXPLAINED BY ATTENUATION OF RECURRENT INHIBITION. R. Dingledine
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When iontophoretically applied into the hippocampus, opiates increase the firing activity of pyramidal neurons. It has been proposed that this response is a disinhibitory phenomenon, being a consequence of inhibition of the tonic activity of inhibitory interneurons (Zieglgänsberger et al., Science, 205:415, 1979). In the present study this hypothesis was directly tested by examining the effect of D-ala^2,D-leu^5-enkephalin (ENK) on inhibitory and excitatory synaptic potentials in an in vitro rat hippocampal slice preparation. Intracellular recordings were made from CA1 pyramidal neurons identified by antidromic activation from the alveus. Stimulation through a microcathode inserted into the stratum radiatum activated the pyramidal cell population orthodromically via synapses made on their apical dendrites.

Bath application of ENK (500nM) enhanced the response to orthodromic stimulation, as manifested by an increase in the size and more prominently the duration of EPSPs. Extracellular field potential recordings showed a larger population spike for a given size field EPSP. These effects of ENK could be entirely reversed by naloxone (500nM). By focal iontophoresis it was shown that the soma and basal dendrite layers were the most sensitive to ENK, even though the test stimulus activated synapses on the apical dendrites. The enhanced orthodromic response was not due to an increased excitability of the pyramidal soma membrane, since membrane potential, input resistance, spike threshold and antidromic field potentials were all unchanged. The enkephalin effect did not appear to be caused by increased excitatory transmitter release or increased excitatory postsynaptic current, because in field potential studies the input-output relationship between the presynaptic fiber volley and the field EPSP remained unchanged. Finally, recurrent IPSP's, evoked by subthreshold electrical stimulation of the alveus, were not detectably reduced in size by ENK when in the same cell the orthodromic EPSP was enhanced.

On several occasions iontophoretic ejection of ENK into the st. pyramidale or st. oriens did affect the intensity of recurrent inhibition, which was monitored in these experiments by the reduction of an orthodromic population spike by a prior antidromic volley. Both potentiation and attenuation of recurrent inhibition were observed in different slices. Thus, although in certain circumstances a reduction of recurrent inhibition by ENK could be demonstrated, it is clear that this effect is not sufficient to explain the enhanced orthodromic responses produced by ENK. Supported by DA02360 and a Sloan Research Fellowship.

210.6 MORPHINE NON-SELECTIVELY DEPRESSES NOXIOUS AND NON-NOXIOUS INPUTS ONTO WIDE DYNAMIC RANGE NOCICEPTIVE NEURONS OF THE SPINAL CORD DORSAL HORN. F.J.Einspahr* and M.F.Piercey. (SPON: R.A. Lahti)
The Upjohn Company, CNS Research, Kalamazoo, MI 49001 .

Microelectrodes were used to record nerve impulses extracellularly from single dorsal horn neurons of unanesthetized, decerebrated, low spinal (L_1) cats. These cells were tested for their responses to noxious cutaneous heat (>45°C), intense cutaneous electrical stimulation, and non-noxious cutaneous air puff stimulation. Intravenous morphine (1-3 mg/kg) depressed the spontaneous activity of purely nociceptive dorsal horn neurons, as well as their evoked responses to cutaneous electrical and noxious heat stimulation. In wide dynamic range nociceptive neurons, morphine also depressed evoked responses to non-noxious air puff stimulation. Morphine was equally efficacious in depressing responses to the non-noxious, as it was to the noxious, stimulation and lowering the initial dose of morphine (0.3 mg/kg i.v.) failed to indicate a difference in threshold for either depression. All of the above effects of morphine were reversed by naloxone (0.3 mg/kg i.v.). Dorsal horn neurons which were purely non-nociceptive were not depressed by morphine in either spontaneous or non-noxious evoked activity. It is concluded that: (1) morphine has a spinal site of action in which both noxious and non-noxious sensory inputs to dorsal horn neurons are depressed, and (2) neurons receiving purely non-noxious sensory input are not depressed by morphine and may represent the only dorsal horn pathway transmitting innocuous sensations during morphine treatment.

210.7 EXCITATORY OR OPIATE-ANTAGONIST ACTION OF NALOXONE DEPENDING ON DOSE AND ROUTE OF ADMINISTRATION IN THE RAT. Y. F. Jacquet, Center for Neurochemistry, Rockland Research Institute, New York, NY 10035.

High or low naloxone doses were injected in rats either intraperitoneally (i.p.), intracerebroventricularly (i.c.v.) or into the CNS site, the periaqueductal gray (PAG) -- a site previously shown (Jacquet & Lajtha, Science 185:1055, 1974) to mediate many of morphine's actions. At low i.p. doses, naloxone antagonized morphine's depressant actions, while at high i.p. doses, naloxone resulted in sedation and immobility. A low i.c.v. or intra-PAG dose of naloxone antagonized the depressant actions of morphine, while a high i.c.v. or intra-PAG dose of naloxone resulted in a fearful hyper-reactivity, similar to the syndrome seen following a high dose of morphine administered i.c.v. or intra-PAG.

In the in vitro assay, the electrically-stimulated rat vas deferens, naloxone (1×10^{-4}M) added to the Krebs-Ringer bath resulted in excitation, and at the same time, blocked the inhibitory action of B-endorphin (1.4×10^{-7}M) added to the bath in its presence. The former excitatory action was non-stereospecific, occurring after both (-)- and (+)-naloxone, while the latter antagonist action was stereospecific, occurring only in the presence of (-)- but not (+)-naloxone.

These results and those of others show that naloxone effects are varied depending on dose. The opiate antagonist action was stereospecific, occurring only after (-)- but not (+)-naloxone (also, cf. Jacquet & Jimenez, Neurosci. Abs., 1979), while the excitatory action was non-stereospecific. These results support our previous proposal (Jacquet, Klee, Rice, Iijima & Minamikawa, Science 198:842, 1977) that opiate effects are mediated by two classes of receptors, one a stereospecific receptor that is antagonized by naloxone and mediates the inhibitory action of opiates, the other a non-stereospecific receptor that is not antagonized by naloxone and mediates the excitatory action. Naloxone at low doses antagonizes the former, while at high doses, exerts an agonist action at the latter. Precipitated abstinence following a high naloxone dose may be due in part to an unmasking of the excitatory action of opiates following selective naloxone blockade of the stereospecific inhibitory receptor, and in part due to an agonist action by naloxone (in addition to the agonist action of opiates) at the non-stereospecific excitatory receptor.

210.8 EFFECT OF MORPHINE ON EVOKED ACTIVITY OF THE HIPPOCAMPUS IN THE FREELY MOVING RAT. Mary Ann Linseman and William A. Corrigall.
Dept. Neurobiology, Addiction Research Foundation, Toronto, Ontario, M5S 2S1.

In previous work (Brain Res., 1980, in press) we have shown that morphine had a consistently stimulant effect on the field potential elicited in the CA_1 cell body layer of the hippocampus by stimulation of the stratum radiatum in the isolated transverse hippocampal slice of the rat. This was manifest as an increase in amplitude of the primary spike including a reduced threshold to stimulation, and a current- and dose-dependent appearance of a second and occasionally additional population spikes. Further analyses showed that both phenomena were probably due to a decrease in GABA-mediated inhibition induced by the morphine.

The present study was undertaken to determine if similar effects could be obtained in the intact, freely-moving animal when the drug was administered by a normal systemic route. Field potentials elicited in the ipsilateral and contralateral CA_1 by stimulation of the stratum radiatum, and in ipsilateral dentate and CA_1 by perforant path stimulation, were recorded prior to and following incremental doses of morphine (2, 6, and 15 mg/kg i.v.) and a subsequent infusion of 2 mg/kg naloxone. Both increases in the amplitude of the primary spike and secondary spikes were observed, frequently following 6 mg/kg, and reliably following 15 mg/kg. All effects were naloxone reversible. These effects were observed in the absence of any convulsive activity, electrophysiological or behavioral, and their occurrence was not consistently related to the presence of high amplitude activity in the simultaneously-recorded frontal cortical EEG.

We conclude that the isolated hippocampal slice is a valid model in this regard for the action of systemically-administered morphine on the hippocampus of the intact freely-moving rat.

210.9 PHYSIOLOGICAL EVIDENCE FOR OPIOID PATHWAYS IN GOLDFISH RETINA. M.B.A. Djamgoz* and W.K. Stell† Jules Stein Eye Inst., UCLA, Los Angeles, CA 90024; *Dept. Zool., Imperial College, London SW7 2BB, England; and †Univ. Calgary Fac. Med., Calgary, Canada T2N 1N4.

Recent studies have shown enkephalin-like immunoreactivity in amacrine cells (ACs), which may use enkephalin as a transmitter in the inner plexiform layer (IPL) (Brecha, N., et al., PNAS, 76: 3010, 1979). We investigated effects of externally applied opioid agonists and antagonists on the activity of ganglion cells (GCs), which are postsynaptic to ACs, and found pathways to all GCs to be specifically responsive to them.

Isolated goldfish retinas were placed receptors up in a chamber under moist 95% O_2 + 5% CO_2 gas at 22°C. GC spikes were recorded with tungsten microelectrodes. Light stimuli, a 1 mm diam. spot and a 1.2 mm i.d., 3.2 mm o.d. annulus, λ=650 or 500 nm, were presented alternately, 1 per 3 s for 0.7 s each. Responses were obtained at several light intensities. Chemicals in physiological saline were sprayed onto the retina through an atomizer system; different solutions could be applied in turn. Opioid agonists at estimated concentrations <1 μM in the IPL induce changes in GC activities.

GC center responses were defined as ON, OFF or ON-OFF to light. A chemical was then applied for a few seconds; after its effect reached a steady state the GC response was tested again. Exogenous D-Ala^2-Met^5-enkephalin (ENK) enhances light-evoked responses and more than doubles spontaneous activity of ON-center cells. In contrast, light-evoked responses and spontaneous activity of OFF-center cells are inhibited, following a transient excitatory period; sometimes their response is changed into ON-center. These effects are reversible; after application of minimum effective ENK concentrations, responses return to their original type in <5 min. In ON-OFF units, ENK either reverses the relative strengths of the two transient light responses or inhibits both of them. The actions of ENK on these 3 types of GCs are consistent with observations that the dendritic arborizations of enkephalin-containing goldfish ACs are multi- or bistratified in the IPL (Brecha, N., and Marshak, D., pers. comm.). Equimolar morphine mimics ENK but normal responses are not recovered in ≥ 20 min. Pre-treatment with a 100-fold molar excess of naloxone blocks the actions of ENK and morphine. After application of Co^{+2}, GCs generally fail to respond to ENK even at excessive concentrations. Physiological saline alone has no effect.

These observations show that opioid receptors are present in goldfish retina but probably not on GC dendrites. They suggest that enkephalinergic ACs synapse upon other ACs or bipolars presynaptic to GCs, rather then GCs directly. (Supported by Research to Prevent Blindness and NIH #EY 01190, EY 00331).

210.10 EFFECT OF ACUTE MORPHINE ADMINISTRATION ON CATECHOLAMINE LEVELS IN THE SUPERIOR CERVICAL GANGLION AND IRIS OF THE RAT PUP. T. G. Mattio* and M. L. Kirby. Dept. of Anatomy, Medical College of Georgia, Augusta, GA 30912.

The effect of morphine administration on catecholamines has been previously studied in monoaminergic brain nuclei. However, this approach has inherent problems in that these nuclei are heterogeneous, have complicated afferent and efferent connections, and are relatively inaccessible for experimental manipulation. In the present study we used the superior cervical ganglion (SCG) and iris of the rat pup to study the effects of acute morphine administration on tissue catecholamine stores. This system has many advantages over brain nuclei due to its relatively homogeneous cell population, well-defined afferent and efferent connections, and easy accessibility for experimental manipulation. Also the morphologic and biochemical development of SCG and iris has been well studied (Coughlin et al., Develop. Biol., 1978; Mytilineou and Black, Br. Res., 1978).

Sixteen day old rat pups were injected subcutaneously (s.c.) in the nuchal region with 10 mg/kg. of morphine in .2cc saline, a dose found to be analgetic by the formalin test (Dubuisson and Dennis, Pain, 1977). Control animals were injected with .2cc of saline. After 1 hour animals were anesthetized with nembutal intraperitoneally and the SCG and iridies were dissected, frozen, and stored in liquid nitrogen until assayed. Two ganglia and two iridies from the same animal were pooled for analysis. Tissue was assayed by using a radiometric assay that utilizes catechol-o-methyltransferase and S-adenosyl-L-(methyl-^{3}H) methionine (Kirby, Br. Res., 1978). Each sample was analyzed in duplicate and the catecholamine values were averaged. Appropriate internal standards and blanks were run with every assay. The sensitivity of the assay was 50pg.

Preliminary results show an increase in norepinephrine (NE) with unchanged dopamine and epinephrine levels in the iris and a slight increase in dopamine, norepinephrine, and epinephrine in the S.C.G. when expressed per mg wet weight. These data are consistent with results found in brain showing a decreased release of NE from neurons of the locus coeruleus after chronic morphine administration (Llorens et al., Nature, 1978). Whether this build up of catecholamines is due to increased synthesis, decreased release, or increased neuronal uptake is not known.

210.11 ALTERED MORPHINE SENSITIVITY FOLLOWING AMYGDALOID KINDLING IN C57BL/6J MICE. A. Mansour, R. Doyle, R. Katz & E.S. Valenstein; Depts. Psych., Neurosci. Lab. & Mental Health Res. Inst., Univ. Michigan, Ann Arbor, Mich. 48109.

Kindling refers to the progressive development of seizures by intermittent, electrical brain stimulation. This phenomenon has been reported to induce a "permanent" change, in that once kindled, a single stimulation will produce a generalized seizure even months after the last stimulation. Pilot experiments indicated that C57BL/6J mice show a marked increase in morphine sensitivity following amygdaloid kindling. The purpose of the present study was to extend these initial results and to examine whether the increase in opiate sensitivity was as "permanent" as the kindling phenomenon itself.

Thirty-five male C57BL/6J mice were implanted with chronic bipolar electrodes in the basolateral portion of the amygdala and divided into four groups. Nineteen animals were kindled to a criterion of 7 consecutive generalized clonic seizures and were subsequently tested with morphine (25mg/kg i.p.) at either 3 (N=9) or 28 days (N=10) following their last stimulation. The other sixteen animals were divided into two equal groups of handled controls.

A high percentage (89%) of kindled animals tested at 3 days exhibited generalized clonic seizures following morphine administration. The testing of kindled animals at 28 days is still in progress, but results indicate that they also exhibit a high percentage of clonic seizures. In contrast none of the control animals responded to morphine with behavioral seizures. Moreover, kindled animals tested at 3 or 28 days showed a greater increase in stereotyped running activity at 20, 40, 60, 80 and 100 minutes post injection compared to respective controls. The kindled mice also showed an exaggerated Straub tail response compared to controls.

These results clearly indicate that kindling produces a long lasting and perhaps permanent change in morphine sensitivity in C57BL/6J mice and suggest that the endogenous opiate system of animals may be altered by the kindling process.

210.12 SYSTEMIC MORPHINE BLOCKS THE EPILEPTIC EFFECTS INDUCED BY CENTRALLY ADMINISTERED MORPHINE. H. Frenk and G. Urca*. Depts of Psychology, and Pharmacology and Physiology, Tel-Aviv Univ., Ramat Aviv, Israel.

Intracerebroventricular (ICV) administration of both morphine and enkephalin produces marked neural and behavioral excitation as manifest by electrographic seizures accompanied by withdrawal-like phenomena such as wet dog shakes (Urca et al. Science, 1977; Frenk et al. Brain Res., 1978). On the other hand, systemic injections of opiates produce a predominantly inhibitory effect. Naloxone blocks both excitatory and inhibitory effects. We therefore sought to examine the question whether these effects are mediated via different, mutually antagonistic, opioid systems.

Male albino rats were prepared for chronic ICV injections and EEG recording. One week later animals were pretreated with 0, 5, 15, and 50 mg/kg of intraperitoneal morphine. Thirty min later morphine (200 μg) was injected ICV and cortical EEG and behavior monitored. Seven of 11 saline pretreated animals showed seizures whereas none were seen in the 7 animals pretreated with 50 mg/kg of morphine. Pretreatment with the lower doses of morphine had no significant effect on the occurrence of seizures. Administration of naloxone (1 mg/kg) 25 min following 50 mg/kg of morphine reversed its anti-epileptic effect such that all animals receiving ICV morphine 5 min after naloxone showed seizures.

An additional group of animals received either saline or 50 mg/kg of morphine intraperitoneally followed at 30 min by ICV leucine-enkephalin (50 μg). Morphine failed to block enkephalin seizures in any of the animals, however, it blocked the occurrence of wet dog shakes.

The ability of systemic morphine to block the epileptic effect of ICV morphine demonstrates the dual antagonistic action of opiates. The fact that both the anti- and pro-epileptic effects of morphine can be blocked by naloxone indicates that two different systems with different opiate receptor populations underly these phenomena. Indeed, the ability of morphine to block morphine- but not enkephalin-induced seizures lends further support to this suggestion.

Supported by a grant from the Israeli Council for Research and Development, Jerusalem.

210.13 ONTOLOGICAL DEVELOPMENT OF OPIATE RECEPTORS IN CHICK EMBRYONIC BRAIN. D. Ann Gibson* and Antonia Vernadakis*. (SPON: Norman Weiner). Departments of Pharmacology and Psychiatry, University of Colorado School of Medicine, Denver, CO 80262.

Infants born to mothers addicted to narcotic drugs exhibit a physiological dependence and develop symptoms of withdrawal during the postpartum period. Stereospecific opiate receptor binding sites have been demonstrated in animal and human brains, and narcotic analgesics are known to cross the placenta. Therefore, the time course for the appearance and binding affinities of the opiate receptors in the developing embryo may be relevant to identifying the periods in development when the nervous system is most sensitive to narcotics. We are studying opiate receptors in the embryonic chick brain at various stages of development (day 10 through hatching) in membrane preparations using the radioligand ^{3}H-etorphine. The competing ligands used to determine specific binding were naloxone, levorphanol and dextrorphan. With naloxone and levorphanol, ^{3}H-etorphine binding was blocked 40-60% and the specific binding was saturable; no reduction in ^{3}H-etorphine binding was observed with dextrorphan, suggesting the binding is stereospecific. Binding sites are present in homogenates of whole brain minus cerebellum as early as 10 days of gestation. Scatchard analysis showed dissociation constants (K_D's) are not significantly different from day 10 through hatching, but the number of binding sites increases during development. At the later stages of development (Days 20, 21 and hatching) several regions of the brain were examined: cerebral hemispheres, midbrain-diencephalon, and optic lobes. The K_D's were similar, although differences in the number of binding sites for the various regions were found. A high affinity site was detected in the cerebral hemispheres in Day 0 hatched chicks. We are presently determining whether a high affinity site is also present in the other regions of the chick brain and when during embryonic development it appears. (Supported by USPHS Research Grant 1 R01 DA 02131-01 from National Institute on Drug Abuse.)

210.14 EFFECTS OF CHRONIC MORPHINE, NALOXONE, AND STRESS ON PREWEANING OPIATE RECEPTOR ONTOGENY. M. T. Bardo, R. K. Bhatnagar, and G. F. Gebhart. Dept. of Pharmacol., Univ. of Iowa, Iowa City, IA 52242.

Evidence indicates that the normal ontogenetic proliferation in number of opiate receptors which occurs from birth to weaning in rats may be altered by various pharmacological and environmental factors. The present investigation assessed the ontogenetic proliferation of opiate receptors in various CNS regions of preweanling rats, and examined the influence of chronic morphine, naloxone, and footshock stress on opiate receptor ontogeny. Starting one day after birth, groups of male and female Sprague-Dawley rat pups were treated with either morphine sulfate (5 mg/kg, s.c.) for 21-22 days, naloxone hydrochloride (1 mg/kg, s.c.) for 23-24 days, or daily 30-min exposures to inescapable footshock (1 mA scrambled, 2 sec duration, presented on a variable interval 30 sec schedule). Shock treatment extended for either 7, 14, or 21 days after birth. Groups of litter-mate control pups were given either saline or exposure to the shock apparatus without shock. The animals were decapitated on the day following the last treatment day and the brains and spinal cords were removed and the brains regionally dissected into pons-medulla, midbrain, hypothalamus, striatum, and cortex. Opiate receptor assays were performed on homogenized tissue incubated with 1 nM ^{3}H-naloxone in the presence or absence of levallorphan (Coyle and Pert, Neuropharmacol., 15:141, 1976).

Morphine treatment for 21-22 consecutive days failed to alter stereospecific binding of 3H-naloxone, although both brain and body weights were significantly decreased by the morphine treatment. Naloxone treatment for 23-24 consective days significantly increased receptor binding in pons-medulla and hypothalamus (12% and 19% increase in fmol/mg wet weight, respectively). Nonsignificant increases in binding following naloxone treatment were obtained in midbrain (8%), striatum (5%), and cortex (11%); no change was observed in spinal cord. Shock treatment also tended to increase binding in all brain areas examined, except these trends were not statistically significant. Analyses of data from 7, 14, and 21 day-old control pups revealed marked regional differences in the ontogeny of binding capacity. While there was no change in binding in spinal cord from 7 to 21 days, age-related increases in binding were in pons-medulla (45%), midbrain (134%), hypothalamus (269%), striatum (698%), and cortex (900%). Supported by USPHS grants NS12121, NS12114 and MH15172.

210.15 SEX AND THE ENDOGENOUS OPIATES: NALOXONE REDUCES POST-COPULATORY BEHAVIOR IN FEMALE CATS. L.M. Johnson* (SPON: J. Roppolo). Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261.

This experiment was designed as a test of the hypothesis (Stein & Belluzi, 1977) that endogenous opiates subserve a function in the regulation of satisfaction and reward behavior. The mating behavior of otherwise drug-naive cats was observed after intravenous administration of naloxone at two doses to determine whether the drug would alter the behavioral "after-reaction" that characterizes a female cat's highly stereotypical mating pattern. During mounting she normally displays lordosis, tail-deflection and treading of the hind paws. At ejaculation she emits a "mating cry", throws off the male and immediately proceeds to roll, pausing occasionally for genital licking. She is unreceptive to the male during most or all of this period.

Eight ovariectomized cats were given a 4 cm subcutaneous Silastic implant of crystalline estradiol-17β to induce behavioral estrus. Six of the animals had a chronically indwelling venous cannula and all were experienced at mating. A maximum of two matings were permitted on a given day, spaced one hour apart. On some days saline or no treatment was given. Naloxone was given either before a single mating or before the second mating, with the first mating serving as a control. After naloxone, no difference was observed in lordotic posture, tail-deflection or treading, nor in the duration and audibility of the mating cry. After control matings, rolling was continuous except for brief pauses to groom. Naloxone-treated cats often did not start rolling immediately after mating. When they did start, the rolling appeared normal for 7-30 seconds, but stopped abruptly or temporarily with the animal moving about or grooming and rolling only occasionally. The amount of time spent rolling was 379 ± 19 (SEM) seconds (n = 19) after non-treated or saline-treated matings; 77 ± 25 seconds (n = 7) after naloxone at 0.1 mg/kg given 10 minutes prior to entry of the male; and 102.5 ± 17 seconds (n = 4) after naloxone at 1 mg/kg given 2 minutes prior to entry of the male. The observations demonstrate a behavioral effect of naloxone in animals not exposed to exogenous opiates and support the hypothesis that affective behavior is mediated by endogenous opiates. (Supported by NIH Grant HD 12240).

211.1 MICROELECTROPHORESIS OF SUBSTITUTED OPIOID TETRAPEPTIDES - COMPARISON OF POTENCY AND DURATION OF ACTION. B. Lamishaw* and R. C. A. Frederickson. The Lilly Research Laboratories, Indianapolis, IN 46285.

This study was undertaken to examine the mechanisms of opioid peptide inactivation at putative enkephalinergic synapses. The natural enkephalins have been shown to be extremely labile to enzymatic degradation. Many modified analogues have been prepared which have protection from such degradation. The enkephalin structure (5AA's) was thought to be the minimal sequence with opioid activity but more recently it has become apparent that the tetrapeptide sequence (tyr-gly-gly-phe) is the minimally active sequence and extremely potent analogues of this structure have been prepared. The action of small opioid peptides applied microelectrophoretically to neurons in brain is terminated very rapidly, presumably due to synaptic inactivation mechanisms. We undertook the present experiments to examine the relative effects of N-terminal versus C-terminal modifications on this synaptic inactivation. Three tetrapeptides with the following structures were microelectrophoresed onto cells in rat frontal cerebral cortex: tyr-gly-gly-phe-NH_2, tyr-D-ala-gly-phe-NH_2, and tyr-D-ala-gly-(N-methyl)phe-NH_2. The peptides were dissolved in 0.05M NaCl at a concentration of 0.05M, pH 4, and were ejected as cations. The effects of tetrapeptides on spontaneous or glutamate-evoked neuronal firing were predominently inhibitory (70% of the cells tested), but excitatory (6%) and biphasic or other complex effects (14%) were observed. Approximately 10% of the cells were unaffected by tetrapeptide electrophoresis. Compared to the unsubstituted peptide, the substituted peptides were somewhat more potent at inhibiting neuronal firing and recovery from their effects was slower. However, there was little difference in the degree of inhibition or rate of recovery between the D-ala^2 analogue and the D-ala^2-4-N-methyl analogue. These results indicate that substitution of gly by D-ala in the second position affords greater protection from synaptic inactivation than substitution at the C-terminal phe residue. This suggests an important role for enzymatic attack at the N-terminus for the termination of synaptic action of the tetrapeptides.

211.2 THE USE OF HPLC COUPLED WITH RADIOIMMUNOASSAYS FOR DETECTING MULTIPLE SPECIES OF β-ENDORPHIN AND α-MSH. Y. Ueda*, H. L. Lin* and H. Akil. Mental Health Research Institute, Univ. of Mich., Ann Arbor, MI 48109.

While a great deal has become known about the structure and localization of the precursor of β-END/ACTH, pro-opiocortin and its products (β-LPH, β-END, ACTH, α-MSH), few studies have addressed the regulation or the normal physiological function of these products in brain. The difficulty lies in achieving highly specific yet sensitive measurements of these substances in various tissues. The problem is exacerbated by the existence of four different species of β-Endorphin (described by Smyth et al.): C, C', N Acetyl C and N Acetyl C', and the presence of at least two species of α-MSH-like substances: N-Acetyl ACTH 1-13 amide, and ACTH 1-13 amide (des-ac., α-MSH) (O'Donohue and Jacobowitz). While HPLC is capable of separating these species, the detection limits are below the amounts normally present in brain, blood or C.S.F. Radioimmunoassays, on the other hand, while highly sensitive, can cross-react with multiple species of peptides. We have therefore established a technique interfacing HPLC with a number of specific RIA's. A single run on a reverse phase column (Ultrasphere ODS, 5μm) separates the four species of β-END and the two forms of α-MSH from other known peptides, such as oxytocin, vasopressin, leucine and methionine enkephalin, or dynorphin 1-13. The peaks are collected and measured by highly sensitive radioimmunoassays. Changes in the concentrations of the peptides and their ratios can then be studied under various physiological states (eg. stress, pain) or after specific pharmacological manipulations.

211.3 REPEATED DOSES OF D-PHE^4-MET-ENKEPHALIN ALTER BLOOD CHEMISTRIES IN SQUIRREL MONKEYS. G. A. Olson, R. D. Olson, A. J. Kastin, R. H. Wolf*, G. M. Brown, and D. H. Coy*. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70122

D-Phe^4-Met-enkephalin, an enkephalin analog with little or no opiate activity, was injected daily over five days in 10 squirrel monkeys (Saimiri sciureus). Half of the animals received a dose of 1.2 mg/kg while the others received 6.15 mg/kg. Behavioral observations and serum specimens were taken the day before the injections began as a baseline measure, the day after the injection series ended, and two weeks after the termination of injections to assess possible residual effects.

Blood samples were analyzed for levels of glucose, BUN, SGOT, SGPT, RBC, Hgb, PCV, WBC, differential, prolactin, and growth hormone. A mixed analysis of variance was performed on each measure and indicated significant changes in several of the tests over time. BUN levels decreased significantly over the injection series but rose again to pretreatment levels by the time of the follow-up measure. This was due primarily to two animals with very high levels at the start of the project who decreased to normal levels near 20 during the study but returned to the pre-treatment levels within two weeks after the termination of the peptide. RBC, Hgb, and PCV increased significantly after receiving the D-Phe^4-Met-enkephalin analog and had not returned to baseline at the time of the follow-up testing, suggesting that these measures might be extremely sensitive indices of the activity of peptides. WBC, prolactin, and growth hormone did not change reliably but the differential was altered as a result of treatment. SGOT also decreased over the injection period and reached a maximum decrease at the two week follow-up examination.

Several behavioral measures were taken to examine the effect of the non-narcotic enkephalin analog on gross motor performance. General activity, gait, posture, dowl grip, dowl bite, poke response (motor), poke response (vocalization), and visual tracking were all tested at each time base. Food intake and gross body weight were also monitored throughout the study on a daily basis. A mixed analysis of variance was performed on each of the behavioral measures but no statistically significant results were obtained for any of the tests, indicating that any effects of this peptide would be relatively subtle.

This study supports the position that opiate peptides have dissociative effects and that the non-narcotic effects may manifest themselves in a wide variety of test situations, both behavioral and biochemical. The data collected in this study indicate that D-Phe^4-Met-enkephalin had no deleterious effects on behavior or blood chemistry in squirrel monkeys, thus suggesting its suitability for potential clinical use.

211.4 EFFECTS OF SEROTONIN ANTAGONISTS ON HYPERTHERMIA INDUCED BY THE INTRACEREBRAL MICROINJECTION OF β-ENDORPHIN. G. E. Martin, C. B. Bacino and N. L. Papp. Merck Institute for Therapeutic Research, West Point, PA 19486.

The intracerebral microinjection of β-endorphin produces a long-lasting increase in the rat's rectal temperature (RT) which can be partially antagonized by the central serotonin (5-HT) antagonist methergoline (Martin, G. E. and Bacino, C. B. Eur. J. Pharmacol., 59: 227, 1979). These data led to the hypothesis that the β-endorphin induced increase in RT might be mediated by the activation of cerebral 5-HT receptors. To test this hypothesis, we examined the actions of methergoline and three additional antagonists of the central 5-HT receptor on the hyperthermia evoked by the intracerebral microinjection of β-endorphin.

In the preoptic/anterior hypothalamus region of each of 54 male rats, a single 24 ga TW injection guide was permanently implanted. β-Endorphin (2.5 μg), dissolved in 0.5 μl of CSF, was microinjected over a 30 sec interval 1 hour after pretreatment. The following drugs and dose levels (mg/kg i.p.) were used: methysergide (7.5,15); cinanserin (4.5,9); cyproheptadine (5); methergoline (1) and haloperidol (0.5). Haloperidol, an antagonist of central dopamine receptors, was used as a control. A counterbalanced design was used in which each rat was given two microinjections of β-endorphin separated by a 1-week interval. Half of the animals were first pretreated with the vehicle solution and half were given the antagonist. As a control for the direct action of the antagonists on RT, each was administered to rats not given a central microinjection. RT was determined by inserting a thermistor probe 6 cm into the unrestrained animal's rectum at selected time intervals. Baseline RT was determined for each rat by averaging the RT read 30 min and just before the pretreatment.

β-Endorphin produced a rise in the rat's RT when injected into the forebrain. However, neither methysergide (7.5,n=5; 15,n=8), nor cinanserin (4.5,n=5; 9.0,n=16), nor cyproheptadine (n=9) produced a marked reduction in this hyperthermia. Cyproheptadine did cause a significant reduction in the magnitude of the rise in RT, but also caused a significant drop in RT in control rats during the same time period. Hence, the diminution of the rise in RT is not due specifically to an attenuation of the endorphin's action. On the other hand, methergoline (n=10) did significantly attenuate the pyrexia induced by the peptide. The reason for this apparent discrepancy in the action of these serotonin antagonists is unclear. The dopamine receptor antagonist, haloperidol (n=11), did not alter β-endorphin hyperthermia. Future research will be required to determine whether there are distinct subpopulations of 5-HT receptors at which these 5-HT antagonists exert their effect.

211.5 ANTICONVULSANT EFFECTS OF OPIATES AND OPIOIDS ON KINDLED SEIZURES IN RATS. S. Caldecott-Hazard*, R.F. Ackermann*, Y. Shavit, J.C. Liebeskind, J. Engel, Jr. Dept. Neurol. & Psychol., UCLA, CA.

EEG afterdischarges (ADs) from intraventricular injections of opioid peptides in rats suggested a role for these substances in certain seizure phenomena (Urca et al.1977). Subsequently, it was found that morphine and naloxone did not affect the ictal phase of seizures in kindled rats, although morphine prolonged and naloxone shortened post-ictal behavioral depression (Frenk et al.1979). It was suggested that opioid peptides are released during ictus and are involved in the post-ictal depressive state. We postulated that if opioids were released by a seizure, they might also affect the elicitation of a subsequent seizure. Therefore, an investigation was made of the effects of morphine, naloxone, an enkephalin analogue, and morphine tolerance and withdrawal on repetitive seizures.

Chronic bipolar stimulating and recording electrodes were implanted in the amygdala and rats were kindled until 3 generalized (stage 5) seizures were produced. Each rat was then injected with either saline, morphine (10 or 50 mg/kg), naloxone (10 mg/kg), or an enkephalin analogue #146104, Eli Lilly (7 mg/kg), and given 7 stimulus trains (60 Hz, 1 sec, 400 μA) spaced at 2 min intervals. The severity of each behavioral seizure was rated according to a modified version of Racine's kindling scale and mean scores were calculated for each rat. Morphine (10 mg/kg) and naloxone did not produce significant changes in seizure severity. Morphine (50 mg/kg)and enkephalin analogue (preliminary results) significantly decreased mean scores of seizure severity. There were fewer behavioral seizures, yet when elicited, they were usually stage 5. A final group of saline-treated kindled rats was tested as before, then made tolerant to morphine (doses increasing from 5-50 mg/kg, given 3 times per day, for 7 days). One group of tolerant rats was tested and compared with another group given naloxone (10 mg/kg to precipitate withdrawal. Whereas tolerant rats showed no significant changes, withdrawal rats had significantly reduced seizure severity scores. Withdrawal rats were retested after 24 hrs of morphine abstinence, and their severity scores were also reduced. These results suggest that opioid peptides are released by the ictal phase of a seizure and that they serve to inhibit the behavioral components of subsequent seizures. Possible mechanisms for these results are discussed.

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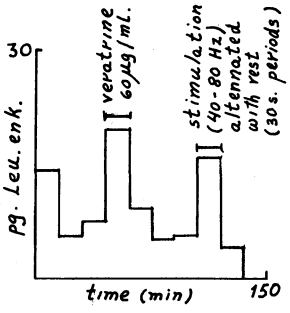
Enkephalin analogue courtesy of R.C.A. Frederickson, Eli Lilly.

211.6 IN VIVO RELEASE OF ENKEPHALIN FROM THE GLOBUS PALLIDUS. EFFECT OF DEPOLARIZING AGENTS AND ELECTRICAL STIMULATION OF THE CAUDATE NUCLEUS. A. Bayon, R. Drucker-Colin, L. Lugo*. Centro de Investigaciones en Fisiologia Celular, U.N.A.M., Apartado Postal 70-600, Mexico 20, D.F. and W.J. Shoemaker, R. Azad* and F.E. Bloom. The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

Guide-cannulae were chronically implanted in adult cats (2-2.5 Kg) and albino rats (150-200 g) in order to allow the stereotaxic placement of a push-pull cannula in the globus pallidus. One week after surgery the push-pull cannulae were inserted into the guides and connected to an infusion-withdrawal syringe pump. The unanaesthetized, freely moving, animals were then perfused with a Krebs-Ringer bicarbonate containing BSA (0.1%) and bacitracin (30 ug/ml). Flow rate was 23 μl/min and 15-min fractions were collected in the withdrawal syringe which contained ice cold 2N acetic acid. The mixed contents were boiled (15 min), lyophilized and redissolved to be assayed in a Leu-enkephalin RIA. (3% cross-reaction with Met-enkephalin). A K+ (50mM)-stimulated, Ca++-dependent release of enkephalin was observed in both cat and rat globus pallidus. Veratrine also enhanced the enkephalin release (see Figure). In rats implanted with a bipolar electrode in the caudate nucleus, electrical stimulation also elicited an increased release of enkephalin in the pallidum (see Figure). These findings support the candidacy of enkephalins as neurotransmitters in the globus pallidus and are consistent with the existence of a striato-pallidal enkephalin pathway, postulated on the basis of lesion experiments (Cuello and Paxinos, Nature 271, 178, 1978). (Supported by NIDA 01785.)



211.7 DISTRIBUTION OF ENKEPHALIN-LIKE IMMUNOREACTIVITY IN THE MOUSE BRAIN: STRAIN COMPARISONS. A.S. Moskowitz, J.C. Liebeskind & L.L. Butcher. Brain Research Institute and Dept. of Psychology, UCLA, Los Angeles, CA 90024.

Several immunohistochemical studies have reported the distribution of enkephalins in rat brain. In the present study, the distribution of methionine enkephalin-like immunoreactivity (met ELI) and leucine enkephalin-like immunoreactivity (leu ELI) was investigated in the mouse brain. Two strains of mice were used: CXBK and C57BL/6BY (Jackson Labs). Baran et.al. (Life Sci., 17: 633, 1975) found fewer central opiate receptors in the CXBK than in the C57BL/6BY strain.

Mice were treated with colchicine (120μg/100g body wt./day, s.c.) on 2 successive days, then perfused with 4% paraformaldehyde. 10μm cryostat sections were cut and stained using the indirect immunofluorescence method. Sections were examined, photographed and counterstained with thionin to verify location of immunofluorescence.

The distribution of ELI in mouse brain correlated well with previous reports on the distribution of enkephalins in rat brain. ELI was observed in cell bodies in anterior olfactory n., frontal cortex, cingulate cortex, n. intersitialis of stria terminalis, n. of diagonal band, caudate-putamen, amygdala, n. reticularis thalami, zona incerta, preoptic area, n. gigantocellularis, lateral n. of medulla, n. centralis caudalis pontis, n. raphe magnus, n. of spinal tract of V, dorsal cochlear n., medial vestibular n., n. of tractus solitarius, medial accessory olivary n., n. commissuralis and ependymal layer of third ventricle. ELI was seen in fibers and terminals in the above areas and was also observed in fibers and terminals in globus pallidus, lateral septal n. and dorsal periaqueductal gray and in fibers in subependymal layer of third ventricle. Met but not leu ELI was observed in fibers, terminals and cell bodies in olfactory tubercle, lateral olfactory area, anterior hypothalamus, dorsal hypothalamus, dorsal tegmental n. and n. raphe pallidus and in fibers and terminals in arcuate n., anterior hippocampus and n. centralis of medulla. Leu but not met ELI was observed in fibers, terminals and cell bodies in anteroventral n. of thalamus, lateral anterior n. of thalamus, ventral and dorsal n. of lateral geniculate, medial and lateral habenular n., areas CA1 and CA2 of hippocampus and granular layer of cerebellum; in cell bodies and fibers in n. reuniens of thalamus and in fibers and terminals in locus coeruleus and main sensory n. of V. No strain differences were observed in the distribution of either met or leu ELI.

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211.8 IMMUNOHISTOCHEMICAL LOCALIZATION OF ENKEPHALINS IN SONG-RELATED BRAIN REGIONS OF A PASSERINE BIRD. Susan M. Ryan, Arthur P. Arnold and Robert P. Elde. Dept. Psychol. and Brain Res. Inst., UCLA, Los Angeles, CA 90024 and Dept. Anat., Univ. Minnesota, Minneapolis, MN 55455

Investigations of several passerine bird species have implicated a number of interconnected brain regions in the control of song. This system includes Area X of the lobus parolfactorius, magnocellular nucleus of the anterior neostriatum (MAN), nucleus interface (NIF), caudal nucleus of the ventral hyperstriatum (HVc), intercollicular nucleus (ICo), robust nucleus of the archistriatum (RA), and tracheosyringeal portion of the hypoglossal nerve nucleus (nXIIts). Most of these areas are sexually dimorphic with respect to size and/or androgen accumulation patterns (Nottebohm & Arnold, 1976; Arnold & Saltiel, 1979). Neurochemical investigations have localized AChE, muscarinic receptors, and catecholamines to various nuclei of the zebra finch song system (Ryan & Arnold, 1979; Lewis et al., 1979).

To investigate further the neurochemistry of the song system, enkephalin distribution was examined by fluorescence immunohistochemistry in adult male and female zebra finches (Poephila guttata). Some animals were pretreated with colchicine 24 to 48 hours prior to perfusion in order to aid perikaryon localization. 12μ cryostat sections were incubated with antisera to methionine or leucine enkephalin. Adjacent sections served as reference sections and absorption controls to insure specificity of staining results.

Both methionine and leucine enkephalin immunoreactivity are present in ICo, MAN, NIF, HVc, and RA of both males and females. Neither is present in Area X or in nXIIts. ICo contains a dense network of enkephalin immunoreactive fibers and perikarya, both dorsomedial to and ventrolateral to MLd. A low density of enkephalin immunoreactive fibers and terminals are distributed throughout MAN, NIF, HVc, and RA. Except the parahippocampal area dorsal to HVc, regions immediately adjacent to MAN, NIF, HVc, and RA do not contain enkephalins. Innervation appears specific to these nuclei-- not to larger regions which include them.

HVc and RA, in particular, are important to song production in the adult song bird (Nottebohm et al., 1976). NIF projects to HVc, and MAN projects to both HVc and RA in the canary (Nottebohm & Kelley, 1978). Thus, localization of terminals in these four nuclei suggests a role for enkephalins in song production and/or song perception in passerine birds.

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211.9 COMBINED MET-ENKEPHALIN-LIKE IMMUNOREACTIVITY AND ACETYLCHOLINESTERASE STAINING IN NEURONS IN THE LATERAL SUPERIOR OLIVE OF THE GUINEA PIG. R. A. Altschuler, J. Fex and M. Parakkal*. Lab. Neuro-otolaryngology, NINCDS, NIH, Bethesda, MD 20205.

Our observations of Met-enkephalin (ME)-like immunoreactive efferent fibers in the guinea pig cochlea led to an investigation to determine the source of the fibers. Warr & Guinan (Brain Res. 173:152 1979) showed that a class of efferents has its origin in acetylcholinesterase (AChE) staining cells in the lateral superior olive (LSO) of the cat. Our studies showed ME-like immunoreactivity in cells in the guinea pig LSO. Coexistence of putative peptide neurotransmitters and traditional transmitters within a single cell has been demonstrated in many combinations. Lundberg et al. (Neuroscience 4:1539 1980) performed immunofluorescence and AChE staining on identical sections to demonstrate vasoactive intestinal polypeptide-like immunoreactivity and AChE staining in the same cells. The same method was used in this study to determine if the ME-like immunoreactivity seen in the guinea pig LSO was in the same cells that show AChE staining.

10 micron cryostat sections were cut through the brainstem of 275-325 gram female NIH stock guinea pigs. Indirect immunofluorescence using antisera against Met-enkephalin was performed on representative sections. The LSO of these sections was then photographed through a Zeiss microscope under epifluorescent illumination. Coverslips were floated off and sections reprocessed to now show AChE activity. The LSO was rephotographed and immunoreactive and AChE positive cells compared.

Strong ME-like immunoreactivity was seen in cells in the LSO without any pre-treatment of animals with colchicine. This population of immunoreactive cells was represented throughout the entire extent of the LSO with greatest concentration rostrally. When AChE staining was examined on identical sections all the cells of the LSO that exhibited ME-like immunoreactivity showed AChE staining. In addition, these were the only cells of the LSO to have AChE activity. There is thus a coexistence of ME-like immunoreactivity and AChE activity in the same cells. These cells have a different size and distribution than those described by Warr and Guinan in the cat LSO. Studies are therefore underway to determine if the cells described in this study project to the cochlea.

211.10 STRUCTURE-ACTIVITY RELATIONSHIPS OF DYNORPHIN PEPTIDES AT THE OPIATE RECEPTOR OF THE GUINEA PIG ILEUM MYENTERIC PLEXUS, C. Chavkin and A. Goldstein, Addiction Research Foundation and Dept. of Pharmacology, Stanford University, Palo Alto, CA 94304.

Dynorphin, an opioid peptide recently purified from porcine pituitary extracts, was found to be 700 times more potent than [Leu^5]-enkephalin in the guinea pig ileum bioassay (Goldstein et al. Proc. Nat. Acad. Sci. 76:6666 (1979)). Since the [Leu^5]-enkephalin pentapeptide sequence corresponds to the first five amino acids in dynorphin (YGGFLRRIRPKLK...), we were interested in determining which amino acids in the carboxy-terminal extension were responsible for the enhanced potency. The interactions of enkephalin analogues with the opiate receptor binding site in guinea pig ileum has been extensively studied (for recent review, see Morley, Ann. Rev. Pharmacol. Toxicol. 20:81 (1980)). The present study explores a hitherto uncharacterized domain of that binding site.

A series of fragments were enzymatically prepared from synthetic dynorphin-(1-13) and dynorphin-(1-9)(Peninsula Labs) by sequentially removing the carboxy-terminal amino acids to obtain dynorphin-(1-12),-(1-11),-(1-10),-(1-8),-(1-7), and -(1-6). These peptides were purified using a μBondapak HPLC column (Waters), and their identity confirmed by amino acid analysis.

A large drop in potency occurs between dynorphin-(1-11) (IC_{50} 0.54nM) and dynorphin-(1-10) (IC_{50} 14 nM) and also between dynorphin-(1-7) (IC50 28 nM) and dynorphin-(1-6) (IC_{50} 780nM), suggesting that Lys^{11} and Arg^7 are critical residues. It would seem that the dynorphin receptor in the myenteric plexus contains two anionic sites able to interact with the basic amino acids in these positions of the dynorphin sequence. Additional dynorphin analogues are being prepared to map the topography of this binding site more completely.

211.11 CHARACTERIZATION OF NEUROTRANSMITTERS MEDIATING INHIBITION OF LOCUS COERULEUS BY HYPOTHALAMIC ARCUATE NUCLEUS STIMULATION: INTERPLAY BETWEEN ADRENERGIC AND ENDORPHIN SYSTEMS. Howard K. Strahlendorf, Jean C. Strahlendorf†, Charles D. Barnes† and James H. Pirch Departments of Pharmacology and Therapeutics and Physiology†, Texas Tech University Health Sciences Center, Lubbock, TX 79430

The locus coeruleus (LC) has been shown to contain a high density of opiate receptors. Systemically administered and iontophoretically applied opiates exert a potent depressant action on coerulear cell firing rate. Immunohistochemical studies have revealed the existence of β-endorphin containing axons emanating from cell bodies localized in or near the hypothalamic arcuate nucleus (AN) which project to various brain sites including LC. Additionally enkephalin containing neurons are present within and surrounding the LC. We have previously reported that electrical stimulation of the AN produced profound inhibition (>1000 msec) of spontaneous coerulear cell discharges in anesthetized cats and rats. In cats, naloxone (NAL) effectively blocked arcuate-induced inhibition when given intravenously (2.5-5.0 mg/kg) or via microiontophoresis; however, preliminary experiments in rats demonstrated NAL to be less effective against suppression of LC unit activity when given by either route.

In order to further investigate this apparent species difference, we have examined the effects of intravenous and iontophoretic NAL and piperoxane (PIP) on arcuate-elicited inhibition of LC cells in chloral hydrate anesthetized rats. In accord with preliminary observations, NAL in doses up to 10 mg/kg frequently evidences only slight (< 20%) blockade of suppression of LC units by arcuate activation. Iontophoretic NAL antagonizes stimulation produced inhibition in some instances and frequently suppresses LC cell discharges similar to opiate agonists. By contrast, PIP given systemically (0.5 mg/kg) or by iontophoresis antagonizes (approx. 50%) arcuate effects in a majority of cells tested. These results suggest that inhibition of LC neurons by arcuate stimulation in rats may result in part from an axon reflex (autoinhibition) and in part via endorphin release on LC-arcuate synapses. The difference in NAL effectiveness between rat and cat may reflect a species difference with regard to opiate receptor types. (Supported by the Tarbox Parkinson's Disease Institute of Texas Tech Univ. Hlth. Sci. Ctr.)

211.12 EFFECTS OF LEUCINE-ENKEPHALIN AND METHIONINE-ENKEPHALIN ON ISOLATED PERFUSED VASCULAR TISSUE. Robert H. Moore, III and Deborah A. Dowling. Dept. of Physiology, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

Preliminary work in our laboratory provided evidence that both leucine-(leu-) enkephalin and methionine-(met-) enkephalin when administered intravenously into pentobarbital-anesthetized cats could alter mean arterial blood pressure and that the responses could be altered by selected pharmacological antagonists. The purpose of this project was to determine the role of vascular tissue in the blood pressure changes seen with the enkephalins and, if the effects can be altered by antagonists. In anesthetized immobilized cats, a femoral artery was exposed and cannulated so that the blood was diverted through a constant flow infusion pump before being reinfused into the artery. Blood flow was maintained at a constant flow for the duration of the experiment; thus, changes in vascular diameter were reflected as changes in pressure. Either leu-enkephalin or met-enkephalin in doses of 0.032, 0.1, 0.32, 1, 3.2, 10, 32, 100 or 320 μg total dose were introduced into the arterial blood as it re-entered the animal distal to the flow pump. Administration of leu-enkephalin produced a dose-dependent drop in the pressure of the perfused limb. A drop of 5% ±1% was seen with a dose of 3.2 μg and extended to a decline of 26% ±4% with a dose of 320 μg. Doses of 1 μg or lower did not induce significant changes. Administration of met-enkephalin also resulted in a dose dependent decline (5±1% with 1 μg; 39±3% with 320 μg). At the three higher doses, met-enkephalin produced a significantly greater decline than did leu-enkephalin. Our results indicate that both enkephalins appear to act on vascular tissue to cause vasodilation. (Supported in part by grants from NIH (#5S08RR091 30-02) and American Heart Association, Missouri Affiliate.)

211.13 ANALGESIA IN MICE AND HUMANS BY D-PHENYLALANINE: RELATION TO BRAIN UPTAKE AND INHIBITION OF ENKEPHALIN DEGRADATION. S. Ehrenpreis, R.C. Balagot*, A.D. Mosanim, K. Kubota*, J. Greenberg*, and C. Okafor*, Chicago Medical School, Chicago, IL 60612.

We have reported that D-phenylalanine (DPA) causes longlasting, naloxone-reversible analgesia in mice (Ehrenpreis et al, in "Endogenous and Exogenous Opiate Agonists and Antagonists", E.L. Way, ed, 1980, p.379). Preliminary studies showed a similar activity in humans. It was postulated that analgesia resulted from inhibition of enkephalin degradation with build-up of the peptides in the CNS. Using a newly developed bioassay procedure for enkephalinases, we have obtained confirmation of this hypothesis by demonstrating that DPA inhibits these enzymes in mouse and guinea pig brain. Met-enkephalin, 3μg/ml, was incubated with suspensions of 20,000xg pellets of brain homogenates. At various times aliquots were boiled to stop the reaction. Residual met-enkephalin was determined from the % inhibition of electrically stimulated guinea pig longitudinal muscle-myenteric plexus preparation. IC_{50} for enkephalinase inhibition by DPA was $3x10^{-2}M$. Other compounds which inhibit enkephalinases (e.g. bacitracin, puromycin) also produce naloxone-reversible analgesia in mice (hot plate method). Order of analgesic potency correlates with potency as enkephalinase inhibitor. Radioactive DPA enters mouse brain slowly, peaking at 30-40 min. and remaining in the brain for very extended periods of time. Uptake into brains of young (3 wk old) mice is much greater than older mice (13 wk). These findings are in accord with the analgesic activity of DPA. DPA provided good to excellent relief in 78% of 43 patients with chronic pain who did not respond to other treatments. Relief was slow in onset and at times of very long duration. The present results provide the pasis for understanding the analgesic properties and mechanism of action of DPA in animals and humans. (Supported in part by a grant from Hoffman-LaRoche and by USPHS Grant 2025).

211.14 DEVELOPMENT OF ENKEPHALIN IN CHICK BRAIN, GUT, ADRENAL AND REMAK'S GANGLION. Miles L. Epstein[o], Iris Lindberg*[+] and June L. Dahl[+]. Departments of Anatomy[o] and Pharmacology[+], University of Wisconsin Medical School, Madison, WI 53706.

We have used sensitive radioimmunoassays to measure the concentrations of met- and leu-enkephalin in the brain, gut, adrenal gland and ganglionated nerve of Remak of the developing chick embryo and the 4-week-old chicken. Both peptides were detectable in brain and gut at 5 days of incubation (d.i.). Enkephalin concentrations, which were greater in brain than gut, increased 10-fold in both tissues by 9 d.i. Concentrations in the medulla-pons and midbrain were twice those in the cerebrum and optic tectum. In all of these brain regions, enkephalin concentrations were highest at 17 d.i. and decreased only slightly thereafter.

A different developmental profile was observed in the gut. There was no significant change in enkephalin concentrations in the midgut and rectum between 13 and 21 d.i. Concentrations in the duodenum were highest at 13 d.i. and decreased gradually to the values observed in the 4-week-old animal. In the adrenal, peak enkephalin levels were observed at 13 d.i., were 50% lower at 21 d.i., and remained constant thereafter. Of all the regions examined, Remak's ganglion contained the highest concentrations of both enkephalins, 10–30 and 2–8 ng per mg protein for met- and leu-enkephalin, respectively. The significance of such high enkephalin levels in this ganglion is presently unclear, but these data suggest a role for met-and leu-enkephalin in the regulation of gut motility.

(This work was supported by the University of Wisconsin Medical School Funds.)

212.1 PROCTOLIN RADIOIMMUNOASSAY ACTIVITY IN ARTHROPOD NERVOUS SYSTEM. T.G. Kingan and M. Titmus*. Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331 and Laboratory of Neurobiology, Boulevard del Valle 201, San Juan, Puerto Rico 00901.

Proctolin is a peptide neurotransmitter candidate in insect visceral muscle (B.E. Brown, Life Sciences 17:1241, 1975) and has been reported to induce rhythmic contractions in locust extensor tibiae (Piek, et al., Comp. Biochem. Physiol. 62C: 151, 1979). We have prepared and characterized an antiserum against proctolin. The antiserum has been used to quantitate proctolin radioimmunoassay (RIA) activity in the Madeira cockroach (Leucophaea maderae) and in three crustaceans. Proctolin RIA activity was found throughout the central nervous system in Leucophaea, being highest in the 6th abdominal ganglion (.68 ± .02 pmole/ganglion) and lowest in the remaining abdominal ganglia (ave. .11 ± .03 pmole/ganglion). Small amounts of proctolin RIA activity could be detected in skeletal muscle and tracheae; none was detected in fat body or in hemolymph. Brains of all three crustaceans contained measurable proctolin RIA activity: Panulirus interruptus, .73 pmole/ganglion; Cancer magister, .31 pmole/ganglion; Pacifastacus leniusculus, .35 pmole/ganglion.

212.2 SUBSTANCE P RELEASE FROM THE NUCLEUS TRACTUS SOLITARIUS AND TRIGEMINAL SPINAL NUCLEUS IN VITRO. Cinda J. Helke, David M. Jacobowitz and Nguyen B. Thoa*. Lab. of Clin. Sci. NIMH, Bethesda, Md. 20205.

Recent studies have suggested that Substance P (SP) may be a neurotransmitter in baro and/or chemoreceptor afferent nerves projecting to the nucleus tractus solitarius (NTS) (Brain Res. 181:476, 1980; Peptides 1:1, 1980) and in afferent pain fibers projecting to the trigeminal spinal nucleus (nV) (Brain Res. 152: 499, 1978) in addition to the well established system of dorsal root SP fibers which terminate in the dorsal horn of the spinal cord. One of the mechanisms for establishing SP as a transmitter in the latter system was the demonstration that depolarization results in a calcium dependent release of SP. In this study, in vitro SP release from the nV and the NTS region of the rat was investigated utilizing slices (225 µm) of the freshly dissected medullary regions. Slices were suspended in 0.6 ml of a modified Krebs-bicarbonate buffer containing 20 µM bacitracin and maintained at pH 7.4 - 7.6. SP released into the medium was analyzed by radioimmunoassay.

Stimulation with K+ (50 mM) in the presence of calcium (2.5 mM) resulted in a marked increase (250-300%) in SP release from both regions. Omission of calcium from the buffer completely abolished SP release by K+.

Capsaicin has also been reported to stimulate SP release from the spinal cord but not the hypothalamus or substantia nigra (Life Sciences 25:629, 1979) while all of these areas release SP with K+ stimulation. This suggests that capsaicin may stimulate release of SP only in those CNS areas containing the nerve terminals of primary afferent neurons. Thus, SP release by capsaicin stimulation (3.3×10^{-5}M) was also assessed in nV, NTS and hypothalamic slices. In the presence of calcium, capsaicin increased SP release (200%) from the nV and the NTS slices but not from the hypothalamic slices. Incubation with capsaicin in the absence of calcium (1 mM EGTA) produced no increase in SP release.

The ability of K+ and capsaicin to release SP from the nV and NTS supports the hypothesis that SP is a neurotransmitter in afferent nerves projecting to these two medullary regions.

212.3 RELEASE OF BOMBESIN-LIKE PEPTIDES FROM RAT HYPOTHALIMIC AND SPINAL CORD SLICES. Terry W. Moody[1], Nguyen B. Thoa*[2], Thomas L. O'Donohue*[3] and David M. Jacobowitz[3]. [1]Dept. Biochemistry, George Washington Univ. Sch. Med., Washington D.C. 20037, [2]Dept. Anesthiology, Uniformed Services Univ. Sch. Med., Bethesda, MD 20014 and [3]Lab. Clin. Sci., NIMH, Bethesda, MD 20205.

Bombesin, a tetradecapeptide isolated from frog skin, is active in the gastrointestinal tract and brain. Upon central administration bombesin induces hyperglycemia, hypothermia and analgesia with a well defined structure activity relationship, similar to that required for receptor binding.

An extensive network of nerves containing bombesin-like peptides has been identified using an antibody which recognizes the C-terminal of bombesin. High levels of bombesin-like immunoreactivity are present in certain hypothalamic, midbrain and hindbrain nuclei. Because these peptides are localized to synaptosomes they may be important neurotransmitters.

Here the release of bombesin-like peptides from rat hypothalamic and spinal cord slices was investigated. 75 mM KCl and 100 µM veratridine released bombesin-like peptides in a Ca^{++}-dependent manner from both hypothalamic and spinal cord slices. Those peptides released by veratridine in a Ca^{++}-dependent manner were blocked by 1 µM tetrodotoxin. The ability of morphine and capsaicin to stimulate release or inhibit KCl-induced release from hypothalamic and spinal cord slices was determined. Also, the released peptides were characterized using high pressure liquid chromatography techniques. Because various depolarizing stimuli release bombesin-like peptides in a Ca^{++}-dependent manner, these peptides may function as physiologically important neuroregulators.

212.4 ANGIOTENSIN INDUCED PHASIC FIRING OF SPINAL CORD NEURONS IN CULTURE. M. Ian Phillips, Phillip G. Nelson, Elaine Neal and James Quinlan*, Lab. Devel. Neurobiol., NICHD, NIH, Bethesda, MD 20205, and Dept. Physiology, Univ. Iowa, Iowa City, IA 52242.

Angiotensin II (AII) has excitatory effects on neurons in the brain and produces drinking behavior, vasopressin release and blood pressure increase. AII-like immunoreactivity has been found in the brain by immunocytochemistry. AII has also been found in the spinal cord in the substantia gelatinosa by the indirect fluorescence technique. Little is known, however, about the physiological action of AII in the spinal cord. We have investigated the electrophysiological action of AII on spinal cord cells grown in culture. Fetal mouse spinal cord (SC) and dorsal root ganglion (DRG) neurons were grown in dissociated cells cultured for 4-6 weeks (Ransom et al., J. Physiol. 40:1132, 1977).

To show the presence of AII in SC neurons, cultures were fixed in Bouin's fixative and reacted with a primary antiserum against AII. Controls were preabsorbed with antigen. The PAP technique of Sternberger revealed several neurons that stained darkly after appropriate tests with antiserum. The cells were possibly SC neurons and not DRG because of their stellate appearance. Intense staining was seen on the varicosities of neuroprocesses which reached and appeared to contact nonreactive soma. This demonstrated the presence of an AII-like substance in SC cells with possible synaptic contact on other cells in culture.

Intracellular recording was made with 4M K+ acetate (pH 7.6) filled pipettes with resistances of 20-60 MOhms. The cells were maintained in a 6 mM Ca++/6 mM Mg++ medium. AII (10^{-6} M) and saralasin (10^{-5} M) were ejected from separate pressure ejection pipettes. Control ejections were Ca++/Mg++ (6:6) medium. Glutamate (0.5 M) was iontophoresed onto the cell.

Two actions of AII were observed. First, a hyperpolarization of -3 to -18 mV with a duration outlasting the stimulus was seen in 10 out of 16 cells. Second, upon recovery from the hyperpolarization, cells which had only random firing patterns prior to testing became phasic with repeated bursts of spontaneous activity lasting for several seconds after stimulation. Glutamate produced single action potentials or excitation that lasted for the duration of the stimulus. Saralasin and controls tended to produce a hyperpolarization but without inducing phasic firing.

The mechanism of the phasic firing remains to be investigated. The results show a) that SC neurons contain an AII-like peptide and b) the random neuronal firing patterns of neurons can be profoundly altered by a peptide. This points to a different action between neurotransmitters and neuropeptides on neuronal membranes.

212.5 THE REGIONAL ACCUMULATION OF CORTICOTROPIN AND α-MELANOTROPIN IN THE MATURING BRAIN. A. Barnea, G. Cho*, and J.C. Porter. Depts. of Ob-Gyn and Physiology, Univ. of Texas Southwestern Med. Sch., Dallas, TX 75235.

The current view is that in the brain ACTH is the precursor of α-MSH, and that both peptides are synthesized and stored within the same neurons. Moreover, the perikarya of these neurons are localized primarily in the arcuate nuclei, whereas the axons of these perikarya are distributed in many regions of the brain. We have previously demonstrated that the content of immunoreactive-like (IL) α-MSH in the rat hypothalamus increases progessively during the first 5-6 months of postnatal life. This increase may be a consequence of increased biosynthesis of the precursors of α-MSH and/or a consequence of increased conversion of ACTH to α-MSH. To address these issues, α-MSH-IL and ACTH-IL were quantified in extracts of the medial basal hypothalamus (a region known to contain the perikarya of the ACTH/ α-MSH neurons) and the preoptic anterior hypothalamus (a region known to contain axons of these neurons). Acetic acid extracts were prepared from brain tissue from young male rats (10-21 days of age) and from adult male rats (90-100 days of age). In the medial basal hypothalamus, the concentration (mol/mg protein) of α-MSH-IL as well as that of ACTH-IL increased with age, but the increase occurred in a manner that the molar ratio of α-MSH-IL to ACTH-IL remained constant at about 2.5 regardless of the age of the animals. In the preoptic anterior hypothalamus, the concentration of α-MSH-IL and ACTH-IL also increased with age, but this increase occurred disproportionately in favor of α-MSH-IL. The disproportionate accumulation of α-MSH-IL in this region of the brain was reflected in the molar ratio of α-MSH-IL to ACTH-IL: being 10 in the adult animal compared to 4 in the young animal. The increase in the concentration of both α-MSH-IL and ACTH-IL with age, noted in the region of the brain containing the perikarya of the ACTH/α-MSH neurons (the medial basal hypothalamus) and in the region of the brain containing only axons of these neurons (the preoptic anterior hypothalamus), is consistent with the possibility that the biosynthesis of the precursors of α-MSH increases with age. The preferential accumulation of α-MSH-IL relative to ACTH-IL that occurs between 21 and 90 days of age in the axons of the ACTH/α-MSH neurons can be a consequence of an increased rate of cleavage of ACTH to form α-MSH. Alternatively, this preferential accumulation of α-MSH-IL could be a result of the formation of new axons or axonal processes that contain mostly α-MSH.

212.6 ONTOGENETIC STUDIES ON THE CELLULAR LOCALIZATION OF TRH IN THE RAT CEREBELLUM. Mauro F. Pacheco, Elizabeth N. Crom* and W. Sue T. Griffin. Dept. of Cell Biology, Univ. of Texas Health Science Center at Dallas, Dallas, Texas 75235.

We have previously postulated a physiological role for TRH in the cerebellum based on studies showing: 1) heterogeneous distribution of endogenous TRH in this organ; 2) parafloccular and flocculo-nodular TRH content more highly concentrated than in the hypothalamus; 3) TRH-immunoreactive neuronal elements in the cerebellar cortex and deep cerebellar nuclei; and 4) the uptake of labeled-TRH by rat cerebellar slices by a process sharing many of the properties of an active transport system. The current work was done to investigate the localization of TRH-containing elements at early developmental stages of the cerebellum. Immunohistochemical localization of endogenous TRH was performed by the indirect immunofluorescent technique in fresh frozen coronal sections from the cerebella of 14-day old rat puppies. Immunofluorescent micrographs of these sections showed three distinctive populations of TRH-immunoreactive neuronal elements in the erebellar cortex. The first group was one of rounded cell bodies lacking neuronal processes and located in the external granular layer. The second population was composed of cells located mainly at the level of the Purkinje cell layer, this cells projected long processes which extended along the molecular layer up to the external granular layer. The third group consisted of cells forming a network in the region of the internal granular layer adjacent to the Purkinje cell layer. This latter population of TRH-immunoreactive neurons closely resembles that found in the cerebellar cortex of adult rats. No TRH-immunoreactivity was found on Purkinje cells, nor on cells of the deep regions of the internal granular layer. These observations suggest that cerebellar TRH is synthesized by cellular elements located within the cerebellum and, therefore, is not of hypothalamic origin. Further developmental and histological analysis is needed to clarify the origin and identity of these peptidergic neuronal elements.

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212.7 CHOLECYSTOKININ DISTRIBUTION IN THE HIPPOCAMPAL FORMATION AND ITS CONNECTIONS. R.S. Greenwood*, S.E. Godar*, T.A. Reaves, Jr. and J.N. Hayward. Dept. Neurology & Neurobiology Program, University of North Carolina, Chapel Hill, North Carolina 27514.

Cholecystokinin-like (CCK-L) immunoreactivity has been detected in the hippocampal formation in all previous immunocytochemical and radioimmunochemical studies of this structure. This study was undertaken to delineate the distribution of CCK-L immunoreactivity in the hippocampal formation, in afferent or efferent hippocampal pathways and in structures receiving axons from or sending axons to the hippocampus.

A well characterized antibody, specific for the COOH-terminal octapeptide common to CCK and gastrin (AB 4562**, Larsson and Rehfeld, Brain Res. 165:201-218,1979) was used with the indirect immunoperoxidase method to localize CCK-L immunoreactivity in neuronal somata and processes in brain sections from colchicine treated rats.

In Ammon's horn, cell bodies were found in regio inferior and regio superior in all layers at all levels of its septo-temporal axis. Cells in the pyramidal layer were most numerous at or near the junction of regio inferior and regio superior. The fewest cell bodies were found in regio inferior at its dentate end. Cell bodies were also found in the dentate, subiculum, presubiculum, parasubiculum and entorhinal cortex. Processes in the hippocampal formation were most prominent in the subiculum and around pyramidal cells in regio superior and regio inferior.

Fibers were also found in the alveus, ventrolateral fimbria, ventral hippocampal commissure and dorsal hippocampal commissure. CCK-L immunoreactive fibers were numerous in many regions receiving hippocampal efferents, including cingulate and perirhinal cortex, ventrolateral portion of lateral septal nucleus, anterior olfactory nucleus, ventromedial hypothalamic nucleus, nucleus accumbens and the bed nucleus of the stria terminalis. Occasional fibers were also observed in the mammillary complex, septofimbrial nucleus, suprachiasmatic nucleus and ventral premammillary nucleus.

Among regions sending axons to the hippocampal formation, dense populations of CCK-L immunoreactive cell bodies were restricted to the cortical nucleus of the amygdala and the prepyriform, the medial frontal and the cingulate cortex. The supramammillary region, the basolateral nucleus of the amygdala and the frontal and temporal cortex each contain sparse populations of CCK-L immunoreactive cell bodies.

These observations suggest that CCK-L immunoreactivity is present in both intrinsic and extrinsic hippocampal formation pathways. (Supported, in part, by Grant No. NS-13411 from USPHS; ** the CCK-L antiserum a generous gift from Prof. Jens Rehfeld.)

212.8 CHOLECYSTOKININ EXCITES DORSAL HORN INTERNEURONS BOTH IN SITU AND IN VITRO. S. Jeftinija*, V. Miletic and M. Randic (SPON: W. G. VanMeter). Dept. of Vet. Physiology & Pharmacology, Iowa State University, Ames, IA 50011.

Immunohistochemical studies have demonstrated the presence of high densities of cholecystokinin octapeptide (CCK-8)-positive fibers in the superficial layers of the dorsal horn of the guinea pig spinal cord. This area is known to contain neurons involved in mechano-, thermo-, and nociceptive information processing. Since the physiological role of CCK-8 in the spinal cord is at present unknown, it was of interest to study the central effects of synthetic CCK-8 (0.8-4.0 mM, pH 7.2; Boehringer Mannheim) by applying the peptide iontophoretically onto dorsal horn interneurons.

In cat spinal cord in situ and in the rat spinal cord slice preparation, CCK-8 caused a moderate excitation of about half of the tested dorsal horn interneurons in laminae I-VI (27/51). Excitation was observed as initiation of firing in a previously quiescent unit, or as an increase in the rate of spontaneous and/or evoked firing. The excitant response to CCK-8 was relatively slow in onset and recovery. The experiments performed in situ showed CCK-8 to possess an excitatory action in all categories of neurons recognized in spinal preparations of cats in this area on the basis of their excitability to different kinds of afferent input. The excitatory effect of CCK-8 remained if the spinal cord slices were perfused with a low-Ca^{++} Krebs solution. This finding indicates that the peptide is probably acting on postsynaptic sites.

These results indicate that CCK-8 should also be added to the ever growing list of peptides having possible roles as transmitters or modulators at synapses in the dorsal horn of the spinal cord.

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212.9 PHARMACOLOGICAL CHARACTERIZATION OF RECIPROCAL HINDLIMB SCRATCHING INDUCED BY INTRACRANIAL SUBSTANCE P. P.J.K.Dobry, M.F. Piercey and L.A.Schroeder*. The Upjohn Company, CNS Research, Kalamazoo, MI 49001.

Male CF-1 mice were injected intracranially (i.c.) with 2 µl drug in saline, given approximately at the midline with a 3.5-mm needle, just anterior to the level of the external auditory meatus. Intracranial substance P (SP) induced the reciprocal hindlimb scratching (RHS) syndrome of Rackham and Share (1979, Neurosci. Abs. 5:615) with an ED50 of 0.03 µg. The C-terminal decapeptide and hexapeptide fragments also induced RHS, but the C-terminal pentapeptide caused no scratching at all. N-terminal fragments (SP 1-10 amide, SP 1-9 amide, SP 1-8 amide) at doses up to 10 µg i.c. induced only one-sided scratching similar to the effect of very low doses of SP. Thus the SP receptor for scratching is similar to that of spinal cord dorsal horn (Piercey and Einspahr, 1980, Brain Res. 187:481-486). SP did not produce scratching when given intravenously (i.v.) or intraperitoneally (i.p.) at doses up to 50 mg/kg. We conclude that peripherally-administered SP does not reach the receptor for RHS.

We confirmed that i.p. morphine (AD50 = 6 mg/kg) antagonized the RHS induced by 0.3 µg i.c. SP, as did i.c. morphine (0.3 µg). However, RHS was also antagonized by a variety of other compounds which can alter motor behavior, amphetamine (4 mg/kg i.p.), baclofen (3 mg/kg), chlorpromazine (5 mg/kg), and cyproheptadine (10 mg/kg). SP's RHS was not antagonized by i.v. sodium cromolyn, nor mimicked by i.v. or i.c. histamine or i.v. Compound 48/80. We conclude that RHS is not caused by histamine release.

Intracranial somatostatin (ED50 = 0.1 µg) induced a weaker, slower onset, one-sided scratching which was indistinguishable from the effect of very low doses of SP. Intravenous somatostatin caused one-sided scratching and was at least as potent as intracranial somatostatin. Thus its site of action may be distant from the i.c. injection site. Morphine (7 mg/kg i.p.) antagonized scratching behavior induced by 1 µg i.c. somatostatin. Thus, consistent with the report that morphine is not an SP receptor antagonist at the SP receptor (Piercey, Einspahr, Dobry, Schroeder and Hollister, 1980, Brain Res. 186:421-434), morphine's effects on RHS seem due to nonspecific disruption of scratching rather than action at the SP receptor.

Intracranial or i.p. kainic acid also causes some weak, one-sided scratching. Intracranial acetylcholine, atropine, bradykinin, DOPA, glutamic acid, histamine, morphine, neurotensin, norepinephrine, and serotonin did not produce scratching.

212.10 CO-EXISTENCE OF DOPAMINE AND CHOLECYSTOKININ (CCK) IN A POPULATION OF MESOLIMBIC NEURONS: IMMUNOHISTOCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES. B.S. Bunney, L.R. Skirboll+, A.A. Grace, D.W. Hommer+, J. Rehfeld+, M. Bodansky+, M. Goldstein and T. Hökfelt+. Depts. of Pharmacology and Psychiatry, Yale Univ. Schl. Med., New Haven, CT 06510; Dept. of Histology, Karolinska Institute, Stockholm 60 Sweden.

In the past several years, extensive gastrin/CCK immunoreactive systems have been identified in mammalian brain. This immunoreactivity has been shown to correspond mainly with the COOH-terminal octapeptide of CCK. In a more detailed examination of CCK immunoreactivity in rat brain, we found a subpopulation of neurons confined to the ventral tegmental area (A10) of rat brain (extending into the zona compacta and pars lateralis of substantia nigra A9)which show immunoreactivity to both CCK and tyrosine hydroxylase (a marker for dopamine neurons.) These A10 neurons appear to project to limbic areas such as nucleus accumbens, tuberculum olfactorium and the bed nucleus of the stria terminalis where overlapping networks are found. This CCK/dopamine projection was confirmed further using retrograde tracing techniques. Following "True blue" injection into the nucleus accumbens, cells were visualized in A10 which contained retrograde dye as well as showed immunoreacitivity to both CCK and tyrosine hydroxylase antibodies. In an effort to explore the functional significance of this peptide/monoamine co-existence, the effects of CCK-7 on single unit activity in area A9 and A10 of rat brain were examined. Recordings were made from cells whose characteristics in terms of waveform, firing pattern and rate matched those identified as dopaminergic. In the A9 area, CCK-7 (4-100 ug/kg iv) increased firing rate and induced bursting patterns of activity in the majority of cells tested; these same effects were observed in most of the cells to which CCK-7 was applied iontophoretically. In the A10 area, ionotphoretic application of CCK-7 produced an increase in rate and bursting similar to that seen in A9. Histological confirmation of A9 and A10 recording sites revealed that the cells which failed to respond to i.v. or iontophoretic CCK-7 were located in areas which show no immunoreactivity to CCK. In control experiments, equivalent currents ejected from the balance channel containing 4M NaCl or Substance P were ineffective in altering firing rates or patterns in either area. The possible functional role of CCK in the modulation of dopaminergic neuron activity will be discussed.

212.11 EFFECT OF NEUROPEPTIDES ON CALMODULIN-INDUCED ACTIVATION OF PHOSPHODIESTERASE. Mary Sellinger Barnette* and Benjamin Weiss, Dept. of Pharmacol., Med. Col. of Pa., Phila., Pa. 19129.

The activity of a calcium-dependent form of phosphodiesterase (Peak II), which is present in high concentrations in the brain, can be increased 10-fold by the calcium binding protein, calmodulin. This activation is selectively inhibited by several different chemical classes of antipsychotic drugs. The mechanism by which antipsychotics block calmodulin's activity is through the direct calcium-dependent binding of the drug to calmodulin. The recent finding that there are endogenous proteins that can bind to, and thereby modify the actions of, calmodulin, and the evidence that certain neuropeptides produce behavioral effects in animals similar to those of antipsychotics suggested that there may be neuropeptides in brain that can interact with calmodulin. Accordingly, we have examined the effects of various neuropeptides on the calmodulin-induced increase in phosphodiesterase activity. An activatable Peak II phosphodiesterase was prepared from rat caudate nucleus by gel electrophoresis and was assayed by the luciferin-luciferase technique. Calmodulin was purified to homogeneity from beef brain. At concentrations up to 25 µM, the peptides ACTH (4-10), bombesin, des tyr γ-endorphin, δ-sleep inducing factor, leu-enkephalin, met-enkephalin, α-MSH, β-MSH, neurotensin, somatostatin, and substance P failed to inhibit either the basal phosphodiesterase activity or the calmodulin-induced activation of phosphodiesterase. By contrast, ACTH (beef brain), β-endorphin and lysine enriched histone blocked completely the calmodulin-induced activation of phosphodiesterase at concentrations that had virtually no effect on the basal activity; the approximate I_{50} values were 2 I.U/ml, 3 µM and 2 µM, respectively. The potency of histone and β-endorphin in blocking the calmodulin-induced activation of phosphodiesterase is similar to or greater than that of currently available phenothiazine antipsychotics. Although we have not as yet examined a sufficient number of peptides to make any definitive statement regarding the biological significance of the binding of peptides to calmodulin, these results suggest that neuropeptides such as β-endorphin may act as endogenous modulators of calmodulin and raise the possibility that they may function as endogenous antipsychotic materials. Supported by USPHS Grant MH30096.

212.12 NEUROTENSIN (NT) IN THE RAT CENTRAL NERVOUS SYSTEM. J. Koenig*, W.K. Samson* and L. Krulich* (SPON: A. Rupert). Dept. of Physiol., Univ. of Tx. Hlth. Sci. Ctr., Dallas, Texas 75235.

The tridecapeptide NT isolated by Carraway and Leeman (J.B.C. 248: 6854, 1973) from hypothalamic extracts has profound effects on thermoregulation and hormone release from the anterior pituitary. This work describes a new NT RIA and examines the identity of the NT found in the rat hypothalamus. Male New Zealand white rabbits were immunized with synthetic NT conjugated to bovine thyroglobulin by glutaraldehyde. Each animal received the equivalent of one mg of NT initially followed by decreasing monthly amounts. After 4 immunizations, antiserum with a titer of 1:50000 bound 60% of the ^{125}I-NT which was added. This antiserum has a sensitivity of 2.5 pg/tube with 50% binding occurring at 38 pg/tube. This antiserum shows no cross-reaction to LRH, TRH, somatostatin, substance P, met-enkephalin, gastrin, CCK, secretin and bombesin in concentrations of 10 µg/ml. Brain areas were removed by gross dissection and homogenized in 1.0 ml of 0.2 N acetic acid. Extracted protein (P) was determined by the Folin method. For comparison, LRH content was measured by specific RIA. Within the CNS, highest levels of NT (2.31±0.48 pg/µg P, n=5) and LRH (2.89±0.7 pg/µg P, n=5) were detected in the hypothalamus. The midbrain and thalamus contained high levels of NT (1.03±0.15 pg/µg P, n=6 and 0.89±0.08 pg/µg P, n=5) and lower, but readily detectable, levels of LRH. Levels of immunoreactive NT in brain stem, cerebral cortex, cerebellum, anterior and posterior pituitary ranged from 0.24±0.03 pg/µg P in the brain stem to 0.03±0.008 pg/µg P in the cerebellum. NT was not detected in the pineal. LRH in these areas was also below the limit of sensitivity (<2 pg/area). The nature of the material within the preoptic area (POA) and median eminence (ME) that reacts with NT antiserum was examined chromatographically. ME and POA areas were removed from the brains of 18 adult male rats and homogenized in 0.2 N acetic acid. Supernatants were applied to a 0.9x96 cm Sephadex G-25 column equilibrated with 0.2 N acetic acid. Under these conditions, NT from the ME and POA coeluted with synthetic NT, implying that endogenous NT in the POA and ME is chromatographically and immunologically similar to synthetic NT. These findings indicate that: 1) the antibody directed toward synthetic NT detects endogenous levels of NT within the CNS, 2) this NT distribution is heterogeneous within the rat CNS, 3) the endogenous immunoreactive NT in the POA and ME is chromatographically similar to the tridecapeptide. Supported by NIH HD-09988 and AM-10073.

212.13 ANALGESIC EFFECTS OF NON-OPIATE PROOPIOCORTIN FRAGMENTS. J. M. Walker*, H. Akil and S. J. Watson. Mental Health Research Institute, Univ. of Mich., Ann Arbor, MI 48109.

β-endorphin apparently arises from a 31K dalton glycoprotein termed proopiocortin, present both in brain and pituitary. This remarkable precursor also contains the sequences of ACTH 1-39, β-lipotropin, and an unstudied 16K dalton polypeptide which itself may contain other biologically active fragments. Since several putative products of this hormone are transported and packaged in a manner consistent with release from terminals, it seems likely that this set of neurons is releasing more than one neuromodulator. Virtually nothing is known about the physiology of neurons with multiple transmitters. Here we address the core issue of whether the effects of two presumed release products, α-MSH and β-endorphin, produce similar effects. The analgesic effects of β-endorphin have been well characterized. Consequently, several substances containing the core sequence of ACTH 4-10 were injected into the periaqueductal grey and the presence or absence of analgesia was observed.

A total of 34 rats were implanted with chronic indwelling cannulae in the periaqueductal grey, under deep anesthesia, using standard stereotaxic methods. Following recovery, these rats were injected with one or more doses of one of the following: ORG 2766 (an analogue of ACTH 4-9), ACTH 1-24, α-MSH and Des(acetyl)-α-MSH, or the vehicle. The tail flick test was used to measure analgesia.

Results indicated that all substances produced a statistically significant degree of analgesia. In all cases, analysis of variance was used to analyze the data. The enzymatically stable analog, ORG 2766, was considerably more potent than the other substances, though all produced analgesia at concentrations in the range needed for morphine analgesia (for ORG 2766 $P < .0001$, ACTH 1-24 $P < .05$, α-MSH $P < .001$, Des(ac)-α-MSH $P < .001$).

Thus, it was concluded that the same neurons may be producing at least two substances with analgesic potency. Moreover, it might be that these compounds are acting synergistically at the postsynaptic sites. Previous data suggest a role for β-endorphin in the modulation of pain sensitivity. α-MSH may have a similar role.

212.14 PRESENCE OF VASOACTIVE INTESTINAL PEPTIDE (VIP) SENSITIVE ADENYLATE CYCLASE IN RETINA AND COMPARISON WITH ACTIVITY IN BRAIN REGIONS Margaret A. Longshore* and Maynard H. Makman* (SPON: R. Katzman). Departments of Biochemistry and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Vasoactive intestinal peptide (VIP) is an octacosapeptide originally isolated from small intestine, and it has been shown to be selectively distributed in nerve terminals in the gastrointestinal tract and central nervous system (CNS). VIP action appears to involve stimulation of adenylate cyclase (AC) activity. The current study was designed to examine the distribution of VIP sensitive AC within the CNS. We report here the presence of VIP stimulated AC in mammalian retina, a stimulation comparable in magnitude to that found in several brain regions. Also this stimulation in retina is comparable to that of dopamine, the only other transmitter, previously known to activate retinal AC.

Fresh bovine and rabbit retinas and rat brain regions were homogenized and incubated 30 min on ice in the presence of VIP, followed by AC assay as previously described (Brain Res., 153:636, 1978). Conditions for incubation of retinal homogenates were also as described (J. Neurochem., 21:477, 1973). The table below indicates that VIP (10 μM) stimulates AC in bovine and rabbit retina and in selected rat brain regions.

Area	-Gpp(NH)p	% increase	1 μM +Gpp(NH)p	% increase
retina, bovine	62.7 ± 12.1	146	-	-
retina, rabbit	329.1 ± 88.6	466	134.1 ± 44.0	91
frontal cortex, rat	29.6 ± 3.5	111	42.2 ± 2.6	136
striatum, rat	14.6 ± 28.6	8	17.8 ± 4.6	50
hippocampus, rat	5.2 ± 17.9	120	29.2 ± 3.9	142
dentate, rat	113.0 ± 4.2	172	96.4 ± 7.2	772
cerebellum, rat	25.8 ± 16.3	76	19.9 ± 17.8	51

Values represent the amount of cAMP formed in pmoles/mg protein/2.5 min ± SE minus the basal cAMP level/(N=2-7).

There is a large amount of VIP-sensitive AC activity in the rabbit retina which does not appear to be enhanced by guanine nucleotides. Preliminary experiments indicate that glucagon and secretin also cause a small stimulation of AC activity in rabbit retina. In rat brain the largest amount of VIP-sensitive AC activity appears to be in the dentate gyrus and frontal cortex with smaller amounts in the striatum, hippocampus and cerebellum. A significant AC stimulation also occurs with 1 μM VIP in retina and brain.

The present study suggests the presence of a VIP-sensitive AC in mammalian retina and in discrete brain areas. While little is known of the specific function of VIP in the CNS, it seems likely that VIP may play a neuromodulatory role in retina as well as in certain other CNS areas.

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212.15 SPECIFIC AMINOTRIPEPTIDASE OF RAT BRAIN. L. Sachs* and Neville Marks. Center for Neurochemistry, Rockland Research Institute, Wards Island, N.Y., N.Y. 10035.

Previously it was shown that rat or pig brain contains a puromycin insensitive aminopeptidase cleaving tripeptides more readily than di-, or oligopeptides but it was never established if a specific aminotripeptidase existed per se. Importance is attached to this question since brain or neurosecretory regions contains number of bioactive tripeptides, or ones that can be derived from putative tripeptide precursors (MIF, TRH). For this study, enzyme was purified from rat cytosol fractions by the procedure of Marks et al (1968) (J.Biol.Chem.243, 2882) with modifications using Leu-Gly-Gly as substrate and detecting cleavage by an automated ninhydrin procedure. Preparations were enriched several hundred fold and were utilised for studies on specificity only when it could be demonstrated that they were devoid of activity towards a range of dipeptides, or a variety of oligopeptides (enkephalin, kinins, angiotensins etc). The specificity was examined using over 30 tripeptides including examples with N- and C-terminal substitutions such as D-amino acids or C-terminal amides or esters. Results showed that aminoacylated analogs of glycylglycyl were among the best substrates; when Leu or Ala were N-terminal, rates were highest (100) followed in descending order by Phe (80), Gly (40) and Pro (35). Presence of N-terminal basic residues, or D-amino acids, or α-Glu, or γ-Glu, or presence of Pro in the second position greatly reduced or blocked hydroiysis by brain enzyme. Enzyme failed to hydroiyse a number of tripeptidyl amides including melanostatin (MIF, Pro-Leu-Gly.NH_2).

Purified enzyme was inhibited by paramericuribenzoate, Zn^{2+}, bestatin, antipain, SQ 14,225 (captopril) and benzothonium chloride. It exhibited a pH optima of 7.6 and had an approx. mol. wt of 78 kilodaltons. The role of a specific aminotripeptidase in the CNS is unclear but may be related to the metabolism of tripeptides generated as a result of protein or polypeptide breakdown, or released by specific aminopeptidase present in pituitary known to release N-terminal tripeptides from growth hormone (Doebber et al., Endocrinology, 103, 1794, 1978). Tripeptides have a number of biological roles that include ones with chemotactic actions, some that have inhibitory properties on spinal motoneurons, in addition to the well known tripeptides with endocrinological actions.

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212.16 EFFECTS OF STRESS ON α-MSH CONCENTRATIONS IN DISCRETE REGIONS OF THE RAT BRAIN. Tichomir Torda*, Thomas L. O'Donohue*, Juan M. Saavedra and David M. Jacobowitz. (SPON: V. John Massari) Laboratory of Clinical Science, NIMH, Bethesda, Md. 20205

An α-MSH (α-melanocyte stimulating hormone) neuronal system has been identified in the rat, cat and human brain. Neurons containing α-MSH appear to emanate primarily from perikarya in the arcuate nucleus and project extensively throughout the forebrain, midbrain and hindbrain. At present, very little is known of the physiological role of this neuropeptidergic system. The only extraneuronal site known to synthesize α-MSH is the pituitary gland where the intermediate lobe synthesizes most of the α-MSH. The fact that the pituitary α-MSH system is influenced by stress (Kastin et al., 1967; Usategui et al., 1976) suggested that the brain α-MSH system might also be involved in the stress reaction and that this involvement may result in changes in α-MSH concentrations in discrete brain regions in stressed rats.

Forced immobilization, a major stress in rats, was accomplished by placing rats in a prone position with heads inserted through steel wire restraining loops fixed on a stainless steel plate and fastening their limbs to four raised metal strips with adhesive tape. The stress procedure was performed acutely, for 2 1/2 hours per day for one or seven consecutive days. Rats were sacrificed immediately after the final stress.

A single immobilization stress resulted in a 40-60% decrease in α-MSH concentrations in the brain regions studied: the medial preoptic nucleus, anterior hypothalamic nucleus, paraventricular nucleus, periventricular nucleus of the thalamus, median eminence, arcuate nucleus and dorsomedial nucleus. This data suggests that stress increased α-MSH release. In contrast, repeatedly immobilized rats had no significant changes in brain α-MSH concentrations suggesting a time or experience related form of neural adaptation. Pituitary α-MSH decreased only slightly after a single but not repeated immobilizations. Previous behavioral studies indicate that α-MSH administration facilitates performance of a number of adaptive behaviors. The increased α-MSH release in stress may reflect a role of that α-MSH neural system in such processes.

212.17 RESPONSE OF IDENTIFIED MOLLUSCAN NEURONS TO α-MELANOTROPIN AND LYSINE VASOPRESSIN. W. Lichtensteiger, D. Felix* and M. Celio*, Insts. of Pharmacol., Brain Research and Anat., Univ. Zürich, Zürich, Switzerland.

Interactions between small molecular weight neurotransmitters and peptides have been studied at the level of individual identified neurons in an in vitro preparation of the c.n.s. of the snail Planorbis corneus. The studies focussed on the circuitry of the giant dopamine neuron (GDN) of the left pedal ganglion.

Results of immunohistochemical studies suggest that the snail c.n.s. contains peptides related to those of vertebrates. α-Melanotropin (α-MSH)-like immunoreactivity was observed in a number of nerve cells and in nerve fibers in several ganglia. Although the connections of this supposedly peptidergic system have not yet been elucidated, we have checked whether α-MSH might interfere with transmission to the GDN. Several neurons afferent to GDN have been identified by electrophysiology. One excitatory input from a cell in the same ganglion is blocked by dihydro-β-erythroidine administered to the bath and hence, probably mediated through a nicotinic mechanism. The existence of a nicotinic excitatory input to GDN (indicated by our earlier data) has further been confirmed by iontophoresis. α-MSH applied to the GDN by iontophoresis prevented the increase in firing and reduction of membrane resistance induced by iontophoretic acetylcholine (Ach); this effect was reversible. A similar interaction was also seen in other neurons. Resting membrane resistance (checked by injection of hyperpolarizing current) appeared to remain unchanged.

Lysine vasopressin (LVP) which was previously found to affect membrane resistance of GDN (Lichtensteiger and Felix, Neurosci. Abst. Vol. 5, 532, 1979), was also capable of modulating the effect of Ach on GDN. When both substances were given by iontophoresis, the activation of GDN by Ach was enhanced by LVP. The possible occurrence of a related peptide in the snail is presently being investigated by immunohistochemistry.

In conclusion, our data suggest that peptides with structural similarities to the pro-opiocortin family and possibly also to vasopressin may be active in the snail c.n.s..

212.18 ACTIVATION OF PEPTIDERGIC NEURONS ENHANCES THEIR SYNTHESIS OF SECRETORY PROTEINS. R.W.Berry and S.Arch. Dept. Anat. Northwestern Med.Sch., Chicago, IL 60611, and Biol.Dept., Reed College, Portland, OR 97202.

Faced with the necessity of maintaining an adequate supply of their secretory products despite fluctuations in secretory rate, peptidergic neurons may have developed a mechanism for regulating synthesis in accord with demand. We have investigated this possibility using the neurosecretory bag cells of Aplysia, which produce and secrete a peptide egg-laying hormone, ELH. ELH and the larger precursors from which it is derived are readily separated from other bag cell proteins by SDS-PAGE, and bag cell activation with consequent ELH secretion is known to follow exposure to elevated external K^+ solutions. Therefore, to determine whether an augmentation of secretory protein synthesis follows massive activation of these cells, we exposed bag cell organs to elevated external K^+ (100mM) for 4 hr, then measured the incorporation of tritiated leucine into ELH precursors over a 2 hr period. The experimental treatment resulted in a 28% increase in ELH precursor labeling, and this persisted for at least 8 hr following the stimulus, resulting in a total increase of 150% over this period. The experimental treatment had no effect on the rate of loss of label from previously-synthesized ELH precursors, indicating that the enhanced labeling reflects an enhancement of synthesis of these peptides, rather than a decrease in their turnover rate. Elevated external K^+ does not enhance ELH synthesis in clusters of bag cell somata which have been surgically isolated from their neurites, indicating that the augmentation of synthesis is not mediated by direct effects of the high K^+, such as membrane depolarization or alterations in internal ionic concentrations. This experiment also suggests that the enhancement of synthesis is mediated by a secretory process, since the clusters are largely isolated from sites of presynaptic input and ELH secretion. A secretion-linked mechanism is also indicated by the fact that low Ca^{++}/high Mg^{++} media block the effect of high K^+ on ELH synthesis.

Therefore, it appears that these peptidergic cells do respond to activation by increasing their synthesis of the secretory peptide. This augmentation is rapid in onset, modest in magnitude, but long in duration, as might be expected of neurons which contain a large reservoir of the secretory product. A secretory event appears to trigger this process, but whether this involves the receipt of presynaptic transmitter or the secretion of ELH itself remains to be investigated.

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212.19 NEURALLY ACTIVE PEPTIDE, ELH, RELEASES EGGS FROM ISOLATED OVOTESTIS OF APLYSIA. B.S. Rothman, G. Weir* and F.E. Dudek. Dept. Physiol., UCSF, San Francisco, CA 94143 and Erindale College, Univ. of Toronto, Mississagua, Ont. Canada L5L 1C6.

Bag cells, a group of neurosecretory cells located in the abdominal ganglion of A. californica, are one of the command elements controlling egg laying behavior. Egg Laying Hormone (ELH), one of several peptides synthesized and released by bag cells, induces egg laying when injected into sexually mature animals. Recent studies have shown that purified ELH excites identified neurons in the abdominal and buccal ganglia. Presumably these effects code for aspects of egg laying behavior.

We are studying another role of ELH in the coordination of egg laying--the release of eggs from the ovotestis. Recent studies in Dudek's laboratory show that a protease sensitive factor in crude extracts of bag cells release eggs in a concentration dependent manner from isolated ovotestis fragments. Superfusates of electrically stimulated bag cells also had egg releasing activity (ERA), and the release of ERA was Ca^{++} dependent. One important question which remains is whether the active factor is ELH and whether other bag cell peptides have ERA. To help answer this question, we purified ELH by a 2-column procedure (Rothman et al, Nsci Abstr 5:260, '79) and tested all fractions for ERA.

The supernate of a bag cell homogenate was applied to a G50 Sephadex column equilibrated with 0.5 M formic acid, the eluate divided into 8 fractions, and the fractions lyophylized. All fractions were assayed for ERA by the method of Tobe and Dudek (Gen. Comp. Endocrinol. 36:618,'78). One fraction, containing material of ca. 4K-10K mw, had 2.8 ± 0.5 (SE) times more ERA than controls (N=10, $p < 0.01$), while the next most active fraction had only half this activity (N=10, $p < 0.1$).

The active material was further purified by cation exchange chromatography using NH_4Ac as an elutant. The peak of material eluting at 0.1 M salt contained 1.6 ± 0.2 times more ERA than controls or the 8 other column fractions (N=7, $p < 0.05$). This material has an amino acid composition in excellent agreement with that of ELH, and is 85 M% pure based on amino acid analysis and TLC. Fluorescamine analysis showed that ELH was present at a concentration of 3×10^{-8} M in the egg release assay. Preliminary dose-response experiments suggest that the threshold for egg release is 4×10^{-9} M.

These data strongly suggest that ELH is the major component with ERA in bag cell extract, and that it acts directly on the ovotestis. They provide further evidence that ELH is the hormone released by bag cells to induce ovulation. Studies

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212.20 THE ORIGIN AND NATURE OF PEPTIDERGIC PROCESSES IN THE BASAL GLIAL LAMINA OF THE RAT SUPRAOPTIC NUCLEUS (SON). W.E. Armstrong and T.H. McNeill. Depts. of Anatomy and Neurology, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

In the present study we sought to determine the origin of peptidergic processes in the basal glial lamina of the SON and were particularly interested in comparing their morphology with dorsally-emerging axons of the neurohypophysial tract. The SON of normal rats was examined in 1) Golgi-Cox preparations; 2) 20-50 um vibratome and 6-10 um paraffin sections stained for neurophysin (NP; anti-NP provided by A. Robinson) or vasopressin (VP; anti-VP provided by E. Zimmerman); and 3) material prepared for routine electron microscopy.

In vibratome sections thin, beaded NP-positive fibers could be seen emerging dorsally from the SON to join the neurohypophysial tract. The ventral NP-positive processes in the glial lamina, however, coursed parallel to the pial surface in both the medial and lateral direction. These processes varied in type from fine, linear profiles to conspicuously large (5-10 um di.) processes with occasional bulbous expansions along their length or at their apparent terminations. None of these processes was seen to join the neurohypophysial tract, nor were any processes of similar shape and size seen to emerge dorsally from the SON. Similar results were found in paraffin sections stained for either NP or VP. In Golgi-Cox material, some 90% of the 75 large (20-30 um di.) SON neurons examined to date possessed at least one ventrally directed dendrite. These dendrites frequently invaded the basal glial lamina where some bifurcated. Bulbous expansions along the course of some of these processes were evident, while other processes tapered distally. Identified axons were not observed projecting ventrally but instead ran dorsally. No clear case of collateral axonal branching was observed. At the ultrastructural level, processes in the lamina characterized as dendrites were sometimes seen to contain large (150-200 nm di.) dense-cored vesicles in varying numbers and were frequently postsynaptic to a variety of presynaptic elements. Further analysis with EM-immunocytochemistry is underway.

The combination of these data allow us to suggest that the ventral NP- and VP-positive processes are SON dendrites, or at least processes different in morphology from fibers of the neurohypophysial tract. The contribution of oxytocin-containing processes to the lamina is as yet unknown. Krisch (Cell Tiss. Res. 197: 95, 1979) has concluded that ventral SON processes secrete VP locally. The presence of synapses upon similar processes may suggest that changes in membrane potential (or Ca^{++} influx?) influencing this release could be induced locally.

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212.21 Terbium Fluorescence Studies of Angiotensin II. Robert G. Canada* Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, NIH, Bethesda, Md. 20205

Angiotensin II (AII) is a potent pressor and has a variety of biological activities. The activity of AII is selectively enhanced *in vivo* and *in vitro* by Na^{+} and Ca^{2+}. In this study, the fluorescent properties of terbium (Tb^{3+}) were used to detect and monitor the metal-peptide interactions of AII. The emission spectrum of Tb^{3+} was enhanced with the binding of AII, and the excitation maximum of the metal shifted to resemble that of the peptide fluorescence. The Tb^{3+} emission spectrum displayed the characteristic quartet at approximately 488,543, 584 and 619nm. The 290nm/260nm intensity ratio of the excitation spectrum was significantly greater for the AII-Tb^{3+} complex than for the free probe. The Tb^{3+} emission intensity at 543nm linearly increased as a function of Tb^{3+} concentration. The dissociation constant for the AII-Tb^{3+} complex was calculated to be about 7×10^{-3}M. The emission intensity of the AII-Tb^{3+} complex at 543 nm was reduced by 0.03M Ca^{2+}, while Na^{+} at the same concentrations had no significant effect. The data suggests that singlet-singlet energy transfer can occur between the hormone and the metal ion, indicating that the Tb^{3+} binding site is in close proximity of the tyrosine residue of AII, and that the hormone has a greater tendency to bind Ca^{2+} than Na^{+}.

212.22 EFFECTS OF NEUROPEPTIDES ON DORSAL HORN INTERNEURONS IN THE RAT SPINAL CORD SLICE PREPARATION. V. Miletic and M. Randic, Dept. of Vet. Physiology & Pharmacology, Iowa State University, Ames, Iowa 50011.

Extensive use of *in vitro* brain slices has been made lately in studies of synaptic function because of a number of advantages this technique offers over the *in vivo* approach. In order to extend our previous *in situ* studies on synaptic function of neuropeptides in the dorsal horn, we have now successfully employed the *in vitro* rat spinal cord slice preparation and demonstrated that the slices are capable of responding to several neuropeptides in a qualitatively similar manner.

Rats (2-35 days old) were anesthetized with ether and, following a laminectomy, a small segment of the lumbosacral spinal cord was quickly removed and cut, by hand with a razor blade, into transverse 300-800 μm thick slices. The slices were then transferred to the recording chamber where they were held on a fine nylon mesh and continuously perfused with a warm (32±1°C), aerated (95% O_2 & 5% CO_2) Krebs solution at a rate of about 3 ml/min. Following an incubation period of 45-60 min, the dorsal horn interneurons were found to be electrophysiologically intact in that they had normal resting potentials and were capable of generating synaptic and action potentials of appropriate size and duration. The tissue can be maintained in good condition for 6-8 hours. Actions of several neuropeptides, known to be present in high concentrations in the dorsal horn, i.e., substance P (SP), methionine-enkephalin (ME), somatostatin (SS), and angiotensin II (AgII), were then examined by applying the peptides into the perfusion medium or by iontophoresis while recording the activity of dorsal horn interneurons extracellularly.

We found that the responses of the dorsal horn interneurons in the rat spinal slices were essentially identical to those observed in previous studies of the cat spinal cord *in situ*. Thus SP powerfully excited almost all interneurons tested (15/16), while ME and SS depressed neuronal discharges in 13/14 and 4/6 units, respectively. AgII had no effect on any of the tested interneurons (n=8) although it was previously reported to be an excitant of hypothalamic neurons, both *in situ* and *in vitro*. In the spinal cord slices perfused with a low Ca^{++}-high Mg^{++} Krebs solution, the observed peptide effects were not modified, suggesting that the peptides were acting directly on postsynaptic sites. Furthermore, it is of interest that in some dorsal horn interneurons the iontophoretic application of ME caused a marked depression of the SP-induced excitation.

These experiments indicate that the *in vitro* rat spinal cord slice preparation can be successfully utilized for further physiological and pharmacological studies of the role of neuropeptides in synaptic transmission processes in the dorsal horn.

213.1 INTERACTION OF EXOGENOUS AND ENDOGENOUSLY RELEASED ADENOSINE WITH SYNAPTIC ACTIVITY IN RAT HIPPOCAMPUS. Thomas Dunwiddie and Barry Hoffer*. Dept. Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

The physiological role of adenosine and related adenine nucleotides was investigated in the hippocampal slice preparation. Two different protocols indicated an interaction between these agents and excitatory synaptic transmission in the CA1 region. First, perfusion of the slice with adenosine and adenine nucleotides reduced the amplitude of synaptically evoked responses, with an apparent EC_{50} of 5 μM. In a second series of experiments, penicillin-induced interictal spiking was slowed by exogenously applied adenosine ($EC_{50} \sim 10$ μM). Perfusion of slices with adenosine deaminase, which catabolizes extracellular adenosine, had the opposite effect on both types of responses. This suggests that the endogenous release of adenosine in the hippocampus in vitro yields extracellular levels which are sufficient to exert a tonic inhibitory effect upon synaptically mediated synaptic responses.

The interaction of adenosine with various adrenergic agents in the hippocampal slice was also investigated. Both adenosine and norepinephrine increase cyclic AMP levels in brain slices, and combinations of these agents have markedly synergistic effects. However, neither the α-adrenergically mediated depression of hippocampal excitability nor the β-adrenergic augmentation of excitability (Mueller, Hoffer and Dunwiddle, this volume) were in any way affected by concurrent application of adenosine.

Taken together, these data suggest endogenously released adenosine is a potent modulator of hippocampal excitatory transmission. In contrast to biochemical studies, however, there is little electrophysiological evidence for the interaction of exogenously applied adenosine with alpha or beta adrenergic agonists.

(This research was supported by ES 02011 to B.J.H and NS 05962-02 to T.V.D.)

213.2 RELEASE OF ATP FROM "AUTONOMIC SYNAPTOSOMES" PREPARED FROM THE MYENTERIC PLEXUS OF GUINEA-PIG ILEUM. T.D.White* and D. Webb* (SPON:R.Leslie). Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

It has been suggested that ATP might be the non-adrenergic non-cholinergic inhibitory neurotransmitter in the myentericplexus of vertebrate intestinal smooth muscle (Burnstock, G., Pharmacol. Rev.,24:509, 1972). Moreover, ATP may be released as a cotransmitter with biogenic amines and acetylcholine to modulate synaptic function. Recently, Miller et al (J. Neurochem., 32:1387,1979) have prepared and isolated "autonomic synaptosomes" from the longitudinal muscle of guinea pig small intestine. We have prepared a crude synaptosomal fraction (P_2) and purified synaptosomal fractions on sucrose density gradients using a modification of their technique and have investigated the ability of depolarizing agents such as elevated extracellular K^+ and veratridine to release ATP from these "autonomic synaptosomes". Release of ATP was directly and continuously monitored by detecting the light produced when the ATP reacted with firefly luciferin-luciferase present in the medium (White, T.D., J. Neurochem., 30: 329, 1978). Both K^+ and veratridine released ATP from these preparations but only the veratridine-induced release was blocked by TTX, indicating the involvement of Na^+-channels. Release of ATP by elevated K^+ and veratridine was restricted to synaptosomal preparations and did not occur from mitochondrial fractions. Elevated extracellular Rb^+ released ATP in a manner similar to elevated K^+, whereas Na^+ and Li^+ did not. Previous studies have shown that both elevated extracellular K^+ and Rb^+ depolarize nerves whereas Na^+ and Li^+ do not (Feng, T.R. and Liu, Y.M., J. Cell. Comp. Physiol., 34: 33, 1949). These findings suggest that depolarization-induced release of ATP occurs from autonomic as well as from CNS nerve-terminals. The nature and function of this release is under investigation. (Supported by the MRC of Canada).

213.3 LOCALIZATION AND CHARACTERIZATION OF A Ca-Mg-ATPase IN RAT BRAIN. R. G. Sorensen* and H. R. Mahler (SPON: R. S. Gurd). Dept. of Chemistry, Indiana University, Bloomington, IN 47405.

We have recently reported the presence of a Ca-Mg-ATPase in a purified synaptic plasma membrane fraction isolated from rat brain cortex [Soc. for Neurosci. 9th Annual Meeting, Vol. 5, p. 309 (1979)]. Maximum activation of this enzyme occurred at a free Ca^{+2} ion concentration of 3-8 μM and the activity required the presence of millimolar concentrations of Mg^{+2} ions. Its activity was further enhanced in the presence of exogenously added calmodulin. We now extend these findings to include a study of the localization of the Ca-Mg-ATPase within various membrane fractions isolated from rat brain cortex and *corpus striatum* and the characterization of this enzyme with respect to its divalent cation requirements and the effects of various known inhibitors of ATPases.

Ca-Mg-ATPase activity was measured in synaptic plasma membrane (SPM), synaptic junctional complex (SJC); postsynaptic density (PSD); P_3B_2, a postsynaptic membrane fraction devoid of PSD, and PSM, a postsynaptic membrane fraction with attached PSD. Studies on the specific and total activities of the enzyme in these various fractions have permitted its assignment to particular structures within the synaptic junction.

Possible assignments of the functional significance of the enzyme in the junction has been aided by the use of specific and characteristic inhibitors such as vanadate, oxalate and DCCD. (Research supported by Research Grant NS 08309 from the NIH.)

213.4 PROSTAGLANDIN MODULATION OF DOPAMINE-MEDIATED NEUROTRANSMISSION IN VIVO AND IN VITRO. R.D. Schwarz*, J.R. Bianchine, and N.J. Uretsky. Dept. of Pharmacology, Ohio State Univ., Colleges of Medicine and Pharmacy, Columbus, Ohio 43214.

Prostaglandins (PGs) have been shown to be involved in normal physiology through-out the body. While PGs have been identified in neural elements, their role in the nervous system is unclear. Current evidence suggests that PGs modulate transmitter release from nerve terminals in the peripheral nervous system. Release of NE has been shown to be reduced by PGE's and facilitated by $PGF_{2\alpha}$ through presynaptic elements which control Ca^{++} flux or binding (Hedqvist theory). A similar role in the CNS has been suggested, but the results are equivocal. The following experiments were designed to examine the role of PGs in modulating dopamine (DA)-mediated mechanisms in the CNS. In vitro studies utilizing the release of ^{3}H-DA from rat, striatal slices were used to study PG modulation of basal and K^+-induced DA release. One hour prior to the experiment, animals were injected with indomethacin (20mg/kg, i.p.) in order to inhibit endogenous PG synthesis. Slices (0.3mm x 0.3mm) were incubated with ^{3}H-DA for 15 min at 37°, washed 4x with media, incubated 15 min with PG present, quickly separated from media by centrifugation, and further incubated for 15 min with PGs in normal media or media with 12.5 mM K^+. Only PGD_2 at 10^{-7}M produced a statistically significant increase in the basal release of ^{3}H-DA. Release by K^+ was significantly inhibited by both PGE_2 and $PGF_{2\alpha}$ at 10^{-6}M. Since release is functionally coupled to synthesis, the effect of PGs on DA synthesis was also measured. Slices were incubated with ^{3}H-tyrosine in the presence of PG amd the formation of both ^{3}H-DA in both tissue and media was measured. In contrast to the release studies, no PG was able to significantly alter the basal or K^+-induced stimulation of synthesis. If PGs are able to alter DA elease, then their administration in vivo should alter DA-mediated behavior. This behavior was evaluated using the mouse-circling model. Male, White Swiss mice, previously lesioned in the left striatum of the brain with 6-hydroxydopamine (16ug/4ul), were administered d-amphetamine (amph; 4mg/kg, i.p.) followed 10 min later by an intraventricular injection of a PG or saline. Other animals received PG or saline without amph. PGE_2 inhibited amph-induced circling at doses of 10 - 1.0ug. $PGF_{2\alpha}$ and PGD_2 also inhibited amph-induced circling at 10 ug, but failed to do so at 1.0ug. None of the PGs produced any circling when administered alone. In summary, it appears that PGs are able to modulate DA-mediated neural mechanisms. The behavioral observation that PGE_2 and $PGF_{2\alpha}$ can block circling may in part be due to their ability to inhibit DA release. (Supported in part by USPHS grant NS 13888).

213.5 HISTAMINE INHIBITS IDENTIFIED NEURONS IN THE SPINY LOBSTER. Brenda J. Claiborne*. (SPON: A. Selverston). Dept. of Biology, University of California, San Diego, La Jolla, Calif. 92093.

Histamine is a putative neurotransmitter in mammals and in the mollusc, Aplysia; however, its role in arthropods is not yet defined. Recent work has shown that the lobster stomatogastric ganglion contains histamine levels that are similar to those in Aplysia ganglia, and exhibits a temperature-dependent uptake system for ^{3}H-histamine (B. Claiborne, unpublished results). The present report characterizes the responses of identified neurons to exogenously-applied histamine.

The stomatogastric nervous system of Panulirus interruptus was prepared for recording as described previously (Mulloney and Selverston, J. Comp. Physiol. 91:1, 1974). Identified neurons in the ganglion were penetrated with two microelectrodes, one for recording and one for current injection. Histamine was applied either iontophoretically to individual somata, or superfused over the ganglion at a concentration of 100 μM. The cells which respond to histamine are the PDs, GMs, LPGs, and EXs. Only one type of histamine response has been found: it is inhibitory, lasts several seconds, desensitizes with repeated histamine applications, and persists in calcium-free saline. The following evidence suggests that the response is due to an increased conductance to chloride: 1) current injection before and after histamine application indicates an increase in membrane conductance, 2) the reversal potential of the response is within 10 mV of the resting potential, 3) the reversal potential becomes less negative following intracellular chloride injection, 4) an increase in external potassium does not significantly alter the reversal potential, whereas 5) a decrease in external chloride does change the reversal potential. Pharmacological studies indicate that the response is not blocked by histamine H_1 or histamine H_2 antagonists, but is blocked by the nonspecific blocker d-tubocurarine. Additional experiments aimed at finding a specific histamine antagonist in this system are in progress.

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213.6 PROPERTIES OF SOLUBLE HISTAMINE H_1 RECEPTORS. Lawrence Toll* and Solomon H. Snyder (SPON: Terry Rogers). Dept. of Pharmacol. Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205.

Specific binding to the histamine H_1 receptor in the soluble state has been demonstrated using (^{3}H)-mepyramine, an antihistamine, as the ligand (Gavish et al., Life Sciences., 25:783, 1979). We had previously shown that the tricyclic antidepressant, doxepin, had the highest affinity of any drug tested on the H_1 receptor. Using (^{3}H)-doxepin as a ligand for the H_1 receptor we are able to further characterize binding properties and regulation of binding to the H_1 receptor in the soluble state, and compare this to the membrane bound receptor.

Of a wide variety of detergents evaluated, only digitonin solubilizes membranes in such a fashion that specific H_1 binding of (^{3}H)-doxepin is retained in the soluble state. The association and dissociation rates as well as saturation kinetics give plots for digitonin solubilized receptor which are virtually superimposable with those derived from the membrane bound receptor. The ability of a variety of antihistamines to displace (^{3}H)-doxepin from soluble and membrane bound receptors is also closely correlated, indicating conservation of the binding site upon solubilization.

Some important regulatory aspects of H_1 receptors have been compared in the membrane bound and soluble states. In brain membranes, Na^+ specifically decreases the affinity of histamine and other agonists for the H_1 receptor. In the soluble state, the Na^+ effect on histamine affinity is conserved, indicating a close association between the Na^+ regulatory and histamine binding sites. Membrane bound H_1 receptors are also regulated by divalent cations and guanine nucleotides, which are thought to act at the same or related sites. However, in the soluble state, guanine nucleotides lose their ability to decrease histamine's affinity, while divalent cations retain their ability to increase histamine's affinity.

213.7 STIMULATION IN VIVO OF BRAIN PHOSPHOLIPID TURNOVER BY HISTAMINE. N. Subramanian*, W.L. Whitmore*, F.J. Seidler* and T.A. Slotkin. Dept. of Pharmacology, Duke Univ. Medical Center, Durham, N.C. 27710.

Membrane phospholipid turnover in the brain is controlled in part by central neurotransmitters, including catecholamines and acetylcholine. This effect has served as a marker for specific receptor-mediated cellular stimulation. Histamine, a putative central neurotransmitter, also is capable of stimulating incorporation of radiolabeled inorganic phosphate (P_i) into brain phospholipids, and the mechanism by which this occurs is the subject of the present study. Histamine or histaminergic agonists were administered to rats intracisternally (i.c.), with or without 5 min i.c. pretreatments with antagonists. Five min after receiving agonist + $^{33}P_i$, rats were killed and brain phospholipids extracted. Histamine produced a dose-dependent increase in P_i incorporation, with a maximal effect (100% increase over control) reached by 10 nmols/g of brain weight. The effect could not be mimicked by compound 48/80 (12.5-50 μg/g brain i.c.), a specific releaser of mast cell histamine, indicating lack of involvement of non-neuronal histamine. The stimulation by histamine was not attenuated by prior depletion of catecholamines with reserpine, indicating that the effect was mediated directly by histamine, and not indirectly through histaminergic release of catecholamines. An H_1 receptor was involved in the effect, since 2(2-pyridyl)ethylamine, an H_1 agonist, produced a similar effect, but 4-methylhistamine, an H_2 agonist, did not. Pyrilamine, an H_1 antagonist, blocked the stimulation, but cimetidine, an H_2 antagonist, was ineffective. Histamine-induced stimulation of $^{33}P_i$ incorporation into brain phospholipids in vivo can be a valuable tool for assessing cellular stimulation evoked by H_1 receptors. (Supported by USPHS HL-24115, HD-09713 and DA-00006).

213.8 HISTAMINE: MECHANISMS AND FREQUENCY SPECIFICITY IN NUCLEUS ACCUMBENS. R. B. Chronister, G. C. Palmer, J. F. DeFrance and R. W. Sikes. Depts. Anat. and Pharm., Univ. of S. Ala., Mobile, Ala. 36688 and Dept. Neurobiol. Anat., Univ. Texas Hlth. Sci. Center, Houston, TX 77025.

Histamine is probably one of the least understood aminergic compounds in the CNS. Yet, evidence is accruing that it functions as a neurotransmitter. Histamine has been applied by iontophoresis onto numerous areas of the brain but never onto a system with a known transmitter substrate and one readily controlled. For this reason, we examined the effects of iontophoretic application of H_2 agonists/antagonists onto fimbrial evoked (glutamate-like) responses in rabbit nucleus accumbens. Additional studies were done to characterize potential sites of neurons giving rise to histamine and receptor mechanisms of the histamine systems in nucleus accumbens.

The sites of the neurons appear to be in mamillary complex and related areas (nuclei gemini). Furthermore, the coupling of histamine to adenylate cyclase activity in accumbens is second only to dopamine. This activation can be readily antagonized by H_2 antagonists (metiamide). Therefore, the physiological studies examined the effects of 4-Me-Histamine (H_2 agonist), combined 4-Me-Histamine-metiamide, and metiamide alone on the frequency spectrum of response in nucleus accumbens.

At low frequency (0.5 Hz) stimulation of the fimbria, iontophoresis of 4-Me-Histamine produces a small amount of depression (20%) to the N component. This inhibition has both a rapid onset and offset. At higher frequencies (6 Hz), iontophoresis produces a greater effect. Here the depression (75%) is striking to the tetanic activation. These results demonstrate that H_2 mechanisms preferentially influence higher frequency (Θ range) activity in nucleus accumbens. These phenomenon are all reversed by metiamide.

Metiamide alone also shows frequency related effects. It depresses the component of the evoked potentials and does so more at 6 Hz than at 0.5 Hz. This P component is bicuculine sensitive suggesting that (H_2) histamine functions through GABA mechanisms in nucleus accumbens. The H_1 effects demonstrated pharmacologically were very variable in physiological effect.

These data in conjunction with our observations with dopamine imply the existence of frequency specific effect of aminergic function. Furthermore, the effects can be complimentary in nature. (Supported by Scottish Rite Schizophrenic Foundation, the Epilepsy Foundation of America and NIH grants 1 R03 MH 32418 and 1 R01 MH 31114-01.

213.9 TECHNIQUES FOR DETECTING AMINES AND TYROSINE CONTAINING PEPTIDES IN THE CNS OF LOCUST AND OTHER INSECTS. C.A. Bishop*, M. O'Shea* and R.J. Dinerstein. (SPON: B. Wainer). Dept. Pharm. & Physiol. Sci., Univ. of Chicago, 947 E. 58th St., Chicago, IL, 60637.

We have used three techniques which permit the detection of specific amine histofluorescence in identified and identifiable neurons in the CNS of the locust and other insects. Together, these techniques allow us to distinguish among dopamine, octopamine, serotonin, noradrenaline and, in addition, allow for the detection of tyrosine and tyrosine containing peptides. The application of these techniques to the much studied locust nervous system will allow for the identification of transmitters in individually identified neurons.

The detection of monoamines, tyrosine and tyrosine containing peptides in the CNS depends on the generation of detectable fluorophores. For this we have used formaldehyde vapor (Falck et al. 1962. J. Histochem. Cytochem. 10, 348-354), glyoxylic acid (de la Torre & Surgeon, 1976. Neurosci. 1, 451-454) and nitroso-naphthol (Sundler et al. 1976, Histochem. 50, 39-46).

We are applying these techniques first to individually identified and isolated cell bodies of neurons which are known to be octopaminergic (DUM neurons, see O'Shea & Evans, Soc. Neurosci. Abstr., 1977). We have shown that these cells produce observable fluorophores when treated with glyoxylic acid or nitroso-napthol. This approach combined with microspectrofluorometry will allow us to characterize the fluorescence associated with neurons known to contain specific amines. A similar approach is being developed for isolated, identified peptide neurons (see O'Shea & Adams, this issue).

In addition, we have applied histofluorescent techniques to sections of tissue. We have detected, in the locust, fluorescing cell bodies, fibers and fiber varicosities which have the spectral characteristic of catecholamines in vertebrates. By comparing the characteristics of the fluorescence found in sectioned locust tissue with that of the isolated identified octopamine-containing cell bodies, we hope to characterize specific octopamine histofluorescence. This approach may aid in the histofluorescent detection of octopamine in the vertebrate brain.

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213.10 HABITUATION AND EFFECTS OF AN OCTOPAMINE AGONIST IN THE DEVELOPING MOTH FLIGHT CONTROL SYSTEM. Sue C. Kinnamon*, Leland W. Klaassen* and Ann E. Kammer. Div. of Biology, Kansas State Univ., Manhattan, KS 66506.

Previous studies have shown that developing Manduca sexta spontaneously produce bouts of activity alternating with periods of inactivity. During activity the flight muscles are excited by patterned motor output similar to that recorded from the flight muscles of flying adults, although the wings are not moved. On the last day of development the amount of spontaneous activity decreases. Thus the developing (pharate) moth provides a suitable preparation in which to examine changes in responsiveness to sensory stimulation with respect to behavioral state. The preparation can also be used to assess the efficacy of a biogenic amine, octopamine, in altering behavioral states.

Wing campaniform sensilla were mechanically stimulated at a rate of approximately 1/sec, and motor output was recorded by fine wires inserted in the indirect flight muscles. In inactive pharate moths tested 1 day before eclosion, the typical response to a stimulus was several cycles of the flight motor pattern. The first few stimuli of a train elicited responses, but with successive stimuli the response usually habituated. In active animals of the same age, stimulation initially increased the amount of motor output. With repeated stimulation the spontaneous activity ceased; then some animals habituated like those in an inactive period, whereas other animals habituated more slowly. Pharate moths tested when quiescent during the last day of development showed greatly reduced responses to sensory stimulation, and the responses habituated more quickly. These results demonstrate that responsiveness to sensory stimulation varies with amount of flight motor pattern being produced spontaneously.

Injection of 6 x 10^{-6} moles of octopamine in active pharate moths (1 day before eclosion) increases the amount of motor output; injection late in an inactive period results in activity. Topical application of 10^{-9} moles of chlordimeform, a putative octopamine agonist, results in continuous flight in adult moths and also in pharate moths examined 1 day before eclosion. Slightly older pharate moths that normally become markedly quiescent before eclosion also respond to treatment with chlordimeform by producing motor output continuously. During the time between treatment and the production of spontaneous activity, sensory stimulation elicited more motor output per response and the response habituated more slowly than in control animals of the same age. The results suggest that octopamine modulates the behavioral state and concomitantly alters the response to sensory stimulation. (Supported by NSF BNS 75-18569)

213.11 MODULATION OF NEUROMUSCULAR TRANSMISSION BY OCTOPAMINE IN DEVELOPING AND ADULT MOTHS (MANDUCA SEXTA). Leland W. Klaassen* and Ann E. Kammer (Spon: Jane A. Westfall). Div. of Biology, Kansas State Univ., Manhattan, KS 66506.

Previous work by several authors has demonstrated that octopamine elicits action potentials and contractures in lobster leg muscles, but in insects it has been shown to have only slight effects on the excitatory junctional potential and tension development of locust extensor tibiae muscle.

The purpose of this study was to examine the effect of octopamine on neural excitation of a developing and mature fast moth muscle.

Stimulating the motor nerve to the dorsal longitudinal flight muscle in developing pharate moths (4 days before eclosion) elicited excitatory junctional potentials (EJPs) that ranged in amplitude from 10-20 mV. Superfusion of 10^{-6} M DL-octopamine increased the amplitude of the EJP to 30-35 mV. This increase in amplitude was sufficient to elicit action potentials in the muscle, which normally does not spike at this age. Superfusion of 10^{-3} M phentolamine decreased the EJP to 5-10 mV. In fully developed adult moth muscle, high concentrations of DL-octopamine had no observable effect on action potentials, i.e. the action potentials of a given fiber before and after treatment were superimposable. Octopamine also had no effect on EJP's which were observed in the absence of spikes by superfusing the preparation with a low calcium saline. Phentolamine treatment of adult muscle, however, reduced the EJP to 10-15 mV, well below the threshold for action potentials in this muscle. These results on the adult muscle can be interpreted to mean that so much octopamine is normally available to the adult neuromuscular junction that exogenous octopamine has no additional effect.

The putative neurotransmitter mediating excitation of this flight muscle is glutamate, as indicated by two observations: the amplitude of the EJP is reduced by applied glutamate (10^{-3} M in the saline) (M. B. Rheuben, 1974), and the muscle membrane is depolarized by 10^{-3} M kainic acid.

Because of the observed effect of octopamine and its antagonist phentolamine on the electrical responses to stimulation of the motor nerve, we suggest that octopamine modulates neuromuscular transmission in both developing and adult fast flight muscle. (Supported by NSF BNS 75-18569)

214.1 INULIN SPACE IN THE SPINAL CORD OF THE SEA LAMPREY. U. Wald* and M.E. Selzer. Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The spinal cords of large larval and feeding stage adult sea lampreys (Petromyzon marinus) were removed by a ventral dissection and incubated in physiologic solution containing ^{14}C-inulin (1.5 μCi/ml). Incubation times were varied from 0-15 min. at 10°C. After a one second dip in regular lamprey solution to wash off surface radioactivity, the cords were homogenized and the radioactivity measured in a scintillation counter. Inulin uptake in larval cords reached a plateau distribution space of about 33% (vol./wt.) within 2.5 min. The inulin space of adult cord was approximately 18%. This probably represents the extracellular space because the time to plateau correlated well with the time (2 min.) for bath-applied tetrodotoxin (10^{-7}M) to abolish all electrical responses in giant interneurons to intracellular or whole cord stimulation. Mannitol was not an efficient marker of extracellular space since its concentration in spinal cords did not reach a plateau even after 30 min. of incubation.

The inulin space increased with temperature. At incubation temperatures of 5°, 10°, and 20°C, the inulin space of larval cord was 26%, 33%, and 42% respectively.

There was no correlation between larval size (and therefore, presumably, age), and the magnitude of the inulin space. Therefore, the large reduction in cord inulin space between the larval and adult stages probably occurs during transformation, and not gradually during the larval phase. This change correlated with morphological changes in the extracellular space in electron micrographs of lamprey spinal cords, although the latter were always much smaller by planimetric analysis than the inulin space.

In magnitude, temperature sensitivity and developmental change the inulin space of the lamprey spinal cord behaves similarly to the view of the extracellular space of mammalian brain which has emerged in recent years. We believe that the inulin space of the lamprey cord is a useful model for the extracellular space of vertebrate CNS because the small diameter of the cord allows rapid distribution of inluin in the extracellular space, and therefore avoids contamination of results by slow intracellular uptake.

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214.2 ASCENDING SPINAL PATHWAYS IN THE SEA LAMPREY. R. G. Northcutt and S. O. E. Ebbesson. Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109 and Institut für Zoologie der Technischen Hochschule Darmstadt, Darmstadt, Federal Republic of Germany.

Hemisection or complete transection at the 5th to 8th cervical spinal level was performed in 7 adult sea lampreys, Petromyzon marinus, under MS222 anesthesia. Animals were housed postoperatively in fresh water (13-15°C) and allowed to survive 7-35 days before being re-anesthesized and perfused transcardially with 0.7% saline followed by 10% formalin. The brains and rostral spinal cords were removed, albumin-embedded, sectioned, and stained for degenerating fibers and terminals by modifications of the Nauta method. All survival times revealed degenerating fibers; however, shorter survival times revealed more degenerating terminals. The degenerating ascending spinal pathways were primarily ipsilateral to the hemisections, but sparse degeneration was observed in the same contralateral pathways and nuclei even in cases where the lesion did not cross the midline. The ascending spinal pathways consist of dorsal and lateroventral funicular systems. The dorsal funicular system ascends medial to the spinal trigeminal tract and consists of large-caliber fibers which terminate in the funicular nucleus at obex levels as well as continue more rostrally where they divide into dorsal and ventral components. The dorsal component courses along the lateral edge of the dorsal and medial octavolateralis nuclei, where terminals may be given off, and can be traced rostrally where it terminates in the molecular layer of the cerebellum. The ventral component courses through the ventral octavolateralis nucleus and also terminates rostrally in the alar grey immediately beneath the cerebellum. The lateroventral funicular system forms a wedge-shaped zone of degeneration from the level of the vagal motor nucleus to midbrain tegmental levels; terminals are likely given off to all branchiomeric motor nuclei as well as the hindbrain reticular system. No ascending spinal pathways appear to reach tectal or thalamic levels in these animals.

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214.3 SPINAL CORD PROJECTIONS IN THE ANURAN BRAINSTEM. C. Diakow and R. Woltmann*. Biology Department, Adelphi University, Garden City, New York 11530.

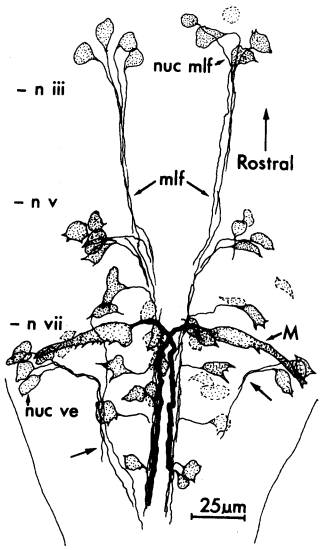
H^3-leucine was injected into the dorsal horns of the spinal cords of 3 female frogs (Rana pipiens) and radioactive label measured in the brainstem.

Injections were performed under Tricaine anesthesia at the level of the brachial enlargement in two frogs and at the sacral enlargement in the third. (Label spread a maximum of 0.4mm and in all cases, the most rostral spread was caudal to the calamus.) Animals were sacrificed 9 or 10 days after injection, the brains fixed in formalin, embedded in paraffin, and sectioned at 10 μ. Sections were coated with Kodak NTB-2 emulsion. After an exposure time of 25 or 31 days, sections were developed and stained with Hematoxylin and Eosin. For each female, five sections at each of 3 levels of the brainstem were studied. Grain counts of several areas on each section were obtained at 1000x. Counts were corrected for background by subtracting counts obtained from the brains of uninjected females processed at the same time as those of experimental females. Corrected grain counts of study areas for each section were compared to counts of a control area in the white matter 0.25mm from the midline. It was concluded that leucine was transported to a study area if the grain count in the study area was significantly higher than that in the control area.

In the caudal medulla at a level which included the nuclei of the tenth and twelfth nerves, there were projections to the tenth nucleus, the reticular nucleus, and in the lateral and medial bundles of the antero-lateral fasciculus. In the medulla at the level of emergence of the seventh nerve, there was also a projection through the lateral and medial bundles. At the midbrain-medulla junction, there were projections to the cerebellar grey, in the spino-cerebellar tract and, in two animals, on the lateral edge of the central grey in the superior reticular nucleus.

214.4 BRAIN NEURONS THAT PROJECT TO THE SPINAL CORD IN YOUNG LARVAE OF THE ZEBRAFISH. Charles B. Kimmel and Susan L. Powell*. Dept. of Biology, Univ. of Oregon, Eugene, OR 97403.

A small number of brainstem neurons project to caudal levels of the spinal cord in the larva of Brachydanio rerio. These cells were identified in animals 6 days after fertilization by backfilling with horseradish peroxidase following transection of the cord at the level of the cloaca. The distribution in the horizontal plane of all cells stained in one animal is shown in the figure. Rostrocaudal levels are indicated by positions of cranial nerve roots. Cells stained in all of the cases were restricted to locations shown in this example. The most rostral cells are within the midbrain nuclei of origin of the medial longitudinal fascicles (nuc mlf). The stained neurons located more caudally also contribute to the mlf. The majority are within the tegmental motor nucleus of the hindbrain, and prominent among them is the pair of Mauthner cells (M), which give rise to thick, crossed axons. All or nearly all of the other axons project ipsilaterally. The other cells in this nucleus are Müller cells, and are arranged in a characteristic ladder-like array. The other contribution of hindbrain neurons is from the vestibular nucleus (nuc ve). The vestibulospinal axons run obliquely through the hindbrain (arrows) before joining the mlf.



The filled cells are the largest ones present. Distinctive cells often have contralateral counterparts. In preparations with the greatest numbers of filled cells a total of 25-30 cells are stained on each side of the midline.

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214.5 PROJECTIONS FROM BASAL TELENCEPHALON TO SPINAL CORD IN THE LIZARD IGUANA iguana. Laura L. Bruce, Donald B. Newman*+ and Ann B. Butler. Dept. Anat., Georgetown Univ., Washington, D.C. 20007; +Dept. Anat., USUHS, Bethesda, Md. 20014.

To determine whether a telencephalo-spinal connection is present in Iguana iguana, horseradish peroxidase (HRP) was applied by insertion of a gelfoam plug into hemisected spinal cord at upper and lower cervical levels. After a survival time of 2 to 6 days, the iguanas were perfused, and the tissue was processed for the HRP reaction product according to the protocol of deOlmos and Heimer (Neurosci. Lett., 1977). Retrogradely labeled cells were found in all cases in two areas of the basal telencephalon in addition to the typical pattern of labeling in brainstem areas. 1) Most of the labeled telencephalic cells are within or closely associated with the nucleus of the anterior commissure (Northcutt, J. comp. Neur., 1967). These cells are pyramidal and fusiform in shape. 2) A small number of labeled cells are present along the ventrolateral edge of the lateral forebrain bundle. These cells are fusiform in shape.

The presence of telencephalo-spinal projections in reptiles has not been reported previously, although they have been found in the salamander Ambystoma (Kokoros and Northcutt, J. comp. Neur., 1977). Determination of the function of these cells in Iguana and whether or not they are homologous to any cell populations in other vertebrates will require further investigation. Studies are currently underway to determine whether or not similar telencephalo-spinal projections are present in other reptiles and to describe the connections of these cell groups in greater detail.

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214.6 DESCENDING INPUTS TO THE OPTIC TECTUM IN RANID FROGS. Timothy J. Neary and Walter Wilczynski. Anat. Dept., Creighton Univ., Omaha, NE 68178 and Sec. Neurobiol. and Behav., Cornell Univ., Ithaca, NY 14853.

Descending inputs to the optic tectum in two species of ranid frogs (R. grylio and R. heckscheri) were examined after 40-60 nl injections of 25% HRP into one lobe of the tectum and processing with TMB histochemistry. Results were similar for both species. In the telencephalon, only one population, the anterior entopeduncular nucleus, ipsilateral to the injections, contained labelled cells. In the diencephalon, several populations contained labelled cells: 1) the anterior thalamic nucleus, primarily ipsilateral to the injections, 2) the nucleus of Bellonci and dorsal ventrolateral nucleus, ipsilateral to the injections, 3) the ventromedial thalamic nucleus, primarily ipsilateral to the injections, 4) the posterior thalamic nucleus, ipsilateral to the injections, 5) the posterodorsal division of the lateral nucleus, primarily ipsilateral to the injections, 6) the ventral hypothalamus, bilaterally, 7) the posterior tuberculum, primarily ipsilateral to the injections, 8) the pretectal grey, ipsilateral to the injections, and 9) nucleus lentiformis mesencephali, ipsilateral to the injections. These results suggest several possibilities: 1) that both anterior thalamic and posterior thalamic/pretectal retinorecipient cell populations influence tectal function (Scalia and Gregory, Brain, Behav., Evol., 3:16 '70), 2) that somatosensory input may influence the tectum after processing in the ventromedial thalamic nucleus (Neary and Wilczynski, Brain Res., 138:529 '77), 3) that olfactory input, carried over both the main and accessory olfactory systems, may influence tectal function after processing in the ventral hypothalamus (Neary, Anat. Rec., 196:135A '80), and 4) that gustatory input may influence tectal function after being processed first in the 2° visceral nucleus and then in the ventral hypothalamus (Neary, loc. cit.). Thus, it appears that tectal function is likely to be influenced not only by direct and indirect visual inputs, but also by inputs from mechanical and chemical sensory systems.

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214.7 SOME CONNECTIONS OF THE TELEOST TELENCEPHALON. Stephen M. Echteler and William M. Saidel. Scripps Instit. of Oceanog. and Dept. Neurosci., U.C.S.D., La Jolla, CA 92093.

Eversion of the pallium during the development of the teleost telencephalon has produced patterns of cellular organization remarkably different from those found in land vertebrates. Afferent and efferent connections of different regions of the teleost pallium should be determined to permit homologies with regions of the telencephalon of land vertebrates. Previous studies have used methods which do not allow adequate resolution of either the source of the efferent fibers from or the termination of afferents to the telencephalon. In the present study small injections of HRP into the telencephalic regions Dm or Dd-Dl in two species of teleosts (Carassius auratus: Cypriniformes, Cyprinoidei and Paralabrax nebulifer: Perciformes, Serranidae) have been used to trace both afferent and efferent projections of these regions.

Unilateral injections of HRP into Dm revealed retrograde cell labelling in ipsilateral preoptic region (nucleus entopeduncularis) and thalamus (posterior dorsal nucleus and nucleus preglomerulosus). Anterograde labeling of axons and terminals revealed ipsilateral efferent projections, via the lateral forebrain bundle, to optic tectum, nucleus preglomerulosus, and inferior lobe of the hypothalamus. Unilateral Dd-Dl injections resulted in heavy retrograde cell labelling in ipsilateral nucleus preglomerulosus. Anterograde labelling revealed efferent fibers which cross to the contralateral telencephalon, via the anterior commissure, and descend bilaterally in the medial forebrain bundle to terminate in the nucleus preopticus periventricularis, nucleus ventromedialis of the thalamus, and midbrain tegmentum.

The connections described for the telencephalic region Dm of the teleosts in this study are strikingly similar to those reported for the striatum in the amphibian telencephalon; furthermore, the connections described for the telencephalic region Dd-Dl of these teleosts are similar to those reported for the medial and dorsal pallium of amphibians (Kicliter, JCN:185, pp. 75-86, '79). Recently, homologies have been proposed between these regions of the teleost and amphibian telencephalon based upon similarities in cytoarchitecture and regional histochemistry (Northcutt and Bradford, Comp. Neurol. Telenceph., S.O.E. (ed.), Plenum, NY, '80). Our results provide additional support for these homologies on the basis of connections.

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214.8 HRP AND GOLGI INVESTIGATIONS OF GOLDFISH NEUROSECRETORY SYSTEMS. W.A. Gregory and C.D. Tweedle, Dept. of Anatomy and the Neuroscience Program, Michigan State University, East Lansing, Michigan 48824.

As part of an ongoing examination of piscine neuronal development, we are examining the morphology and projections of the magnocellular preoptic nucleus of the common goldfish (Carassius auratus). A topographical segregation of neurons has been shown to exist within the homologous magnocellular nuclei of rats (Armstrong, et al., Neuroscience, in press). The nature of regional differentiation within the hypothalamic magnocellular neurosecretory system of lower vertebrates is less clear. Horseradish peroxidase (.6-2mg Sigma VI) in .1-.2ml of .75% NaCl and 2% DMSO was injected IP into fish weighing 2-5grams and having total lengths of 5-8cm, in order to fill hypophyseal extravascular spaces (Abraham, et al., Cell Tiss. Res., 167:289-296, 1976). Following survival times of 2-10 days, fish were sacrificed, brains removed, sectioned and reacted for the presence of HRP using diaminobenzidine and cobalt intensification, with or without the addition of glucose oxidase (Itoh, et al., Brain Res., 175:341-346, 1979).

Preliminary results show lightly labeled neurons distributed within the preoptic and tuberal nuclear complexes. Since the control of pituitary secretion has been generally attributed to these nuclei, it is likely that many or all of the HRP-labeled cells project to the pituitary. Injections of HRP will be made into the brainstem and spinal cord, in order to determine the location of the preoptic neurons with descending projections (Hoheisel, et al., Cell Tiss. Res., 189:331-345, 1978). Brains from similar fish were processed with a Golgi procedure. Multipolar, bipolar and apparent unipolar neurons of various sizes were seen within the magnocellular preoptic region. Tendencies for neuronal morphological regional specializations were noted.

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214.9 MONOAMINE NEURONS IN THE BRAIN OF THE LONGNOSE GAR, Lepisosteus osseus. A. Parent and R.G. Northcutt, Lab. Neurobiol., Fac. Méd., Laval Univ., Québec, Canada and Div. Biol. Sci's, Univ. of Michigan, Ann Arbor, MI 48109.

The morphological organization of monoamine (MA)-containing neurons in the brain of Lepisosteus osseus, one of the few living representatives of the holostean group of ray-finned fishes, was studied by means of fluorescence histochemical methods. The classic Falck-Hillarp procedure (Falck et al., '62) and the more recent cryostat glyoxylic acid method of de la Torre and Surgeon ('76) were used. In this species by far the largest number of MA cells is found within the preoptico-hypothalamic complex. A multitude of small-sized MA cells with short club-like processes protruding into the III ventricle is present along the ependymal wall of the medio-basal hypothalamus and inferior lobes. Both catecholamine (CA) and serotonin (5-HT) type cells occur at this level. However, the 5-HT cells appear to represent only about 30% of the total population. Numerous fibers arise from these CSF-contacting MA cells. Some of them proceed rostrally toward telencephalon while other course dorsally to reach the optic tectum. Many fibers, however arborized directly within hypothalamus, particularly around blood vessels where they form patches of highly fluorescent material. Small CA cells are also scattered along the preoptic recess wall. They do not contact directly the CSF but appear to contribute also to the CA innervation of telencephalon. At brainstem levels CA cells are scattered at the basis of rostral midbrain, within the central portion of rostral medulla and in the area of the vagal lobe (nucleus solitarius). The latter neurons give rise to dense CA innervation of the periventricular gray of IV ventricle. One of the most striking feature of MA systems in Lepisosteus is the remarquable development of the 5-HT neuronal network. There is a prominent 5-HT cell column which extends rostrocaudally, in the raphe region, from caudal midbrain to upper spinal cord levels. In caudal midbrain and isthmus the 5-HT cells also invade the lateral tegmentum. The 5-HT neurons innervate profusely various brainstem structures as well as large portions of telencephalon. In telencephalon the 5-HT innervation is strikingly dense in the dorsal nucleus of area ventralis (Vd) and moderately so in the medial zone of area dorsalis (Dm, rostral pole). By comparison, the CA innervation of telencephalon is weak, except for the olfactory bulb where numerous CA varicosities are found, particularly in the glomerular layer. These findings in Lepisosteus suggest that the organization of the MA neuronal systems in holosteans is significantly different from that found in more advanced fishes such as teleosteans.

214.10 HISTOCHEMICAL STUDY OF THE MONOAMINE-CONTAINING NEURONS IN THE BRAIN OF THE SALAMANDER (Necturus maculosus). L. Dubé* and A. Parent (SPON: L. Larochelle). Lab. Neurobiol., Fac. Méd., Laval Univ., Québec, Canada.

A study of the morphological organization of monoamine (MA)-containing neurons in the brain of the mudpuppy was undertaken by means of the Falck-Hillarp paraformaldehyde histofluorescence method and the cryostat glyoxylic acid procedure of de la Torre and Surgeon ('76). Numerous MA neuronal somata and axonal varicosities are present in this amphibian brain. In the diencephalon a large number of catecholamine (CA) type cell bodies occurs in hypothalamus and thalamus, particularly along ependymal wall. Some of these are small bipolar cells with one short process protruding into the lumen of the ventricle. Other CA cell bodies are closely-packed together within more lateral regions of diencephalon. In the mesencephalon a small group of large-sized CA cells is lying ventromedially in the tegmentum, near the N III root fibers. These neurons give rise to numerous varicose fibers which ascend along the medial and lateral forebrain bundles to arborize profusely within the neuropil of the corpus striatum. In the medulla a few CA cells are scattered in the area of nucleus solitarius. Serotonin (5-HT) type cell bodies are found in the raphe region from caudal midbrain to upper medulla levels. Numerous MA fibers and varicosities can also be visualized at all levels of the neuraxis in Necturus. For example, CA varicosities are found in large number in the olfactory bulb. The corpus striatum, nucleus lateralis septi and the accumbens nucleus also receive a massive CA innervation. CA varicosities occurs in pars dorsalis of the primordium hippocampi whereas 5-HT varicosities predominate in pars ventralis of the same structure. Varicose CA fibers are scattered in the primordium piriforme. At diencephalic levels CA varicosities are encountered in habenula, preoptic area and pars ventralis hypothalami. At brain stem levels MA fibers ascend within the fasciculi ventralis and dorsalis tegmenti. Strong AChE activity can be observed in the neuropil of the corpus striatum, and in the cellular areas of nucleus lateralis septi, the viscinity of N III root fibers and the raphe nuclei. A moderate, AChE activity is detected in the glomerular layer of the olfactory bulb and in the primordium piriforme. These findings suggest that the central MA neurons in salamanders are organized according to a pattern which is in many ways similar to that disclosed in the brain of amniote vertebrates. For example, the meso-striatal CA projection disclosed in Necturus appears equivalent to the nigro-striatal CA pathway present in reptiles, birds and mammals.

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214.11 TELENCEPHALIC PROJECTIONS FROM CATECHOLAMINE CELL GROUPS IN THE PIGEON. Cheryl A. Kitt and Steven E. Brauth. Dept. of Psychology University of Maryland, College Park, Maryland 20742.

In pigeons, the telencephalic projections of the locus ceruleus (LoC), nucleus tegmenti pedunculo pontinus (TP) and area ventralis of Tsai (AVT) were traced by autoradiography.

LoC projections terminate principally in the paleostriatal complex. Specifically, the lobus parolfactorious (LPO) receives the heaviest projections from the LoC with the densest accumulations of silver grains lying over its central and caudal portions. Silver grain label was also evident over the paleostriatum augmentatum (PA), especially over its dorsomedial segment, albeit to a much lesser degree than that found in LPO. The paleostriatum primitivum (PP) and nucleus intrapeduncularis (INP) contained only small amounts of silver grain label in these experiments. Very light accumulations of silver grains could also be seen in the nucleus accumbens (Ac), lateral and medial septal areas (SL and SM), hippocampus (Hp), parahippocampal area (APH) and the hyperstriatum dorsale (HD). The hyperstriatum ventrale (HV) contained weak silver grain label. The neostriatum (N), contained only very light label, primarily at caudal levels. In addition, a patch of light silver grain label could be observed within the dorsal portion of the intermediate and posterior divisions of the dorsal archistriatum, as well in the olfactory bulb and tubercle (BO and TO). LoC projections were bilateral, but all projections were far heavier in the hemisphere ipsilateral to the injection site.

TP projections reach primarily the PA, particularly its rostral and lateral components. Silver grain label was also found in LPO, but label was light here compared to that found following the LoC injections. The LPO label in TP cases was most pronounced in its rostral and lateral segments. Accumulations of silver grains could also be seen over INP, Ac, SL, SM, HV, HD and BO neurons. In addition, a consistent patch of silver grain label was seen ventral to PP, INP and LPO, mixed among the fibers of the lateral forebrain bundle. A streak of fine silver grain label could also be seen in the dorsal portion of the intermediate and posterior dorsal archistriatum.

AVT injections revealed a different pattern of telencephalic projections. In these cases the heaviest silver grain label accumulations were observed in the medial and ventral components of LPO, within Ac, in the diagonal band of Broca (FDB), the region ventral to PP and INP and the olfactory tubercle (TO).

Although the projections of LoC, TP and AVT neurons do overlap, each of these cell groups shows unique and distinct patterns of projection upon the avian telencephalon.

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215.1 ONTOGENY OF SPONTANEOUS UNIT ACTIVITY IN THE BASAL GANGLIA OF THE CAT. N.A. Buchwald, R.S. Fisher, M.S. Levine, and C.D. Hull. MRRC and BRI, Sch. Med., UCLA, Los Angeles, CA 90024.

In the course of a series of studies on the development of the basal ganglia in the cat, spontaneous extracellular single unit activity was recorded from neurons located in the caudate nucleus (498 units, 15 cats), internal and external pallidum (entopeduncular nucleus and globus pallidus, 138 units, 23 cats) and substantia nigra (249 units, 24 cats). Data were obtained from acutely prepared anesthetized (Halothane-H_2O) cats ranging in age from neonates to adults. Results indicate that 1) neuronal activity in the cat basal ganglia increases as a direct function of age, 2) spontaneous neuronal activity becomes more patterned as a direct function of age, and 3) maturation of spontaneous unit activity in the pallidum and nigra precedes maturation of spontaneous unit activity in the caudate nucleus.

Spontaneous unit activity was analyzed by computation of unit mean interspike intervals (ISI's). In all nuclei of the basal ganglia, ISI's showed a two to threefold decrease between 1-20 days of age and adulthood. Caudate units fired slowly throughout life (group $\bar{X}$ ISI = 4435 msec in 41-80 day old cats, 1694 msec in adults). In contrast, pallidal and nigral unit ISI's decreased markedly prior to 41 days of age, and adult ISI's were much lower in these structures than in the caudate nucleus. In 1-20 day old kittens, pallidal units had a group $\bar{X}$ ISI = 889 msec (346 msec in adults) while nigral units had a group $\bar{X}$ ISI = 1897 msec (589 msec in adults). Burst (3 spikes/10 msec) occurence was analyzed as an indicator of firing patterns. Proportions of units that displayed bursts increased with age in all nuclei of the basal ganglia. In the caudate nucleus, bursts were found in 23% of units in 40-180 day old cats (48% in adults). Pallidal and nigral units showed both a greater tendency to burst in adults, and a greater maturational increase in the amount of bursting. In the pallidum, 5% of units had bursts in 1-20 day old kittens (65% in adults). In the nigra, 13% of units had bursts in 1-20 day old kittens (72% in adults).

When group $\bar{X}$ ISI's were compared across the basal ganglia at each age level, the pallidum and nigra developed in a parallel fashion and were functionally mature by 40-180 days of age. The caudate nucleus lagged behind its target nuclei in rate of maturation since adult ISI levels were not attained prior to 180 days of age. However, the ontogeny of burst patterns was similar for all structures tested and was immature prior to 180 days of age. This suggests that the development of unit firing patterns in the basal ganglia is dependent on the maturation of neuronal activity in the caudate nucleus. (Supported by USPHS HD 05958 and 1T32MH15345).

215.2 BEHAVIORAL EFFECTS OF CHRONIC D-AMPHETAMINE ADMINISTRATION IN DEVELOPING KITTENS. M.S. Levine, C.D. Hull, N.A. Buchwald. Mental Retardation Research Center and Brain Research Institute, School of Medicine, UCLA, Los Angeles, CA 90024.

These experiments were designed to determine the behavioral effects of chronic administration of d-amphetamine in kittens. Fifteen kittens (from 5 litters) received injections of d-amphetamine (5mg/kg i.p.) every other day from about 10 days of age until 60 days of age. Five control animals (1 from each litter) received a similar schedule of saline injections. One of the first effects observed was reduced weight gain by the drug-treated animals compared to saline-treated controls. The d-amphetamine-treated animals were accepted by their mothers and seemed to nurse normally but they did not gain as much weight as their littermate controls. This effect was transient and by 35 days of age there were no differences in body weight between the groups. The behavior of all kittens was monitored after the drug injections to determine if amphetamine produced differing responses when administered at different ages. The types of behaviors occurring after the injections were rated every 3 min for a 15 min period 45 min after the injection. The type of behavior evoked by the d-amphetamine differed according to the age of the kitten. In kittens under 30 days of age the most consistent behavioral response was licking of the forelimbs or floor of the observation chamber. Another response occurring frequently in this age range was slow side-to-side head movements. Saline-injected kittens under 30 days vocalized intensely and frequently for the first 5-10 min of the observation period after which time they became inactive and usually slept. D-amphetamine-treated kittens did not vocalize as frequently but remained active for the duration of the test and for several hours after the injection. After 3 weeks of age d-amphetamine produced marked increases in locomotor activity accompanied by rapid side-to-side head turns. The kitten would walk in one direction, turn its head to one side, then walk in that direction, etc. Behavioral effects of amphetamine up to 5 weeks of age were not observed on the day after injection. After 5 weeks of age d-amphetamine-treated kittens were showing behavioral alterations (stereotypies, locomotor activity increases) on the day following the injection. Thus, there appeared to be a prolonged sensitivity to the repeated injections with increasing age. At about 7 weeks of age d-amphetamine induced vertical and horizontal head movements accompanied by visual tracking. This response is similar to that observed in adult cats. These experiments demonstrate that the behavioral effects of amphetamine administration are age-related in the developing kitten. The adult-like response does not emerge until the animals are almost 7 weeks of age. (Supp. by USPHS HD05958, RR05756, NS12324).

215.3 DEVELOPMENTAL LOCOMOTOR ACTIVITY IN RATS TESTED OVER CLEAN VS. HOME CAGE BEDDING. Judy Buelke-Sam and Carole A. Kimmel* Division of Teratogenesis Research, National Center for Toxicological Research (FDA), Jefferson, AR 72079

Many species that undergo extensive postnatal development display a characteristic pattern of locomotor activity during the developmental period. Campbell and associates have described the normal sequential increase and decrease in activity that occurs in developing rats under a variety of environmental conditions. It is also established that perinatal exposure to agents such as lead, carbon monoxide, x-irradiation and ethanol can result in enhanced and/or prolonged juvenile hyperactivity. These findings suggest that measurements of developmental activity could provide a sensitive and early indicator of toxicity following perinatal insult. In this study, litters from 23 CD rats were used to characterize developmental activity patterns obtained in stainless steel chambers equipped with a single, height-adjustable photobeam detector. The chambers had wire mesh flooring 2.5 cm above a tray which contained clean shavings (C) or an equal volume of shavings removed from each pup's litter cage (HC). They also contained removable partitions, and measured 20 cm x 26 cm with partitions in place, 38 cm x 26 cm with partitions removed. At birth (day 1), litters were culled to 8 pups, 4 of each sex, and 2 of each sex were assigned to each testing condition (C vs. HC). Six litters were tested in the small chamber every second day from postnatal days (PND) 10-28. The other 17 litters were tested on PND 12, 16, 20 and 24. All offspring were tested at 30 and 60 days over C in the large chamber. Animals were weighed following each 30 min session and also checked for the appearance of physical landmarks.

A developmental hyperactivity phase in isolated juvenile rats tested repeatedly in a new apparatus has been confirmed in this study. In C rats of both sexes, and in HC females, short-term activity levels peaked at PND 16. However, HC males displayed a prolonged and even greater elevation in activity from PND 12-16. This overall pattern was found in rats tested either every second day or every fourth day. These data contrast with those reported by Campbell and associates in two respects. First, they reported that the degree of activity was inversely related to the similarity between testing and home environments. We found an increase in activity in the presence of HC olfactory cues, and a shift to earlier, rather than later ages in peak activity levels in a "more familiar" environment (c.f., Campbell and Raskin, J. Comp. Physiol. Psychol., 1978). Second, in this study different patterns of hyperactivity were noted in male vs. female rats; males were more activated in the presence of HC olfactory cues. Testing over HC bedding in this apparatus vs. on HC litter itself may account for the differences reported here.

215.4 HOME ORIENTATION IN NORMAL AND IN D-AMPHETAMINE TREATED RAT PUPS. B. Weston* and J.A. Sechzer. Dept. of Psychiatry, Cornell Univ. Med. College and E.W. Bourne Behavioral Research Lab., The New York Hospital, White Plains, N.Y. 10605.

Home orientation by rat pups has not been investigated. Fleischer and Turkewitz (1978) measured the frequency of returns to the home area of the nest by rat pups. They did not observe the home orientation response where the rat pup either enters the home area of the nest and remains there for a long period or does not leave the home area if placed there initially. This is different from the behavior of other developing altricial species, like kittens, which show highly specific orientation to the home area of their cage.

The present study investigated orientation to the home area of the nesting cage by normal, saline treated, and d-amphetamine treated rat pups. Amphetamine treated pups were injected daily with 2.5 mg/kg/b.w. I.P. of d-amphetamine from 3-21 days of age. Home orientation was individually tested in each rat pup for 2 minutes a day and the pattern and locus of their activity was traced. Home orientation by both normal and saline treated pups was absent and they showed a diffuse pattern of exploration throughout the test sessions. The amphetamine treated pups, by 9-10 days of age, showed a highly specific pattern of home orientation which was maintained until they were 14-15 days old.

These observations suggest that home orientation may be a normal, adaptive behavior in developing rats but that exploration and/or hunger may conflict and interfere with its performance. D-amphetamine, which inhibits exploration in rats and also produces anorexia, may have diminished or suppressed these behaviors and facilitated home orientation behavior.

215.5 NEURAL AND BEHAVIORAL TOXICITY OF TRIETHYLTIN IN DEVELOPING RATS. Patricia Ruppert*, Glendora Heavner*, Karen Kidd* and Lawrence Reiter* (SPON: M. Gage). Neurotoxicology Division, Health Effects Research Laboratory, U. S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Triethyltin (TET) is a highly toxic biocide and industrial catalyst which inhibits oxidative phosphorylation and produces cerebral edema and myelinopathy in the adult animal. We have shown that TET exposure in the adult rat produces a reversible hypoactivity, impairment of neuromotor function, and, at higher doses, hindlimb paralysis. However, little is known about the behavioral and neurotoxic effects of TET in the developing animal. In the present study, rat pups received a single injection of either 0, 3, 6, 9 or 12 mg/kg of triethyltin bromide on Day 5 of postnatal life. Most of the pups receiving 9 or 12 mg/kg died before weaning; histological examination of the brains of survivors indicated extensive damage to the pyriform cortex and amygdala, with shrinking of the dorsal hippocampus and enlargement of the lateral ventricles. For 0, 3 and 6 mg/kg TET-treated pups, whole brain levels of tin, zinc and calcium were analyzed 12 hrs, 24 hrs, 5 days and 10 days after injection to determine the kinetics of tin in developing pups. Pups receiving these lower doses were also tested for behavioral toxicity. Postnatal exposure to TET resulted in permanent hyperactivity in a figure-eight maze which persisted from day 28 through day 258 of age. These morphological and behavioral data indicate that developing animals are uniquely susceptible to the toxicity of TET: acute exposure on Day 5 produces specific brain lesions which are not seen even at lethal doses in the adult, and also produces permanent alterations in behavior.

215.6 EFFECTS OF PERINATAL EXPOSURE TO CHLORDIAZEPOXIDE ON LOCOMOTOR ACTIVITY AND ACTIVE AVOIDANCE IN THE RAT. P. M. Adams. Deptment of Psychiatry & Behavioral Sciences, University of Texas Medical Branch, Galveston, Tesas 77550.

The effects of perinatal exposure to chlordiazepoxide (1 mg/kg) on post-weaning active avoidance acquisition and extinction were studied in F344 rats. Pregnant primaparous females were treated daily on Days 1-21 of pregnancy with chlordiazepoxide or saline (IP). Lactating females were treated with chlordiazepoxide or saline on postnatal Days 1-21. Active avoidance learning and extinction were examined in all litters at thirty days of age. Open field activity was assessed on Days 14 and 21.

Prenatally exposed offspring of either sex were significantly more resistant to extinction compared to the saline-exposed pups. This difference was not due to an increase in spontaneous activity as there were no differences between the prenatal chlordiazepoxide and saline exposed pups. The postnatally exposed chlordiazepoxide pups were also significantly more resistant to extinction than post-natally exposed saline pups. Open field activity was significantly lower on post-natal Days 14 and 21 in the post-natally exposed chlordiazepoxide pups.

There was a significantly greater number of avoidance responses in extinction and faster extinction latencies in the post-natally exposed chlordiazepoxide pups compared to the prenatally exposed chlordiazepoxide pups. These findings support the hypothesis that perinatal exposure to chlordiazepoxide can cause significant alterations in the post-weaning behavior of the offspring regardless of gender.

215.7 EFFECTS OF FETAL TASTE/ODOR CONDITIONING ON NIPPLE PREFERENCE TESTING. G. Stickrod* , D.P. Kimble, Department of Psychology, University of Oregon, Eugene, OR 97403 and W.P. Smotherman, Department of Psychology, Oregon State University, Corvallis, OR 97331.

Taste and odor aversion conditioning have been demonstrated in rats in the first few days of postnatal life. Data to be presented demonstrate that such associations can be formed when fetuses are presented with a compound taste/odor stimulus via its injection into the amnionic fluid, and then injected with lithium chloride (LiCl). Conditioning took place with 20 day old fetuses from Sprague-Dawley female rats. Dams were anesthetized at 20 days of gestation and the uterine horns elevated and exposed through a midline laparotomy. A 26 ga needle was placed within 1 mm of the nose/mouth of the fetus and the amnionic fluid was injected with either saline or apple juice (.04ml). Following the last amnionic injection, each fetus was injected i.p. with LiCl (.02 ml/.15M) or saline. In the Experimental Group (2 mothers) N= 19 pups received apple juice followed by LiCl (AL), while Control Groups (1 mother) N=8 pups received apple juice followed by saline (AS) or (2 mothers) N=22 pups received saline into the amnionic fluid followed by saline i.p. (SS). All injections were accomplished under visual guidance. Females were sutured to close the incision and subsequently delivered pups on the 22nd or 23rd day of gestation. Nipple preference testing of the conditioned fetuses took place on postnatal day 16. Pups were removed 8 hr prior to testing. Dams were anesthetized and placed in a goal box at the end of a 7 x 15 cm plexiglass runway maintained at 35° C. Pups were allowed to approach and suckle until attached to one nipple for 15 sec. Approach trials were repeated until 10 were complete at which time the preferred nipple and at least four others were painted with apple juice. All other nipples were painted with saline. Pups were then run an additional 10 trials and scored for preferences for either apple juice or saline painted nipples. Results indicated that the AL Experimental Group preferred the saline painted to the apple juice painted nipples whereas both the AS and SS Control Groups showed preferences for apple juice over saline painted nipples. Preference for the saline painted nipples shown by AL pups when compared to AS or SS pups was significant by a t-test ($p < .05$) whereas the AS and SS pups did not differ. These data demonstrate in utero conditioning of an aversion to a compound taste/odor stimulus in the fetal rat. This association is a lasting one as evidenced by the nipple preference data collected on day 16 of postnatal life.

215.8 PRENATAL ADMINISTRATION OF KANAMYCIN: EFFECTS ON BEHAVIORAL DEVELOPMENT IN MICE. V. P. Perdue, P. J. Donovick and R. G. Burright*. Dept. of Psychology and Center for Neurobehavioral Sciences, SUNY Binghamton, Binghamton, N.Y. 13901.

The aminoglycoside antibiotics, including gentamicin and kanamycin, are widely prescribed to humans. Most research has focused on their major side effects, ototoxicity and nephrotoxicity, but have ignored possible concomitant behavioral consequences. We have recently shown significant changes in a variety of behaviors including aggression and maze performance. The present investigation examined the behavior of mice from birth to weaning at 21 days of age following prenatal administration of kanamycin.

Virgin female Binghamton heterogeneous stock (HET) mice were housed with sexually inexperienced HET males in standard cages containing nesting material. Females received daily, subcutaneous 400 mg/kg injections of kanamycin, or equal volume (.1ml/10 gm of body weight) injections of normal saline from the day following introduction of the male into the breeding cage until pups were born (mdn=21 days). Neither males nor pups were injected at any time.

Litters were culled to not more than 8 pups with an equal number of males and females wherever possible. On even days postpartum, litters were weighed and their righting reflex was tested. On days 8, 10, 12, and 14 postpartum, each pup's latency to return to the nest in the mother's absence was assessed. At 21 days of age, a 3 min. open field test was conducted under red light with a white strobe light during the second minute. The animals were then tested on a rotating rod to assess vestibular functioning.

Kanamycin administration during gestation altered pup behavior during the first 3 weeks of life. For instance, pups whose mothers were given kanamycin returned to the nest in the absence of their mothers at an earlier age than did their control counterparts. This finding parallels the nest return behavior of genetically deaf pups. Disruption of vestibular functioning following prenatal administration of kanamycin was suggested by later acquisition of the righting reflex and shorter latency to fall from the rotorod. In addition, male mice whose mothers were given kanamycin crossed fewer squares in the open field than did the other groups.

Thus, prenatal administration of kanamycin appears to affect several aspects of behavioral development in mice. In particular, behaviors related to vestibular functioning and exploration of the environment seem to be altered. Our findings point to the importance of continued investigation of the behavioral consequences of this medically important antibiotic.

215.9 OLFACTORY AND VOMERONASAL SYSTEM MEDIATION OF MATERNAL RECOGNITION IN THE DEVELOPING RAT. Martin H. Teicher, Bennett A. Shaywitz, and Augustus R. Lumia*. Section of Pediatric Neurology Yale Univ. School of Medicine, New Haven, Ct. 06510 and Skidmore College, Saratoga Springs, N.Y. 12866.

Recently developed procedures have permitted the precise quantification of a rat pup's immediate arousal response to its' mother or other test stimuli (Teicher & Shaywitz, Physiol. Behav. 23: 481, 1979). Using this apparatus, we demonstrated that 8-9 day old pups emitted 16% more vibrations (a measure of activity) toward their natural mother's ventrum than toward the ventrum of a lactating female with pups of the same age. They also emitted 36% fewer vibrations to a virgin females ventrum, 61% fewer vibrations to a male rat's ventrum, and 88% fewer vibrations to a wooden block or plastic container. Thus, indicating that this was a useful means for studying maternal recognition.

The contribution of both main olfactory bulb (MOB) and vomeronasal (VMN) systems to this capacity were assessed on a set of stimuli that normal pups grade with greater than 85% accuracy (lactating female's ventrum > lactating female's dorsum > heating pad). Pups receiving bilateral olfactory bulbectomies (BOB) (see Teicher et. al., Physiol. Behav. 21: 553, 1978) failed to grade these 3 stimuli when non-deprived, and they emitted many fewer responses to the conspecifics then sham or unilaterally lesioned pups (UOB). When deprived for 24 hours BOB pups responsed differentially to these stimuli, but they emitted their greatest response to the dorsal surface. Sham and UOB pups invariably emitted their maximal response to the ventral surface. Furthermore, the maximal response of the BOB pups was always less than half the maximal response of controls.

Pups receiving selective VMN nerve sections at 5 days of age graded their responses in the same fashion as BOB pups; emitting their maximal response toward the dorsal surface. However, unlike BOB pups, their maximal response was much more vigorous, and was comparable in magnitude to the maximal response emitted by the sham controls. Further studies suggested that the dorsal surface preference of these lesioned pups was tactually mediated, and based on the presence of longer hairs on the dorsal surface.

Overall, destruction of the VMN system with preservation of the MOB system disrupted only the pups' discriminative abilities, without affecting their level of responsiveness, or their ability to suckle. Destruction of both systems, as seen in BOB pups, disrupted discrimination, markedly diminished maternally-directed arousal levels, and produced serious impairments in suckling behavior.

215.10 DEVELOPMENTAL CHANGES IN HEART RATE CONDITIONED RESPONSES IN THE CAT (FELIS CATUS). G.E. Wolfe* and S.S. Soltysik*. (SPON:) S. Eiduson. Mental Retardation Research Center and Department of Psychiatry, UCLA, Los Angeles, CA 90024.

Heart rate (HR) responses to a 5 sec conditioned stimulus (CS, air blow to the back of the cat) paired with a brief electric shock (300 msec, 4 ma, 60 HZ), delivered to the left foot and the tail, show a single pattern in 1 week (N=5) and 4 week (N=7) old kittens, but several patterns in 12 week old (N=9) and adult cats (N=12). Control subjects (N=3, 4, 6, and 4, in the same age groups), which received only unpaired air CS and shock on separate trials (pseudoconditioning), showed much less (in younger subjects) or no HR responses (in adults) to the air blow CS. The infantile pattern of a HR response consists of a deceleration reaching a peak after one sec, followed by a return to or above baseline before the shock. Several patterns seen in adult cats included: pure acceleration, pure deceleration, and combinations of both. These individually specific patterns appear after a few days of training and remain stable over many months.

The emergence of individual specificity in conditioned HR responses to the signal of shock occurs between the 4th and 12th week of postnatal development.

The diversity of conditioned HR responses to the aversive CS in cats has not been noticed in previous studies. It is not known in other experimental animals (except for dogs when weak shock or a trace conditioning procedure is used: Black, A.H. et al., Psychol. Monogr. 76: No. 548, 1962). Cats may be unique animal subjects responding, like Humans, with a variety of autonomic (emotional) patterns to stressful stimuli and thus provide a useful model for developmental studies on genetic and experiential contributions to individual specificity. (Supported by USPHS HD05958)

215.11 BEHAVIORAL EFFECTS OF AMPHETAMINE IN ANIMALS TREATED NEONATALLY WITH 6-HYDROXYDOPA OR 6-HYDROXYDOPAMINE. Jack H. Mclean, Dorothy N. Morgan and Richard M. Kostrzewa. Department of Psychology, University of New Orleans, New Orleans, LA 70122.

Litters of rats were treated within 4 hr of birth with IP injections of either diluent saline (0.85%)-ascorbic acid (0.1%), 6-hydroxydopamine (6-OHDA) (60 µg/g), or 6-hydroxydopa (6-OHDOPA) (60 µg/g). The 6-OHDOPA group received a second injection 48 hr later. The animals were tested for general activity in 11 15-min sessions, beginning on Day 5 of life and continuing at three-day intervals through Day 35. Amphetamine sulfate injections (2 mg/kg, IP) preceded each activity session by 30 min. Activity was compared to animals from a previous study (1), treated identically, except for the amphetamine injections.

Treatment with amphetamine resulted in large increases in activity for all groups. When general activity was broken down into exploratory vs self-directed behavior, it became evident that the activity increment was due primarily to increased exploratory behavior. Number of self-directed responses, however, was decreased by the amphetamine treatment.

References:
1. Dorothy N. Morgan, Jack H. McLean, and Richard M. Kostrzewa. Effects of 6-hydroxydopamine and 6-hydroxydopa on development of behavior. Pharmacology, Biochemistry, and Behavior, 11, 1979, 309-312.

215.12 PRENATAL DEVELOPMENT OF THE SPINAL TRACT OF V AND ITS BEARING ON SPONTANEOUS AND EVOKED ACTIVITY IN RATS. C.H.Narayanan and Y. Narayanan*. Dept.Anat., LSU Sch.Med., New Orleans, LA 70119.

The spinal tract of the trigeminal nerve in rat fetuses was studied in order to understand the neural basis for motor activity patterns involving the head, neck and forelimbs during normal development. Albino rats (Holtzman) from 12-20 days of pregnancy were used. Maternal spinal anesthesia was accomplished by chemomyelotomy at the level of L_1-L_2. Laporotomy was performed and the uterine horns were exteriorized. Recordings of the frequency of movements of the head, forelimbs and oral regions in various combinations were made using an Esterline Angus Event recorder. Evoked activity was studied at each stage using an esthesiometer with fetuses 'ex utero' (Narayanan et al 1971). Fetuses from each age group were sacrificed and processed for paraffin section ing and stained according to the reduced silver method.

The response to exteroceptive stimulation of the perioral region is weak at 16 days of gestation. From then on the fetuses show a progressive increase of response to stimulation of the tri geminal area with clear head extension and forelimb flexion. At 19 days, stimulation of the perioral region produces pronounced trunk movements combined with head movements. The head is turned towards the side of stimulation. Fasciculus gracilis can be iden tified at upper cervical levels beginning from 16 days onwards. At 19 days it is prominent and clearly delimited by the dorsal intermediate septum. At 16 days, the maxillomandibular and ophthalmic divisions of the spinal tract of V appear to have grown into the first and second cervical segments, and at 19 days, the spinal tract is proportionately large and extends as far as C_4. The changing nature and extent of these responses to stimulation can be correlated with the progressive development of the spinal tract of V and of the motor neurons of cervical levels. The morphological changes observed in these centers during development are now being studied at light and EM levels. Supported by the National Institutes of Health-National Institute of Child Health and Human Development. RO1 - HD12064.

215.13 EFFECTS OF CHRONIC CAFFEINE IN DRINKING WATER OF RATS ON NEONATAL DEVELOPMENT AND ON MATERNAL AND OFFSPRING BEHAVIOR. G. L. West†, T. J. Sobotka, R. E. Brodie† and J. M. Beier† Div. of Toxicology, Bureau of Foods, FDA, Washington, D.C. 20204.

Caffeine in concentrations of .0125, .025, or .05% in drinking water was given to pregnant female rats (FDA Osborne-Mendel) throughout gestation and lactation and to their offspring after weaning. Based on body weights and water intake data, dose levels were 17, 31, or 58 mg/kg/day for dams during gestation; 31, 63, or 115 mg/kg/day for dams during lactation; 13, 29, or 54 mg/kg/day for postweanling males; and 20, 41, or 84 mg/kg/day for postweanling females.

Behavior was assessed in dams during gestation and lactation. Physical and neurobehavioral profiles of development were obtained for preweanling pups and subsequent behavioral tests were made on postweanling adolescents.

Water intake was increased ($p<.05$) in the .0125% dams during the second and third weeks of gestation only. Body weight and food intake were relatively unaffected in dams. Photocell activity (PAC) was increased ($p<.05$) during gestation in .025 and .05% dams and was decreased ($p<.05$) during lactation in .0125% dams. Open field (OF) activity was increased ($p<.05$) in .05% dams during gestation only. Pup-retrieval latencies decreased with dose on day of parturition and increased with dose on day 12 of lactation, but neither was significant ($p>.05$). Adjunctive water intake during absence of the pups increased ($p<.05$) in the .025% and .05% dams.

Caffeine at the levels used did not affect length of gestation, litter size, sex ratio, or pup mortality, but did decrease ($p<.05$) body weights in .05% male offspring from birth throughout the 18-wk study. Incisor eruption was delayed ($p<.05$) in both sexes at .025% and .05% and in females only at .0125%. Onset of auditory startle was delayed ($p<.05$) in .05% males and females. Other indices of physical (pinnae, eyes, testes descent and vaginal opening) and behavioral (righting, PAC, OF, swimming) development were relatively unaffected.

Postweanling water intake was increased ($p<.05$) in .025% males and females. Shuttlebox avoidance (CAR) performance was modified ($p<.05$) in the .05% male offspring but was not affected in the females. Postweanling activity measures (PAC, OF, circadian) were relatively unaffected while hypermotility induced by d,l-amphetamine (AMP) was reduced with 1.5 mg/kg AMP and enhanced with 3.0 mg/kg AMP.

Results will be discussed in terms of reported effects of caffeine on endocrine and metabolic systems.

215.14 BEHAVIORAL AND BIOCHEMICAL EFFECTS OF INFANTILE NUTRITIONAL AND ENVIRONMENTAL TREATMENTS. L.S. Crnic, J. Bell*, R. Mangold*, M. Gruenthal*, J. Eiler* and S. Finger. Stolinsky Metabolic Resch Lab.,U. Colo. Med. Sch., Denver, CO 80262 and Dept. Psychol., Washington Univ., St. Louis, MO 63130.

Rats experiencing equivalent malnutrition but different environments were used to answer the question: To what extent are the behavioral and brain effects of infantile malnutrition due to the confounded environmental alterations? Pups separated from their dams for half of each day during the 1st 20 days of life were kept either in an incubator (I) or with a non-lactating, maternally primed female (A). At 20 days, the deficit in body weight was 38% compared to both normals (C) and a group which controlled for the handling involved in deprivation (HC) but declined to 11% in adulthood. Brains at 20 days revealed deficits in the amount of DNA and protein in cerebral cortex and brainstem in both experimental groups. Cerebellar protein was unaffected and DNA decreased only in the A pups. Cell size was reduced in both experimental groups in cortex and cerebellum but only for the A group in the brainstem. The two experimental groups differed from each other in protein concentrations in all brain parts. Thus, both nutritional and environmental manipulations affected brain biochemistry, with the A group the most affected. Although the direction of the effect was the same in the A and I groups, the environmental condition often determined whether the effect reached significance. Adult brains of similarly treated rats have revealed persisting effects.

Open field behavior (at 150,260,300 days in males, 150 and 300 days in females), response to sucrose solutions (males only), shock response thresholds, passive avoidance and spatial alternation maze performance were tested in adulthood. For males, the only behavioral effects were less rearing up on the hind legs in the open field by the A rats in the 1st and 2nd but not 3rd test with more vocalization on handling, and greater suppression of activity by the presence of a social stimulus in the open field compared to other groups. The A females were less active on the 1st day of open field testing, and, along with C females, more hesitant to respond prior to shock on the passive avoidance task The I females were less hesitant than C rats to respond on the 30 min. retention test on this task.

We conclude that the environmental variable influenced both behavior and brain biochemistry, with those more severely impaired biochemically showing more behavioral alterations. However, the magnitude and consistency of the behavioral effects wa unimpressive, especially in the light of the biochemical effects The extensive testing may have moderated any nutritional effects (Supported by grant HD08315)

215.15 ALTERATIONS IN ADULT BRAIN AMINE LEVELS RESULTING FROM NEONATAL HYPOXIA. M. K. Shellenberger, Dept. of Pharmacology and Kansas Center for Mental Retardation, Kansas Univ. Med. Center, Kansas City, KS 66103

The brain of the five day old rat is at approximately the same stage of development as the full term human fetus. Norton and Culver have shown that hypoxia, induced by carbon monoxide (CO), in the rat pup at this age produces hyperactivity in the maturing animal (Exp. Neurol. 50: 80, 1976) and altered caudate neuron morphology (Brain Res. 132: 455, 1977) when the rat reaches adulthood. This hypoxic model is now being used to determine if permanent changes in proposed neurotransmitter agents accompany the morphologic changes.

Infants were bred in our animal facilities. Litters born within 12 hrs were cross fostered with equal numbers of males and females and reduced to 8 pups within 48 hrs. At 5 days of age, groups of 4 pups were exposed to 0.5% CO in a constant flow of air at 4 L/min for 2 hrs at a chamber temp. of 24°C. Other animals were exposed to air alone under the same circumstances. A third group of animals were handled in the same way from birth but were not isolated from the dam nor placed in the chamber. All animals were sacrificed between 16 and 24 weeks of age. The brains were sampled and assays were performed for dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) in frontal cortex, striatum, midbrain and pons-medulla.

Both treatments were found to produce alterations in biogenic amine levels relative to the untreated group. The 2 hr isolation from the dam in air at 24°C caused increases in DA in frontal cortex and pons-medulla while NE was reduced in cortex, midbrain and pons-medulla. On the other hand, 5-HT was reduced in cortex and pons-medulla but increased in midbrain. CO exposure under these conditions generally tended to reverse or prevent the isolation-induced effects with notable exceptions. Cortical DA levels remained elevated and midbrain NE remained low in CO-exposed females. Effects on striatum were particularly striking as DA was reduced 28% in males and 33% in females while both NE and 5-HT were increased only in females.

These results indicate that a 2 hr isolation from the mother under hypothermic conditions is a sufficient stress in 5 day old rat pups to alter the course of neurochemical maturation in widespread brain areas. This proved to be a confounding factor in studies of hypoxia except in the striatum where CO-induced hypoxia produced clearly different effects. The relationship of these effects to other hypoxia-induced functional, morphologic and neurochemical changes remains to be established. (Supported by USPHS Grant MH 27739).

215.16 ALTERATIONS IN ADULT BRAIN AMINO ACIDS FROM NEONATAL TRAUMA. A. S. Kimes and M. K. Shellenberger, Dept. of Pharmacology, and Kansas Center for Mental Retardation, Kansas Univ. Med. Center, Kansas City, Kansas 66103.

Published studies have shown that hypoxia in the five day old rat, induced by carbon monoxide (CO), results in hyperactivity in the developing animal and persistent changes in caudate neuronal morphology. We have utilized 5 groups of five day old male and female rats to determine if there are persisting neurochemical changes as well. Pups were cross fostered and litters reduced to 8 within 48 hrs of birth. (1) Pups were exposed to 0.5% CO in a constant flow of air of 4 L/min for 2 hrs; chamber temp. 24°C. (2) Pups were exposed to air only for 2 hrs as above. (3) Pups were anesthetized with hypothermia and light ether exposure. The brain was exposed from the coronal suture to the olfactory bulbs and the cortical surface removed by suction from the midline laterally to the orbital area and arteriorly to the frontal poles. (4) Pups were anesthetized as above and the scalp and calvarium opened (sham operated group). (5) A control group was handled as above but not subjected to other traumas. All rats were sacrificed between 16 and 24 weeks old. Brain samples (cortex, striatum, midbrain and pons-medulla were assayed by the o-pthaldialdehyde reaction for Glu, GABA, Asp and Gln.

Significant treatment related effects were seen primarily in Glu and Asp with no significant alterations in GABA levels. The majority of significant alterations were confined to striatum and midbrain and occurred with greater frequency in the females. All treatments tended to increase striatal Glu, compared to the untreated group, in both sexes, with females showing a consistently larger response. Midbrain Glu was increased by operative procedures but not by the exposure conditions. Striatal Asp levels were markedly increased in both sexes by CO exposure and to a lesser extent by operative procedures in females. Exposures, particularly CO, increased midbrain Asp but lesions increased Asp only in males while the sham operation caused an increase in females which was reversed by lesioning. Significant alterations of Gln also occurred in striatum and midbrain.

The metabolic and/or morphologic bases of these changes in amino acid levels are not known. It is clear, however, that situations such as 2 hrs isolation from the mother with accompanying hypothermia are adequately stressful to result in permanent changes in brain amino acid levels. Furthermore, these effects are confounding when other treatments are superimposed. (Supported by USPHS Grant MH 27739. ASK was supported by an RSA (HD-07066).

215.17 BEHAVIORAL AND DENDRITIC SPINE DEFICITS IN MONOCULARLY DEPRIVED RATS: THE ROLE OF REDUCED PHOTIC STIMULATION. M.L. Schwartz and L.A. Rothblat. Dept. of Psychology, George Washington Univ., Washington, D.C. 20052.

Monocular deprivation in the rat significantly reduces the density of dendritic spines in visual cortex contralateral to the deprived eye (Fifkova, J. Comp. Neurol., 140, 1970) and severely impairs the ability of these animals to discriminate complex visual patterns (Rothblat, Schwartz and Kasdan, Br. Res., 158, 1978). Whether these deficits are the result of a reduction in the pattern content of visual stimulation or a decrease in the level of photic stimulation of the retina remains to be determined. The present study was designed to examine the role that levels of retinal stimulation play in the production of these deficits.

All behaviorally tested rats (n=40) were unilaterally lid sutured at 12 days of age (DOA), and then assigned to one of three identical rearing environments which varied only in the level of ambient illumination provided during the light portion of the 12h light/dark cycle. Groups 1 and 2 were reared in an environment with an ambient illumination of 200 lx (normal laboratory level) while groups 3 and 4 were exposed to illumination levels which were 1 and 1.5 log units greater, respectively. These levels were chosen based upon earlier measurements which revealed that lid suture in this species reduces photic retinal stimulation by 1 to 1.5 log units and as such the deprived eye of animals in these groups should receive a "normal" level of photic stimulation in the absence of pattern stimulation. At 45 DOA the rats in groups 2, 3 and 4 were reverse sutured and began behavioral testing using the originally deprived eye. Group 1 served as a control and began testing at the same time using the non-deprived eye. All animals were trained to discriminate between columns and rows of 5mm squares.

The results revealed that rats in groups 2, 3 and 4 were sig. impaired in the acquisition of the discrimination when compared with group 1, however, no difference in performance was found on this task between groups 2, 3 and 4.

Dendritic spine density was examined in four groups (n=20) of rats. Groups 2S, 3S and 4S were MD litter mates of behavioral groups 2, 3 and 4, while rats in group 1S were non-deprived and reared in the 200 lx environment. Spine density comparisons between group 1S and the deprived hemispheres of groups 2S, 3S and 4S revealed a sig. reduction in density for group 2S, but no sig. reduction was found for groups 3S and 4S.

These results suggest that while spine density in visual cortex may be sensitive to the level of retinal photic stimulation, perceptual capacity may be more dependent upon the level of exposure to visual pattern during development. (Supported by NIH Grant 9-R01-EY03304)

215.18 RECOVERY OF FUNCTION AFTER NEUROTOXIC DAMAGE TO THE HIPPOCAMPAL CA3 REGION: IMPORTANCE OF POSTOPERATIVE RECOVERY INTERVAL AND TASK EXPERIENCE. Gail E. Handelmann* and David S. Olton, Dept. of Psychology, The Johns Hopkins Univ., Baltimore, MD 21218

Rats with kainic acid induced damage to hippocampal CA3 pyramidal cells show impaired postoperative retention of spatial maze tasks, with eventual relearning. This experiment evaluates the importance of the length of the postoperative recovery interval and the extent of task experience in facilitating the relearning.

Rats were trained on a T-maze rewarded alternation task, and then given either kainic acid (experimental) or saline (control) injections into the hippocampal CA3 region. Half the rats in each group began postoperative testing 5 d. after surgery; the rest began 30 d. after surgery. Of the rats beginning retesting 5 d. after surgery, half received one daily test session and half received two daily test sessions. The same was true for the rats beginning retesting 30 d. after surgery.

	Days to Criterion			
	5 d. Recovery Interval		30 d. Recovery Interval	
	Kainic Acid	Control	Kainic Acid	Control
One Test Session Per Day	27.7	3.0	16.6	2.0
Two Test Sessions Per Day	13.0	2.9	15.2	2.7

All groups of control rats required a similar number of days to reach criterion. Neither the length of the postoperative recovery interval nor the number of daily test sessions affected the amount of time necessary to reach criterion performance. The number of days of experience was more important than the actual amount of experience.

All experimental groups performed worse than the controls, but all rats eventually relearned the task. The worst performance was shown by the group tested once a day beginning 5 d. after surgery. Performance could be improved significantly by either increasing the amount of task experience or by lengthening the recovery interval. For added experience to improve performance, however, it had to be administered immediately after surgery.

215.19 EFFECTS OF HIBERNATION AND DIFFERENTIAL ENVIRONMENTS ON WEIGHTS AND NUCLEIC ACIDS IN BRAINS OF BELDING'S GROUND SQUIRRELS. Mark R. Rosenzweig, Edward L. Bennett, Paul W. Sherman*, Marie H. Alberti*, Dept. Psychol. and Lawrence Berkeley Lab., Univ. of Calif., Berkeley 94720.

We studied brain development in juvenile Belding's ground squirrels during hibernation in a cold room at 5° C and awake in a laboratory at 20° C. The animals were live-trapped near Tioga Pass, CA. 9/13-14/79 at about 80 days of age. Some were sacrificed for baseline values on 9/18. Others were put into the experimental conditions on 9/21/79 and were sacrificed for brain analyses 2/20/80 at 8 mo of age. During the 5 mo in experimental conditions, the squirrels that remained awake continued to grow in brain weight (+ 5%), body weight, and skeletal development; the hibernators lost large amounts of body weight and decreased only slightly in brain weight (- 2%). The differences in brain weights between hibernators (H) and non-hibernators (NH) were not statistically significant in cerebral cortex but were significant in the rest of the brain and in several specific regions, including the hypothalamus, caudate nucleus, and medulla. Weight differences in brain regions between hibernating and non-hibernating conspecifics do not appear to have been reported previously. In nucleic acid measures, H were significantly lower than NH in total DNA of the olfactory bulbs, hypothalamus, caudate nucleus and medulla, indicating loss of cells; in the latter 3 regions the percentage loss in DNA equalled the percentage loss in weight. Total RNA did not differ between H and NH, but in RNA/wt H exceeded NH significantly in almost every brain region measured. This greater specific RNA of the hibernators runs contrary to some published reports, and this indicates the need for further research which we are pursuing.

We also substantially replicated our study reported at the 1979 Neuroscience meeting (abstract no. 2160) showing that laboratory-born juvenile Belding's ground squirrels assigned to an enriched laboratory environment (EC) developed brain measures similar to those of feral squirrels and significantly greater than those of conspecifics assigned to an impoverished environment in individual cages (IC). Thus, both hibernation and differential experience are demonstrated to produce significant effects on brain weights and brain nucleic acids.

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215.20 ENRICHED ENVIRONMENTAL REARING INCREASES NEURAL EXCITABILITY AND IMPAIRS BRIGHTNESS DISCRIMINATION LEARNING. S. Kilchenmann*, P.B. Feuerstein*, V. Wolfson*, and W. Fishbein. Psychobiology Laboratory, Dept. of Psychology, The City College of the City University of N.Y., New York, N.Y. 10031.

Considerable research has focused on behavioral, morphological and chemical differences as a function of differential rearing, with the general assumption that enrichment leads to increased complexity and behavioral adaptability. The present experiments add another dimension, that of neural excitability changes resulting from differential rearing.

One hundred thirty four male albino mice (Carworth Farms) are reared for 5 weeks post-weaning in either enriched (EE), social (SE), or isolated (IE) environments. At 56(±2) days of age, subjects are removed. Experiment 1: Subjects are trained on 5 consecutive days in a brightness discrimination task (Y-maze). Results indicate that EE's perform significantly worse on 3 recorded measures (Correct avoidances, Avoidances and Correct discriminations) beginning on day 3 of training, as compared to both SE's and IE's, which do not differ from each other. Experiment 2: Immediately after removal from their respective environments, subjects are administered transcorneal electroconvulsive shock. Duration of tonic and clonic phases and seizure thresholds (mA, rms) are determined. Results reflect the findings in Experiment 1; EE's show significantly lower seizure thresholds compared to SE's and IE's, which do not differ among themselves.

These findings challenge the notion that enrichment increases behavioral adaptability and indicate a correlative relationship between observed behavioral differences and brain excitability in differentially reared animals; the findings further suggest that enrichment in all likelihood leads to higher baseline excitation levels which, when combined with a task that engenders excitation, leads to an overload and subsequent breakdown of task performance.

215.21 PARADOXICAL DEOXYGLUCOSE UPTAKE IN LAYER A OF CAT LATERAL GENICULATE NUCLEUS (LGN) AFTER OPTIC CHIASM SECTION. C. Shaw*, S. Kirby*, F. Leporé, J. P. Guillemot and M. Cynader (SPON: J. Szerb). Dept. of Psychol., Dalhousie Univ., Halifax, N. S. and Dept. of Psychol., Université de Montréal, P. Q., Canada.

Optic chiasm section severs optic nerve fibers crossing to the opposite side of the brain. If this manipulation is performed in young kittens and one eyelid is sutured at the same time, however, the opened eye becomes the major route for effective visual stimulation of neurons near the 17/18 border on both sides of the brain. We sought an anatomic correlation of this phenomenon by applying the ^{14}C-2 deoxyglucose (2 DG) method to this preparation. Two kittens were subjected to optic chiasm section and monocular eyelid suture at 3 weeks of age. They were reared normally until 110-116 days of age when the deprived eye was removed in one of the kittens. The next day, 2 DG (100 µg/Kg I.V.) was injected. As expected, layer A_1 of the LGN ipsilateral to the normal eye was heavily labelled in this kitten. In the other hemisphere, however, layer A of the LGN was instead densely labelled. This labelling was nearly as dense as that found in layer A_1 of the LGN ipsilateral to the normal eye. This is surprising because the combination of enucleation and chiasm section should abolish all retinal input to the LGN ipsilateral to the deprived eye. To rule out incomplete chiasm section as the mechanism underlying these results, we repeated the experiment in another kitten but, in addition, injected ^{3}H-proline into the normal eye. 2 DG results were the same as previously and tritium label was found only on the side of the brain ipsilateral to the normal eye (layer A_1), confirming the completeness of chiasm section.

Two possible sources of the heightened glucose consumption in layer A of the LGN contralateral to the normal eye were considered. One candidate mechanism is a pathway for visual input as follows: normal eye-ipsilateral LGN-ipsilateral cortex-opposite cortex (via corpus callosum)-layer A of LGN (via corticothalmic fibers). To evaluate the functional efficiency of such a pathway, we recorded in the LGN contralateral to the normal eye in another kitten. Visual responses could not be elicited from layer A of this kitten although many cells showed spontaneous activity. The lack of visual responses makes it unlikely that the polysynaptic pathway mentioned above is the source of the heightened 2 DG uptake in layer A. Alternatively, the difference in activity between LGN layers A and A_1 ipsilateral to the deprived eye may reflect differences in the time after denervation for cells in the two layers. Since layer A was denervated in infancy (by chiasm section) and layer A_1 was denervated in adulthood (by enucleation) the increased activity in layer A may reflect different metabolic consequences of neonatal versus adult (or early vs. recent) denervation. Experiments are underway to test these hypotheses.

216.1 POSTNATAL DEVELOPMENT OF THE RAT ACCESSORY OLFACTORY BULB. L. Rosselli-Austin, K. Hamilton* and J. Williams*. Chicago State University, Chicago, Il. 60628.

The rat accessory olfactory bulb (AOB) was studied quantitatively under the light microscope during its development from birth to postnatal day 60. The following parameters were evaluated: total volume of the AOB and its component layers, mitral and internal granule cell numbers, cellular growth, cellular packing densities and granule to mitral cell ratio.

The total volume of the AOB increased 600% from the day of birth to its peak at postnatal day 18. Thereafter the volume declined: the 25- through 60-day age groups had significantly smaller volumes than the 18-day group (Newman-Keuls, $p < .05$). By day 60, the AOB volume showed a 34% reduction from its maximum value. Volumes of the individual layers of the AOB followed this same pattern of growth and decline with the exception of the internal plexiform layer whose volume did not decrease during the period studied. It grew at a moderate rate until day 18 when it reached a plateau, and then underwent a second period of growth between days 25 and 30.

The total number of mitral cells declined dramatically after day 18; by day 60 only 60% of the mean number of mitral cells extant from days 0 through 18 remained. Mitral cell density decreased rapidly during the first postnatal week and then more slowly during the following two weeks. Areas of projected outlines of mitral cell perikarya followed a pattern inverse to that of cell density.

The total number of granule cells rose 300% from days 0 to 18. There was a 24% reduction in number after day 18 but this was not significant. Cell packing density declined during the first week as granule cell diameters increased. Cell diameters of the 16- and the 18-day old groups were significantly larger than the 0- through 3- and the 60-day age groups. The granule to mitral cell ratio rose throughout the early postnatal period and reached an asymptote around day 20.

The postnatal development of the AOB contrasts in many respects with that of the main olfactory bulb (MOB)(Rosselli-Austin, L. and Altman, J. J. Develop. Physiol., 1:295, 1979). While the MOB exhibits tremendous growth and neuronal acquisition, the AOB shows more moderate growth. Moreover, the AOB appears to undergo involutionary changes around the time of weaning while the MOB does not. However, both the MOB and the AOB undergo mitral cell losses at about the same time.

216.2 DEVELOPMENTAL CHANGES IN THE TOPOGRAPHICAL DISTRIBUTION OF CELLS CONTRIBUTING TO THE LATERAL OLFACTORY TRACT. Marjorie R. Grafe and Christiana M. Leonard. Dept. of Neuroscience, University of Florida, Gainesville, Florida 32610.

Developmental changes in the locations of cells in the olfactory bulb which contribute axons to the lateral olfactory tract (LOT) were investigated using retrograde transport of horseradish peroxidase (HRP). HRP was placed in various parts of the olfactory tubercle, LOT, and/or piriform cortex of golden hamsters ages 3, 7, 8, and 20 days. The animals were allowed to survive 24 hours, and frozen sections were cut at 50μ, reacted with TMB, and counterstained with neutral red. The number of labeled mitral and tufted cells were counted on an average of 12 equidistant sections through the olfactory bulb.

In the younger animals (Days 3 and 7, n=10), there was a distinct localization of labeled cells in the medial quadrant of the bulb. A Friedman two-way analysis of variance showed a significant difference in the distribution of labeled cells among the quadrants ($p<.02$). A Wilcoxon test on this group showed that the number of labeled cells in the medial quadrant was greater than in each of the other quadrants ($p=.05$). The number of cells in the medial quadrant was approximately equal to that of the other three quadrants combined. Paired comparisons of the other quadrants showed no differences between them. In animals Day 8 and older (n=7) the cells were distributed equally over all quadrants. Between Days 3 and 20, the average perimeter of the mitral cell layer doubled, and the average number of cells labeled per section increased from 4.7 to 22.4. When pairs of brains from the different age groups with HRP injections of similar size and location are compared, the older brain always has more labeled cells. There was no relation of the number of labeled cells or the distribution of labeled cells to the location of the injection. In contrast, the number of labeled cells does correlate positively with the size of the injection.

We believe this is the first report of a developmental topographical gradient of the cells of origin of the LOT. No such gradient has been reported for cell birth day. This localization may be related to an uneven distribution of the olfactory nerve fibers entering the bulb, or to early topographical differences in functional activity.

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216.3 SENSORY DEPRIVATION AFFECTS THE DEVELOPING OLFACTORY SYSTEM OF MICE. T.E. Benson and J.W. Hinds. Dept. Anat., Boston University School of Medicine, Boston, MA 02118.

Frühwald (Arch. f. Ohren-, Nasen- u. Kehlkopfh., 139: 153-173, 1935) has reported striking qualitative differences in the olfactory epithelium and olfactory bulb (OB) consequent to unilateral nasal occlusion in newborn rabbits. The present study using unilateral, neonatal naris cauterization augments the work of Frühwald and updates our previous communication (Benson, T.E. and D.K. Ryugo, Soc. Neurosci. Abstr., 4: 108, 1978.)

Histological preparations of OB's from 30 day old experimental and control CD-1 mice have been used for morphometric analysis. The volumes of the left control OB's (C_L) do not differ from the right (n=6, paired t-test). In contrast, in the experimental animals the mean volume of the OB's on the deprived (D) side is 26% smaller than that of the non-deprived (ND) side ($P<0.001$, n=7, paired t-test). The volumes of the ND bulbs do not differ from those of the C_L. This suggests there is no compensatory hypertrophy of the ND bulbs. Based on paired t-tests of volumes, the following layers of the D bulbs are significantly smaller than the ND: Glomerular (GL), 24%, $P<0.01$; external plexiform (EPL), 35%, $P<0.01$; mitral cell (MCL), 23%, $P<0.01$; and granule cell (GCL), 29%, $P<0.02$. The olfactory nerve layer (ONL) is 12% smaller and the ventricular/subependymal zone (V) is 11% larger on the D side but not significantly. An analysis of variance comparing the effect of deprivation among layers indicated significant differences ($P<0.001$). An a posteriori analysis (Tukey (b)) showed that V differs from all other layers and EPL and GCL differ from ONL.

Preliminary counts of receptor knobs in 1 µm plastic sections of nasal septum show a magnitude of effect similar to that seen in the ONL. Cell counts in OB's will also use 1 µm sections. So far, in each of two OB's 14 mitral cell nuclei from comparable positions have been reconstructed from serial 1 µm sections. Mean nuclear volume is 639 μm^3 on the D side and 763 μm^3 on the ND with 5.0 and 3.2 nucleoli per nucleus respectively.

Tritiated deoxyglucose radiography shows decreased labeling of the D bulbs on postnatal days 20 and 30.

These results suggest a retardative effect of deprivation on development. For example, the smaller volume of OB layers compared with the large relative size of V, and the smaller size of mitral cell nuclei with a greater number of nucleoli is similar to an earlier developmental stage in control OB's or other developing systems. In addition, our examination of OB's embedded in paraffin and in plastic suggests there is a significant potential for undercounting mitral cells in paraffin sections, especially in the D bulbs. (Supported by U. Vermont GRS grant PHS 5429-40 and USPHS Research grant AG-00001.)

216.4 DEVELOPMENT OF OLFACTORY BULB ELECTRICAL ACTIVITY RECORDED CHRONICALLY FROM 3 TO 90 DAY OLD RATS. C. Robert Almli, William B. Forbes, Mark A. Henault,* Craig A. Velozo,* and Peter J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, Ma. 01545 and Psychol. Dept., Ohio Univ., Athens, Ohio 45701.

Bipolar stainless steel electrodes were chronically implanted into the main olfactory bulb (MOB) in male albino rats at 3, 4, 8, 10, 12, 14, 17, 19, 30, or 90 days of age. Electrical slow-wave activity was recorded from the MOB for approximately two hours each day for three successive days. The rats were returned to their home cages between recording sessions. Infant rats with chronic implants continued to suckle from their dam while in the litter cage, and rats of all age groups displayed normal growth over the three day recording period. The electrically shielded recording chamber was equipped with a removable wood-chip floor and a plexiglas window for observation of behavior. Fine wire leads were plugged into the electrode carrier and attached to a multicontact commutater. The electrical signal was recorded on a polygraph and FM tape recorder for computer analysis. The EEG signal was analyzed for frequency content using the Fast Fourier transform. Various 8-sec epochs were digitized at 256 Hz yielding power spectral values with .125 Hz resolution over a frequency range of 0-128 Hz. The electrical signal was also analyzed for respiration using filtering procedures. The behaviors of rats were scored on the polygraph trace as locomotion, moving in place, quiet resting-sleep, and active-REM sleep. Olfactory stimulation consisted of urine soaked home-cage nest material, an anesthetized lactating dam, and powdered rat food.

With and without specific olfactory simulation, two major frequency components were present within the MOB electrical signal, both of which increased in frequency during development. The lower of the two frequency components (which increased from approximately 14 Hz at 10 days of age to 25 Hz at 21 days of age) was correlated with respiration and sniffing suggesting that these were induced waves as defined by Adrian. The upper frequency component (which increased from approximately 40 Hz at 10 days of age to 63 Hz at 21 days of age) was not linked to respiration and therefore appeared to represent intrinsic waves of Adrian. The ontogeny of these frequency components of spontaneous electrical activity recorded from the MOB parallels the neurogenesis of MOB granule cells and the postnatal development of granule-to-mitral cell synapses in the murine MOB.
(Supported by NICHD grant HD 06364)

216.5 FUNCTIONAL ORGANIZATION OF MOUSE & RAT BARREL CORTEX FOLLOWING WHISKER DAMAGE ON DIFFERENT POSTNATAL DAYS. D.J. Simons, D. Durham and T.A. Woolsey. Dept. Anat. and Neurobiol., Washington U. Sch. Med., St. Louis, MO. 63110.

In mice and rats neonatal lesions of the facial vibrissae alter the anatomical organization of barrels in the contralateral SmI cortex. These effects depend on the pattern of whiskers damaged and the developmental age of the animals. To understand the functional correlates of these anatomical changes, the middle row of vibrissae (Row-C) was damaged in mice and rats on postnatal days 1,3 and 5 and the animals were studied as young adults. Using methods previously described (Simons, '78) over 700 single units were characterized in penetrations parallel and perpendicular to the pia; selected recording sites were marked with the dyes Alcian blue, fast green or HRP; electrode tracts were reconstructed from cortical sections with respect to the layer IV barrels and other cortical layers; and the extent of peripheral damage was verified histologically.

In general, the results from both species demonstrate that, as in normal animals, there is an orderly representation of the periphery which coincides with the cytoarchitectonic organization of the barrels. Specific findings were: 1) Units in the enlarged Row-B or Row-D barrels responded primarily to Row-B or -D whiskers (those next to the damaged Row-C). 2) In layer IV units in the altered Row-C zone either could not be driven from the periphery or were driven from tissue associated with the scar. This Row-C zone is wider the later the whiskers are damaged. 3) Units in supra- and infra-granular layers - which in normal animals are activated by a complement of adjacent whiskers - either behaved as if Row-C were absent or incorporated scar tissue in their receptive fields in a topologically correct fashion. Further, responses of these units to scar tissue were qualitatively similar to those elicited from the intact whiskers (e.g. excited or inhibited). 4) Units in SmII which responded to whiskers had receptive fields which paralleled the representation of the periphery found in SmI. 5) In no case was there mapping of non-mystacial pad representations in the barrel cortex, nor were there units with abnormal multiwhisker interactions when laminar boundaries were taken into account.

We conclude that although neonatal damage to the whiskers predictably alters somatotopy, the functional organization within barrel cortex remains largely unchanged.

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216.6 CHANGES IN MODALITY CONVERGENCE IN CELLS OF PARTIALLY DEAFFERENTED DORSAL HORN. M.J. Sedivec*, J. Ovelmen-Levitt*, L.M. Mendell, Dept. Physiol., Duke Univ. Med. Ctr, Durham, N.C. 27710

We have analyzed properties of identified spinocervical units in adult cats anesthetized with α-chloralose. Our aim was to examine whether changes in modality convergence would occur in segments subject to chronic partial deafferentation by extradural dorsal rhizotomy. In intact preparations we observed a proportion of units which could be activated by hair stimulation only (38%) and a similar proportion of units which could be excited by hair and by noxious mechanical stimulation (47%). In spinal segments subject to acute dorsal rhizotomy there was a marked increase in the proportion of units which respond to hair only (71%) and a complementary decrease in the number which responded to both hair and noxious pressure (21%). We hypothesize that the functional projection from afferent fibers mediating the noxious pressure response is more completely restricted to the segment of entry than is the projection from afferent fibers responsive to hair displacement. The acutely deafferented spinal cord provides a more appropriate control population of units for the evaluation of the effects of chronic deafferentation than does the intact preparation. In chronically deafferented segments of the spinal cord we noted a virtual reversal in the proportions of units responsive to hair displacement only (17%) and hair and noxious pressure (68%). An increase was also observed in the percentage of neurons responding to temperature changes in chronically deafferented segments (70% of the units) when compared to acutely deafferented segments (10% of the units). These findings support the hypothesis that afferent connections to the chronically deafferented dorsal horn are altered such that units have a much greater probability of exhibiting convergence from multiple peripheral receptor types. Penetration of neurons studied by extracellular recording in intact and acute deafferented preparations did not reveal a substantial subliminal fringe, e.g. "hair only" cells did not have subthreshold responses to noxious stimulation. Therefore, it seems unlikely that the increase in responses following chronic deafferentation reflects a strengthening of previously weak connections on spinocervical neurons. However, we cannot conclude whether the increase in modality convergence reflects appearance of new functional afferent connections onto spinocervical cells or whether the functional convergence from peripheral receptors onto neurons (e.g. in the substantia gelatinosa) which project to these relay cells is altered by the deafferentation. (Supported by NIH grant NS 14899.)

216.7 PATTERNS OF CORTICOCORTICAL FIBER DEVELOPMENT IN THE NEONATAL RAT. H.P. Killackey and R.M. Akers. Dept. of Psychobiology, Univ. of Calif., Irvine 92717.

The granular parietal cortex of the rat (SmI) projects to three ipsilateral cortical sites: the second somatosensory area (SmII), motor cortex (MC), and the surrounding agranular parietal cortex (APC). Autoradiographic analysis of ^{3}H-leucine transport in neonatal rats revealed that each of these projections follows a different developmental schedule.

Rat pups ranging in age from birth (PND 0) to PND 9 received unilateral cortical injections of isotope and were sacrificed 24 hours later. Intracortical transport of label is evident in animals sacrificed as early as PND 1; conspicuous bands of transported label radiate from the injection site, extending through layer VI to presumptive target areas in SmII, MC and APC. Transport of label into the superjacent cortical layers occurs subsequent to PND 1 in each projection site. In SmII transported label appears in the lower one-half of layer V by PND 2, extends to the upper border of layer V by PND 3, and reaches the superficial cortical layers (I-III) by PND 4. From PND 5 to PND 8, labeling in layers I, III and IV increases; by PND 8 the pattern of label distribution is identical to that seen in adults. In MC transported label is confined to layer VI at PND 2, extends through layer V from PND 3 to PND 5, and does not reach the superficial layers until PND 6. Accumulation of label in layers I-III occurs subsequent to PND 6 and continues through PND 9-10. In APC layers V and VI are heavily labeled from PND 2 through PND 4, but transported label does not extend into the superficial cortical layers until PND 5. The adult pattern of label distribution is established in APC by PND 8.

These findings suggest that: 1) the corticocortical axons are located beneath their cortical target areas on the day following birth and subsequently ascend to terminal fields in the superficial layers; and 2) fibers destined for MC invade their terminal fields one to two days after those destined for SmII and APC.

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216.8 FUNCTIONAL REORGANIZATION AND SENSORY INTERACTIONS IN CAT SOMATIC SENSORY-MOTOR AND STRIATE CORTEX. Jacqueline Metzler. Sect. of Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT 06510.

Recent studies have demonstrated the effects of selective experience on the functional characteristics of cells in the developing nervous system. This study examined the effect of selective multi-sensory experience on the functional properties of cells in both somatic sensory-motor (SmI) and striate cortex (VI) of adult cats. Four cats were trained to flex one foreleg (FL) when 2 vertical or horizontal bars were presented to the ipsilateral eye. Failure to respond within 2 sec resulted in a mild electric shock to the ipsilateral hindleg (HL) ankle region. Orthogonally oriented bars presented to the other eye required no response and no shock was administered. Each animal, therefore, served as its own control. All cats performed to criterion (90% correct responses on 3 consecutive days) within 8 wks of training, at which time extracellular single-unit activity was recorded in VI and SmI.

Data from the 85 cells examined in VI revealed ocular dominance (OD) and orientation selectivity distributions to be modified as a a result of training. There was a 5-10% increase in the number of units in OD categories 2 and 6, and a corresponding decrease in categories 3-5, relative to controls. Moreover, 10-16% of the cells activated by the eye associated with the FL flexion/HL ankle shock had receptive-field orientations coincident with the bar pattern the eye had been exposed to during training, relative to those driven by the other eye. More striking changes were found in SmI. In all 4 animals, areas in the hemisphere receiving projections from body regions associated with training and responsive to FL and HL stimulation increased significantly relative to the control hemisphere. The area responsive to FL stimulation expanded about 7 mm^2 (30%) relative to the control SmI, shifting its borders an average of 1.75 mm toward the midline. While the region containing cells activated by stimulation of the HL ankle was, at most, 3-4 mm^2 and adjacent to the midline in the control hemisphere, it now averaged 12 mm^2 in the trained SmI, extending up to 5.5 mm lateral to the midline. The majority (66%) of the 63 cells recorded from the trained hemisphere were also responsive to visual stimuli, with the orientation of the bars associated with training generally the most effective, compared to only 19% of the 56 cells examined in the control hemisphere which responded to all stimulus orientations.

Recent results in the spinal cord (Dostrovsky et al., 1976), DCN (Millar et al., 1976), and SmI (Metzler and Marks, 1979; Franck, 1980) indicate that widespread afferent connections may be present throughout the somatosensory system, but are usually ineffective. The changes observed here may be mediated, at least in part, by local strengthening of such previously latent synapses.

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216.9 FUNCTIONAL ORGANIZATION OF RACCOON SOMATOSENSORY CORTEX: EFFECTS OF EARLY PERIPHERAL INJURY. A.M. Kelahan, R.H. Ray, L.V. Carson* and G.S. Doetsch. Depts. of Physiology and Surgery (Neurosurg), Med. Coll. Ga., Augusta, Ga. 30912.

The effects of neonatal peripheral lesions on the functional organization of primary somatosensory (SmI) cortex were studied in the adult raccoon, a species noted for it's anatomically well-demarcated forepaw digit representations (Welker et al., Am. Zool., 4:75-94, 1964). Recordings of primary cortical evoked potentials and single or multiple neuron responses were used to map the SmI forepaw area of normal animals and of animals which had the third digit of the left forepaw amputated several weeks after birth.

In normal animals, the focus of the maximum primary response to "punctate" mechanical stimulation of each digit and pad was localized in a separate gyral region. However, considerable overlap of digit and pad responses was observed; peak-to-peak voltages as great as 25% to 50% of the maximum response at one digit focus could sometimes be recorded from an adjacent digit area. These findings were generally consistent with receptive field (RF) maps derived from single or multiple neuron recordings. Threshold RFs were typically small, usually confined to one claw, one palmar segment of a digit or to one pad. However, RFs mapped with suprathreshold stimuli (Force = 2g and 15g) were larger, often including the palmar aspect of an entire digit and sometimes including an adjacent digit and/or pad.

In animals with left digit 3 amputations, normal somatotopic organization seemed to characterize the left (control) SmI cortex. In contrast, dramatic alterations in somatotopy were found within the right (experimental) SmI area. The primary response foci for the intact digits and the pads retained their usual cortical locations, but the entire area normally containing the digit 3 focus was invaded by inputs from all remaining digits and adjacent skin regions. The site normally representing the digit 3 focus gave large responses to stimulation of the digit 3 stump, the four intact digits and the pads. No unresponsive regions were found. In agreement with these results, threshold RFs of neurons within this tissue were generally larger than normal, often including continuous and discontinuous parts of digits 2 and 4 and/or a pad. Suprathreshold RFs were even more extensive, ranging from two digits and adjacent pads, to all four digits plus the digit 3 stump, to the entire paw; these RFs included dorsal paw regions much more often than normal. Finally there was greater variability in RF location as a function of distance across the cortex and of depth within the cortex. These findings demonstrate that early digit amputation has profound effects on the organization of SmI cortex. The cortical area normally representing a digit that was amputated receives widely overlapping inputs from adjacent skin regions, resulting in local disruption of normal somatotopy.

216.10 LONG TERM VISUAL DEPRIVATION IN A HUMAN. B. Gordon and J. Moran*. Dept. of Psychology, Univ. of Oregon, Eugene, OR 97403.

We have made rough preoperative and postoperative measurements of visual function in a patient (D.C.) with a monocular congenital cataract that was removed at 19 years of age. Acuity was measured with square wave patterns which D.C. identified as horizontal or vertical. Preoperatively, he was virtually blind in his deprived eye, failing to resolve 0.1 c/deg. With his normal eye he resolved about 30c/deg. For postoperative testing we refracted D.C. by retinoscopy because he never obtained a corrective lens. One month postoperatively he resolved 0.4 c/deg, and 4 months postoperatively he resolved 0.6 c/deg. His acuity did not improve further over the next 7 months.

For visual field measurements D.C. detected a small light moved behind a plastic hemisphere from its periphery to its center. Both pre- and postoperatively the visual fields of D.C.'s deprived eye were smaller than were the visual fields of his normal eye. Very high contrast conditions were used (light,17 cd/m^2; background, less than 0.0/cd/m^2) because D.C. could not see the light preoperatively under lower contrast. The deficit was greatest in the contralateral field (temporal retina) which was virtually nonexistent preoperatively and immediately postoperatively. By 3.5 months postoperatively the temporal field had increased to 38°, but the other fields showed no clear increase.

After the fields had stabilized, we tried to detect further improvement by making monthly measurements of D.C.'s visual thresholds as a function of field position. D.C. judged whether or not he could see a light behind a window in a cardboard hemicylinder. The lights were placed every 5° along the horizontal meridian. Thresholds did not change over 7 months, but the thresholds of the deprived eye, in contrast to those of the normal eye, varied markedly with field position. By 50° in the ipsilateral field thresholds were about 2X their value 10° from the fixation point. At 40° in the contralateral field thresholds had risen by 30X this value.

A surprising form of binocular inhibition convinced us that D.C.'s deficits were primarily central. Light reaching D.C.'s normal eye elevated deprived eye thresholds as much as 19X at 10° from the fixation point. Thirty-five degrees from the fixation point this inhibition had disappeared.

These results indicate that many of the effects of monocular deprivation are similar in cat, monkey, and man. They also suggest that the failure of cortical cells to respond to the deprived eye might be modified by rigorously excluding all visual stimulation from the normal eye.

216.11 GOLDFISH RETINA HAS TWO KINDS OF GERMINAL CELLS: ONE IS SPECIFIC FOR RODS. P. R. Johns. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

New cells are added to the retina in growing adult goldfish by germinal cells at the retinal margin. This study describes a second type of germinal cell, located in the outer nuclear layer, which only generates new rods.

Dividing cells were identified using ^{3}H-thymidine autoradiography. Goldfish were injected intraperitoneally six times at 8 hour intervals with 5 μCi/g of ^{3}H-thymidine of very high specific activity (95 Ci/mmole). Retinas were prepared for light microscopic autoradiography using JB-4 plastic. Retinas from normal, uninjected fish were prepared for electron microscopy.

At 1 to 5 days after injection, the marginal germinal cells were heavily labeled. By 10 to 20 days they had produced heavily and weakly labeled neurons in all retinal layers. Electron microscopic examination of the newly-formed retina immediately adjacent to the germinal zone revealed that the outer nuclear layer (ONL) was incomplete -- it contained no rods, but only a single row of immature cones. Rod nuclei (smaller, rounder and darker than cone nuclei) first appeared several cell diameters more centrally. This suggests that rods may be produced outside the marginal germinal zone. Autoradiographs revealed a dispersed population of dividing cells in the ONL, lying along the border of the outer plexiform layer (OPL). More than half of these labeled cells were within 0.5 to 1 mm of the margin; the rest were spread out, sometimes in groups, along the central 3 to 4 mm length of retina. In electron micrographs of peripheral retina these cells were tentatively identified; they appeared very similar to marginal germinal cells. By 10 to 20 days the labeled cells in the ONL had divided: there were more of them and many were weakly labeled. Some had moved away from the OPL border and were now among the rod nuclei and, in light microscopic autoradiographs, the labeled cells were indistinguishable from rods. Electron microscopic autoradiographic studies are in progress to confirm that the dividing cells in the ONL are producing rods.

As the goldfish grows, the ratio of rods to ganglion cells increases several fold across the entire retina. This is difficult to explain if new rods are produced only at the margin of the retina; to do so requires that the layers of retinal cells be allowed to slide past one another as the retina grows. The presence of a dispersed rod precursor provides an explanation for the higher proportion of rods in larger fish retinas -- as the retina grows, new rods are inserted into already differentiated regions of retina, where the population of ganglion cells and other retinal neurons is stable.
(Supported by EY 03301).

216.12 DEVELOPMENT OF SINGLE-FIBER TASTE RESPONSES TO MONOCHLORIDE SALTS IN THE RAT'S CHORDA TYMPANI NERVE. D. L. Hill, R. M. Bradley, and C. M. Mistretta. Dept. Oral Biol., Sch. Dent., U. Mich., Ann Arbor, MI 48109.

Integrated recordings from the rat's chorda tympani nerve change developmentally in response to lingually applied monochloride salts (Neurosci. Abst., 5, 127 & 128). Responses to NaCl and LiCl are smaller relative to NH_4Cl in the young rat. Throughout development, though, NaCl and LiCl become more effective stimuli. To learn if young fibers respond to NaCl and LiCl, and to identify possible developmental changes in response frequencies and response-concentration functions, we recorded from single chorda tympani fibers. Solutions of 0.1 M and 0.5 M NH_4Cl, NaCl, LiCl, KCl, and a concentration series of NH_4Cl (0.005-1.0 M) were applied to the anterior tongue for 15 seconds. Each solution was followed by a distilled water rinse.

Data from 19 single fibers in rats 14-20 days old, 16 fibers in rats 24-35 days old, and 20 fibers in adults were similar to integrated data after responses were expressed relative to the NaCl response. All fibers responded to each stimulus. For 0.1 M solutions, response frequencies to NH_4Cl and KCl did not differ for age; frequencies to NaCl and LiCl significantly increased between 20 days of age and adulthood. Response frequencies to 0.5 M NH_4Cl also did not differ with age while frequencies to 0.5 M NaCl, LiCl, and KCl significantly increased between 20 days of age and adulthood. Mean responses for rats 24-35 days of age were intermediate to those for 14-20 day rats and adults. Most young fibers responded maximally to NH_4Cl solutions and most adult fibers responded maximally to NaCl or LiCl, whereas, the majority of fibers in rats 24-35 days old responded similarly to all equimolar salts. No fiber responded maximally to KCl. Response-concentration functions from 6 young and 6 adult fibers for NH_4Cl were similar, and the binding constants calculated for both groups were nearly identical to those reported by Beidler (Prog. Biophys. & Biophys. Chem., 12, 107-151).

In summary, developmental changes in integrated chorda tympani responses cannot be explained by an absence of young fibers responding to NaCl and LiCl. However, they may be explained by developmental increases in frequencies to NaCl and LiCl while frequencies to NH_4Cl remain constant, and by changes in the number of fibers responding maximally to NH_4Cl, or NaCl and LiCl. Since response-concentration functions for NH_4Cl are similar throughout development and NH_4Cl response frequencies do not change, taste receptor membrane sensitivity to NH_4Cl may be stable during development. Conversely, membrane sensitivity to NaCl and LiCl probably changes. (Supported by NIH Postdoctoral Fellowship NS06423 to D.H. and N.S.F. Grant BNS77-09920 to R.M.B. & C.M.M.)

216.13 ULTRASTRUCTURAL CHANGES IN DEVELOPING TASTE BUDS ACCOMPANY FUNCTIONAL CHANGES. Robert M. Bradley, Charlotte M. Mistretta and Soo D. Lee*. Dept. Oral Biol., Sch. Dent., U. Mich., Ann Arbor, MI 48109.

Previous work from this laboratory has demonstrated that electrophysiological taste responses to salts and acids change during development in fetal and newborn sheep. To learn about taste receptor morphology during the period of functional changes, we have studied the ultrastructure of developing taste buds in sheep fungiform papillae. Eight fetuses aged 75-135 days of gestation (term = 147 days), 1 lamb and 1 adult sheep were perfused with buffered glutaraldehyde, the tongues removed, and fungiform papillae dissected and post-fixed in buffered OsO_4. The papillae were embedded in epon and sectioned for electron microscopy.

In the youngest fetuses (75 days), no taste pits or pores are observed. The taste bud consists of a complex arrangement of cells covered by one or two layers of epithelial cells. Between the taste bud cells are large numbers of nerve profiles. In most cells the nucleus has a dense appearance and the cytoplasm contains dark granules. However, the nuclei of a small number of cells are less dense in appearance, and these cells do not have dense cytoplasmic granules. Two cell types are therefore observed --'dark' and 'light'. At 80 days of gestation, taste pits are present. These consist of a shallow depression in the surface of the papilla; the base of this depression is composed of the apices of the taste bud cells. Cells lining the pit terminate either in short microvilli or blunt, club-shaped processes. All cell apices are joined by tight junctions, and nerve profiles are seen close to the pit area. Both light and dark cells are present.

The microvilli have increased in length (but not in number) in fetuses of 100 days. Some club-shaped apical processes are still present. No differences are observed in the apical cytoplasmic contents of those cells that end in either microvilli or club processes. In term fetuses, lambs, and adults, the structure of the taste pit is similar to that in the 100 day fetus. However, the pit now communicates with the surface of the papilla by a narrow taste pore filled with a dense amorphous substance.

In conclusion, taste buds at all ages studied possess at least two cell types, and when a taste pit is present, microvilli and other processes are observed. The apices of the cells are joined by tight junctions from at least 80 days of gestation. Since microvilli and tight junctions are present, there is no reason to propose that chemicals penetrate into the taste bud in fetal animals. Rather, taste stimulation is probably via interaction with microvilli membranes as in adults.

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216.14 DEVELOPMENT OF SPINOCEREBELLAR PROJECTIONS IN THE POUCH YOUNG OPOSSUM. James C. Hazlett, Department of Anatomy, Wayne State University, Detroit, MI. 48201.

The present study is part of our ongoing analysis of the development of long ascending pathways in the opossum. Spinal cord lesions (tractotomies or hemisections) were performed at C-2 in seven litters of pouch young opossums ranging in age from 26 to 75 days in the pouch. Results from the three youngest litters will be reported here. The approximate ages of each litter were based on measurements of snout-rump length with subsequent reference to the growth curves determined for the opossum (Cutts et al.,'78). The spinal cord lesions were performed under hypothermic anesthesia and survival times of 6,12,18,24 and 30 to 36 hours were selected in an attempt to increase the successful identification of degenerating axons. Brains were embedded in egg yolk, sectioned in the transverse plane and reacted by the Fink-Schneider technique for degenerating immature axons. In the youngest group studied (26 days in the pouch) axonal debris is present in the brainstem underlying the cerebellum and a very sparse accumulation of axon fragments is observed in the cerebellar peduncles. These latter structures are not well demarcated at this age. Within the cerebellum the only area containing unequivocal degenerating fragments is located in the posterior lobe vermis. At this age it is not certain if this area corresponds to the pyramis. Furthermore, the degenerating fragments are located just below the external granular layer. This lamina of cells is still in the process of inward migration at this time. No degeneration was observed in the anterior lobe. The second group (33 days in the pouch) present approximately the same degeneration picture as observed in the youngest group. However, folial development has progressed sufficiently to permit the identification of major cerebellar subdivisions. In the oldest group (41 days in the pouch) more axonal debris was present in the brainstem than was observed at earlier ages. It is now possible to identify the cerebellar peduncles as separate structures which contain scattered degenerating axon fragments. One folium in the posterior lobe (assumed to be the pyramis) contains an accumulation of degenerating axon fragments on the dorsolateral periphery, bilaterally. In addition, a few widely scattered clumps of axonal debris were identified in the middle of the anterior lobe. This is the first stage in which we observed degeneration in this area. Earlier and later litters of pouch young opossums are being examined in an attempt to 1. determine the time at which spinal axons first encounter the cerebellum and 2. establish the time when the well- known longitudinal orientation is first encountered. (Supported by the National Science Foundation- Grant # BNS 79-14072).

216.15

Withdrawn by Author

216.16 EFFECTS OF SYNAPTIC ACTIVITY ON THE SIZE OF SOMATIC SPINES AND ON THEIR CONTENT OF SMOOTH ENDOPLASMIC RETICULUM. L. Maler, E. Sas*. Dept. of Anatomy, Sch. of Med., Univ. of Ottawa, Ontario K1N 9A9, Canada.

The size and number of dendritic spines have proven to be a sensitive indicator of synaptic activity in a number of systems. This is especially apparent in the visual system where a number of workers have demonstrated that the integrity of certain spines on the apical dendrites of striate cortex pyramidal neurons is dependent on visual input. We have been studying the posterior lateral line lobe of gymnotid fish - this region of the brain receives a direct input from the electroreceptors distributed over the fish's body surface. Electroreceptor afferents make monosynaptic contacts with interneurons of the posterior lobe, and these interneurons in turn project to the somata, somatic spines of large pyramidal cells of the posterior lobe (Maler, L., J. Comp. Neurol., 183:323, 1979;Maler, L., Sas, E. and Rogers, J., J. Comp. Neurol. in press). The somatic spines of pyramidal neurons are a good system for analyzing the effect of synaptic input on spine structure because i) the peripheral nerve to the electroreceptors is readily accessible to resection or stimulation, and ii) electroreceptor afferents in these fish discharge continuously at very high frequencies (50-250 Hz), which means that deafferention produces a drastic drop in synaptic input.

We have sectioned the electroreceptor afferent nerve peripheral to its ganglion and examined thin sections of the posterior lobe following survival times of 6 hr to 6 days. The overall cytology of the posterior lobe does not change following this treatment; in particular the axon terminals of the local interneurons shows very little alteration. One to three days after nerve section the somatic spines upon which these terminals synapse become shrunken by about 30%-50%; they also show a dramatic decrease in their content of smooth endoplasmic reticulum (SER; 90% loss). After 6 days survival time half the somatic spines remain shrunken and lack SER; the rest return to normal in both size and SER contents. As regeneration proceeds all the spines return to their presurgical appearance. The cell biological and functional implications of these findings will be discussed.

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217.1 ANTICONVULSANT TOXICITY: CHRONIC PHENYTOIN EXPOSURE DECREASES NEURONAL NUMBER IN CELL CULTURES FROM FETAL MOUSE CEREBRAL CORTEX. E.A. Neale, K.F. Swaiman,* B.K. Schrier, W.H. Habig,* and P.G. Nelson. Lab. Devel. Neurobiol., NICHD, NIH, Bethesda, MD 20205, Div. of Ped. Neurol., Univ. of Minn., Minneapolis, MN 55455, and Div. Bact. Prod., Bureau of Biologics, NIH, Bethesda, MD 20205.

Phenytoin is an anticonvulsant frequently administered for seizure therapy of pregnant women, neonates, infants and children. Studies were undertaken to evaluate the effect of chronic phenytoin treatment on developing central neurons. Dissociated cell cultures were prepared from 16 day fetal mouse cerebral cortex. After growth for 11 days, phenytoin (mid-therapeutic to toxic levels--15 µg/ml, 25 µg/ml, and 50 µg/ml) was added to the culture medium for an additional 7 days.

Phenytoin-treated and control cultures were assayed for neuronal cell number by phase microscopy, and for ^{125}I-tetanus toxin binding and ^{3}H-γ-aminobutyric acid (GABA) uptake by both scintillation spectrometry and radioautography. In control cultures, morphologically identified neurons grew as networks or aggregates. These cells displayed ultrastructural features typical of neurons, and synaptic profiles were common. After incubation for 30 min. at 37°C in 0.1 uM ^{3}H-GABA and subsequent radioautography, approximately 10-15% of the neurons were heavily labeled with silver grains.

Analysis of phenytoin-treated cultures indicated a drug-related loss of neurons. At the concentrations of phenytoin tested, neuronal cell counts were depressed to approximately 70%, 60%, and 15% respectively, of control values. Decreases in neuronal cell counts were paralleled by decreases in both ^{125}I-tetanus toxin binding and ^{3}H-GABA uptake. The number of neurons radioautographically labeled by high affinity GABA-uptake was depressed to the same extent as total neurons, indicating that the presumed GABAergic neurons were not preferentially spared. In similar preparations (see abstract this volume, D.W. Gallager, et al.), clonazepam-displaceable ^{3}H-diazepam binding (an index of neuronal benzodiazepine receptors) was also significantly depressed.

These studies suggest that therapeutic concentrations of phenytoin may alter numbers of developing neurons. The use of ^{125}I-tetanus toxin binding and ^{3}H-GABA uptake are reliable means of assessing this effect. The utilization of this culture system, in conjuction with quantitative morphological and biochemical techniques, provides a useful approach to the evaluation of drug effects on developing nervous tissue.

217.2 ENHANCEMENT OF SYMPATHETIC NERVE DEVELOPMENT IN THE FETAL ALCOHOL SYNDROME. J.V. Bartolome*, S.M. Schanberg and T.A. Slotkin. (SPON: W.A. Wilson). Dept. of Pharmacology, Duke Univ. Medical Ctr., Durham, N.C. 27710.

Exposure of the fetus to ethanol produces abnormalities in maturation of the nervous system, with potentially serious consequences for subsequent development. Long-term damage can occur in behavior and in function of various organ systems. Previous studies have shown that cardiovascular development may be compromised, and the current work examines whether disturbances in the ontogeny of peripheral sympathetic nerves are involved in the cardiovascular effects. The fetal alcohol syndrome was induced by providing 6.8% ethanol in a liquid diet to pregnant rats from the 13th day of gestation; at parturition, all rats were cross-fostered to normal mothers to ensure adequate maternal care. Development of sympathetic neurotransmission in the heart and adrenal medulla of the offspring was monitored by eliciting reflex central stimulation with insulin-induced hypoglycemia. Stimulation was assessed by measuring secretion of adrenal catecholamines and β-adrenoceptor mediated stimulation of cardiac ornithine decarboxylase activity evoked by the insulin treatment. In control neonates (offspring of mothers given isocaloric liquid diet without ethanol), sympathetic neurotransmission was absent at birth and was first operable toward the end of the first week of postnatal age. In contrast, pups whose mothers received ethanol displayed functional sympathetic reflex responses as early as 2 days of postnatal age. The development of peripheral sympathetic neurotransmission thus occurs precociously in the fetal alcohol syndrome; *this should not be viewed as a beneficial, nor even an innocuous event, since premature exposure of developing tissues to neuronal input may be responsible for the subsequent abnormalities of cellular maturation in those tissues.*
(Supported by USPHS DA-00465, DA-00006 and AA-02935).

217.3 THE IMPORTANCE OF PRENATAL LEAD(Pb) EXPOSURE TO POSTNATAL BRAIN DEVELOPMENT IN THE RAT PUP. Paul T. McCauley, Annette Tonti and Richard J. Bull, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268

Previous reports from this laboratory, McCauley et al. (Neuropharmcol. 18:93-101, 1979) document delayed synaptogenesis in the cerebral cortex of male offspring of female rats that were exposed to 200 ppm Pb in drinking water from two weeks prior to breeding until pups were weaned at 21 days of age. Subsequent experiments have demonstrated that the prenatal exposure to Pb played the major role in delayed behavioral development of rat pups (Crofton et al. Life Sciences 26:823-831, 1980). To determine whether the prenatal exposure to Pb played the same role in delaying synaptogenesis, cross-fostering experiments were initiated. Photographs were made (10,000 x mag) of layers 1-3 of the parietal contex and synaptic figures visualized by ethanol-phosphotungstic acid stain were counted blind by four different observers.

The table shows observed synapse counts and blood Pb for 15 day old pups (Prenatal/Postnatal exposure to pup):

Exposure			Synapse/mm^3	Pup Blood Pb Concentration in mg%
Prenatal	Postnatal			
Control	Control	CT/CT	4.15 ± .35	4.6 ± 0.9
Control	Lead	CT/Pb	3.97 ± .64	24.8 ± 1.3
Lead	Control	Pb/CT	2.78 ± .52	6.9 ± 0.9
Lead	Lead	Pb/Pb	2.92 ± .42	30.3 ± 2.9

Synaptic counts in groups Pb/CT and Pb/Pb were statistically different from group CT/CT (P = .026 and P = .025 respectively. CT/Pb pups were not significantly different from CT/CT (P = .82).

The data demonstrates that gestation period is the critical period for low level lead exposure to produce delays in synaptogenesis. Synaptogenesis is clearly less sensitive to low level Pb exposure during lactation despite the fact that pup blood lead levels in the CT/Pb group are considerably greater than the Pb/CT group at 15 days of age. Parallel effects on the development of exploratory and locomotor activity using the same cross-fostering design were seen by Crofton et al. As in the delays in synaptogenesis, delays in behavioral development produced in Pb/Pb animals were almost entirely accounted for in Pb/CT animals. These data strongly suggest that development of the cerebral cortex, a largely postnatal event, is more sensitive to in utero exposure to lead than postnatal exposure to Pb. Current data suggest this result may be related to a peak in blood Pb concentrations in the dam and pup just prior to birth (Bull, et al. Pharmacologist 21:210, 1979.

217.4 SHAKING STIMULATIION ACCELERATES HATCHING TIME IN CHICKEN EGGS. J. H. Gassler*, M. L. Kirby, S. D. Stoney. Depts. of Anatomy and Physiology, Medical College of Georgia, Augusta, GA 30912.

The avian embryo is sensitive to different types of sensory stimulation during development. It is known that light and auditory stimuli given during incubation will accelerate hatching time. The vestibular system matures early in the development of the chick embryo. Its integrity is necessary for hatching to occur. It is not known what effects vestibular stimulation would have on the developing chick embryo. To address this question, White Leghorn chicken eggs were incubated at 37.5±.5°C and 60% humidity in a forced draft incubator, modified to allow shaking of experimental eggs. The experimental egg tray was shaken linearly with a 1.5 inch excursion, 70 cycles per minute on an intermittent schedule (2 hours on, 2 hours off). Shaking began on day 10 (240 hours) and continued until pipping, when the eggs were transferred to a brooder. Control eggs were incubated on a separate stationary tray in the same incubator. Motility was recorded in 13 to 19 day embryos by placing platinum electrodes into holes pierced in the egg and recorded on a Beckman Dynograph. Recordings were made during the following time periods: a) the last 15 minutes of the resting portion of the cycle; b) the first 15 minutes after the shaker was turned on; c) the last 15 minutes of the shaking portion of the cycle; and d) the first 15 minutes after the shaker shut off. Results indicate that hatching time is decreased by 7 hours in experimental eggs. There is no difference in body weights between the two groups. Motility recordings indicate that beginning on day 15, shaken eggs show increased motility when shaking begins, but after 2 hours of stimulation motility has decreased to levels recorded when the shaker is off. Control eggs maintain a constant level of motility during all recording periods.

217.5 EFFECT OF MATERNAL DIETARY PROTEIN RESTRICTION ON ONTOGENY OF OLFACTORY BULB ELECTRICAL ACTIVITY IN THE RAT. W. B. Forbes, C. R. Almli, M. A. Henault,* and C. A. Velozo*. Worcester Foundation for Experimental Biology, Shrewsbury, MA, 01545, and Ohio University, Athens, OH, 45701.

Ontogeny of main olfactory bulb (MOB) EEG was studied in male rats whose dams were fed one of two isocaloric diets containing 8% or 25% casein for five weeks prior to mating, throughout gestation and lactation. Bipolar stainless steel electrodes were chronically implanted in the MOB of rats 3 - 19 days of age. EEG was recorded for two hours on each of 3 consecutive days. Between recording sessions, rats were returned to their home cages. Rats of all dietary and age groups exhibited normal body growth over the three day recording period. Behavior of the pups during recording sessions was monitored visually. Urine-soaked nest material and an anesthetized lactating dam served as specific olfactory stimuli. Multiple 8-sec epochs of MOB electrical activity were digitized at 256 Hz and analyzed using fast-Fourier techniques yielding power spectral values with a resolution of .125 Hz over a range of 0 - 128 Hz.

MOB electrical activity was found to contain two frequency components in rats 10 - 21 days of age. In control (25% casein) rats, the lower frequency component increased from about 14 Hz at 10 days of age to about 25 Hz at 21 days of age. The higher frequency component increased from about 40 Hz at 10 days of age to about 63 Hz at 21 days of age. These two components were interpreted as Adrian's "induced" and "intrinsic" waves, respectively. The effect of maternal dietary protein restriction was to retard this developmental pattern by 1 - 2 days. Since normal EEG ontogeny closely parallels the morphological development of the granule cell population of the MOB, the retardation associated with dietary restriction may be associated with retarded structural ontogeny of this specific class of neuron in the MOB.

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217.6 RETARDED BRAIN GROWTH IN NEONATAL RATS EXPOSED TO HYPERPHENYLALANINEMIA IN UTERO

D.A. Spero* and M.C. Yu* (SPON.L. Laemle) Department of Anatomy New Jersey Medical School, Newark, New Jersey 07103.

High levels of phenylalanine in maternal plasma are in most cases caused by a defect in phenylalanine hydroxylase, an hepatic enzyme necessary for the conversion of phenylalanine to tyrosine. Excessive phenylalanine in maternal plasma has been shown to be transported across the placenta and to interfere with the normal processes of prenatal development resulting in a condition known as maternal hyperphenylalaninemia. In the present study hyperphenylalaninemia was induced in 2 groups of pregnant rats from the 14th through the 21st days of gestation in the following manner: Group I received subcutaneous injections of L-phenylalanine (Phe) (250mg./100g.B.W.) plus methylphenylalanine (mPhe) an inhibitor of phenylalanine hydroxylase at a dosage of 300mg./100g.B.W. dissolved in carboxymethycellulose (CMC) two times daily at 12-hr. intervals. Group II was injected with Phe (400 mg /100g.B.W.) in CMC three times daily at 8-hr. intervals. For control, another group of animals were injected with CMC and pair-fed during the treatment period. mPhe/Phe treatment resulted in a 30-fold increase in fetal plasma Phe for approximately 10-hr. postinjection. Phe treatment alone resulted in a 24-fold increase in plasma Phe for 3-hr. postinjection. At day 20 of gestation (D20G) and day 1 (D1) and day 5 (D5) postnatal, the body and brain weights of the mPhe/Phe and Phe treated rats were significantly lower than the control. Light and electron microscopic observations of the cerebral cortex revealed distinct morphological alterations in both treated groups which were not present in the control. These include a decrease in thickness of the cerebral cortices and increased cell packing density, an increase in cell death and in the number of phagocytic cells. The number of pyknotic cells reached a peak at birth in both treatment groups. These changes were more severe in the mPhe/Phe treated group than in the Phe group. Biochemical determinations revealed that the DNA content of the cerebral cortex of both the mPhe/Phe and Phe treated groups were significantly less than the control at D20G, D1 and D5, respectively. These findings suggest that the retarded brain growth in the treated groups may be due in part to increased cell death.

This work is supported by New Jersey Medical School General Research Support Fund.

217.7 EFFECTS OF PRENATAL ETHANOL EXPOSURE IN RATS. Francine Lancaster, Bina Mayur* and T. Sámorajski. Dept. of Biology, Texas Woman's University, Houston, TX 77030.

Pregnant (days 5-19 gestation) Long-Evans rats were fed ethanol (6.7% v/v) in a modified Lieber-DeCarli liquid diet. Control groups were: (1) pair-fed liquid diet (2) liquid diet ad libitum (3) lab chow and water ad libitum. After day 19 of gestation, all animals received lab chow and water ad libitum. All litters were culled to seven pups at birth. Biweekly weight records were maintained for all animals. At ages 16, 21, and 30 days experimental pups were injected intraperitoneally with ^{3}H-leucine and control pups with 1-^{14}C-leucine. Synthesis of brain subcellular proteins and quantitative myelin were measured. At the age of 30 days organ/body weight ratios of brain, pituitary, adrenals, heart, spleen, liver, and kidneys were determined. Myelin levels of ethanol animals were lower than those of controls. Spleen/body weight ratios of ethanol animals were higher than all control groups. Supported by grants from TWU and Texas Research Institute for Mental Sciences.

218.1 ADAPTIVE COMPENSATORY RESPONSES AFTER ADRENAL DEMEDULLATION IN RATS. P. Christopher Coburn*, Jean A. Hosutt*, Edward M. Stricker, Michael J. Zigmond, (Sponsor: R. Keller). Departments of Psychology and Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Bilateral removal of the adrenal medulla in adult male rats was found to greatly impair their maintenance of arterial blood pressure (ABP) during hypovolemia. For example, intact animals did not become hypotensive despite plasma volume deficits of 20-30% induced by subcutaneous treatment with hypertoncotic solutions of polyethylene glycol (PEG). Two days after adrenal demedullation, however, PEG treatment lowered ABP by 30-50 mm Hg while heart rate decreased. These effects were not seen either when PEG-treated rats could compensate behaviorally by drinking isotonic NaCl solution, or when animals were examined 2 wk after adrenal demedullation in the absence of drinking fluids.

Adrenal demedullation also interfered with the maintenance of blood sugar during insulin-induced hypoglycemia. For example, in normal rats blood glucose was depressed to 30-50 mg/100 ml after iv injection of 2U insulin/kg but returned to normal levels of 100-110 mg/100 ml within 90-120 min; in contrast in rats tested 2-3 days after adrenal demedullation blood glucose usually remained depressed throughout the 5-hr period of observation or dropped to such low levels that rats succumbed to hypoglycemic shock. When food was available, adrenal demedullated rats ate so rapidly after insulin treatment that the induced hypoglycemia was blunted. When these experiments were repeated 3 wk later, adrenal demedullated rats maintained a stable hypoglycemia after insulin treatment whether food was available or not. This improvement was abolished after sympathectomy by 6-hydroxydopamine (75 mg/kg, iv). Since epinephrine-induced hyperglycemia was not altered, these findings collectively suggest that a gradual increase in the capacity for NE release from the sympathetic nerves permits the recovery of function after adrenal demedullation. As such, these results parallel our recent findings that the synthesis and release of NE from residual neurons increases following subtotal destruction of noradrenergic nerve terminals in the brain (Acheson et al., Science 207: 537-540, 1980).

218.2 CHANGES IN ADRENAL TETRAHYDROBIOPTERIN (BH_4) INDUCED BY INSULIN AND RESERPINE AND ITS ROLE IN BH_4-DEPENDENT HYDROXYLATION REACTIONS. Martha Abou-Donia*, S. P. Wilson and O. H. Viveros. Dept. of Medicinal Biochemistry, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709.

BH_4, the cofactor for tyrosine-hydroxylase (TH) and other aromatic amino acid hydroxylases, is present in monoaminergic cells and nerve terminals at limiting concentrations (Mandell, A. J., Ann. Rev. Pharmacol. Toxicol., 18: 461, 1978). Neurogenic stimulation and catecholamine (CA) depletion induce TH synthesis in the adrenal medulla (Viveros et al., Mol. Pharmacol. 5: 69, 1969). TH induction can be functionally expressed only if the concentration of cofactor is maintained or increased. Two days after addition of reserpine (100 nM) to primary cultures of bovine chromaffin cells, total TH activity from lysed cells doubles, while BH_4 levels and tyrosine hydroxylation in intact cells fall to 23 and 72% of control, respectively. Addition of BH_4 (100 μM) to the medium increases tyrosine hydroxylation such that reserpine treated cultures have 38% more activity than controls. In contrast to the effect in culture, administration of reserpine to rats (5 mg/kg, i.p.) increases adrenal gland BH_4 from 287 ± 32 ng/g to 1,044 ± 129 ng/g 24 hr later, and to 768 ± 52 ng/g 24 hr after hypoglycemia, induced by insulin, 10 U/kg i.v. (values are means ± S.E., N=5 for each group). BH_4 levels return to normal 3 days after reserpine. These effects are only partially blocked by splanchnic denervation or chlorisondamine administration (2 x 5 mg/kg). When BH_4 was determined separately in medulla and cortex, insulin and reserpine increased medullary BH_4 by 40% ($p<0.05$ vs control); cortical BH_4 increased by 245% ($p<0.001$). Denervation prevented the increase in medullary BH_4 but not the increase in cortical BH_4 induced by insulin. Both insulin-induced hypoglycemia and reserpine are known to induce ACTH secretion from the hypophysis. This is the first demonstration that increased cellular activity can lead to significant and rapid increases in endogenous cofactor levels. Regulation of BH_4 concentration may occur through changes in the activity of biopterin synthetic enzymes (see these Abstracts: J. C. Nixon et al.). The same stimuli that increase BH_4 also induce TH, dopamine-β-hydroxylase, CA uptake and storage, and opioid peptide synthesis in the adrenal medulla. Control of BH_4 levels has obvious implications for the regulation of monoamine synthesis and the activity of other BH_4 dependent enzymes.

218.3 INDUCTION OF GTP-CYCLOHYDROLASE BY THE STIMULI THAT INCREASE TETRAHYDROBIOPTERIN (BH_4) IN RAT ADRENAL MEDULLA AND CORTEX. J. C. Nixon*, C.-L. Lee*, M. Abou-Donia*, T. Fukushima*, C. A. Nichol*, E. Diliberto*, Jr. and O. H. Viveros. Dept. of Medicinal Biochemistry, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709.

The activation of adrenal medullary chromaffin cells by reflex neurogenic stimulation and/or catecholamine depletion (reserpine and insulin hypoglycemia) of adrenal cortical cells is accompanied by rapid and significant increases in total biopterin and in the active cofactor, BH_4 (see previous abstract: Abou-Donia, Wilson and Viveros). GTP-cyclohydrolase is the first enzyme in the BH_4 biosynthetic pathway. The activity of this enzyme was determined by a direct measurement of neopterin formed in a reaction utilizing saturating levels of GTP as substrate. The immediate product of this reaction was then oxidized and enzymatically dephosphorylated to yield neopterin, which was quantitated by high-performance liquid-chromatography (Fukushima and Nixon, Anal. Biochem., 102, 176-188,1980). The enzyme was extracted from adrenal medulla and adrenal cortex homogenates and partially purified through a G-25 Sephadex column. While adrenal medulla GTP-cyclohydrolase has a 6-fold higher specific activity [313 ± 17 pmole/hr/mg protein (mean ± S.E., n=5)] than the cortex, the total enzyme activity in the adrenal is 41% cortex, 59% medulla. Reserpine (5 mg/kg i.p., administered 24 hr before sacrifice) increases GTP-cyclohydrolase activity to 147 ± 26 pmole/hr/mg protein in cortex and 722 ± 67 pmole/hr/mg protein in adrenal medulla. Simultaneous injection of reserpine with cycloheximide (1 mg/kg) decreases GTP-cyclohydrolase activity in medulla to 255 ± 35 pmole/hr/mg protein and cortex to 63 ± 15 pmole/hr/mg protein, while cycloheximide does not affect the enzyme activity in the cortex of non-reserpine-treated animals and decreases it by 34% in the adrenal medulla. The activity of dihydropteridine reductase, the enzyme that recycles quinonoid dihydrobiopterin to BH_4, is not altered by reserpine treatment. This is the first demonstration that GTP-cyclohydrolase activity can be controlled and amplified, apparently by an induction of enzyme synthesis, and supports our previous report that endogenous BH_4 levels are regulated by changes in cellular activity. Activation of adrenal medullary cells leads to a coordinated induction of both the synthesis of chromaffin vesicles to take up and store catecholamines and induction of the enzymes necessary for catecholamine synthesis including tyrosine hydroxylase, dopamine-β-hydroxylase and GTP-cyclohydrolase for adequate supply of BH_4.

218.4 PRODUCTION AND RELEASE OF ACETYLCHOLINESTERASE BY ADRENAL CHROMAFFIN CELLS IN CULTURE. Fumio Mizobe* and Bruce G. Livett, Division of Neurology, The Montreal General Hospital and McGill University, Montreal, Canada.

Histochemical studies of the bovine adrenal gland have shown that acetylcholinesterase (AChE) activity occurs both in association with the splanchnic nerve and also in the target chromaffin cells. The origin of this cellular AChE in the chromaffin cells, however, has not been firmly established. We have previously shown that bovine adrenal chromaffin cells can be isolated and maintained as primary cultures under conditions free of non-neuronal cells. The absence of splanchnic nerve influence on these cells in culture therefore provided a convenient system for investigating the origin of the cellular AChE in the adrenal medulla.

The cells when freshly isolated by collagenase perfusion contained only low levels of AChE activity (3.4 ±0.3nmol/min/10^6 cells), but synthesized AChE during the culture period. The total AChE activity increased 6-fold from days 2-15. The non-specific cholinesterase activity of the cells remained at less than 6% of AChE activity. It was also found that AChE activity appeared in the culture medium and increased in activity proportional to the period during which the medium was in contact with the cells. These findings show that chromaffin cells produce and release AChE into their immediate environment. The cumulative amount of AChE activity released into the medium over 15 days reached 60 nmole/min/10^6 cells.

We have examined the role of the nascent AChE on cholinergic stimulus-secretion coupling in the cultured chromaffin cells.[1] A progressive decrease in the efficacy of ACh ($5x10^{-5}$M) to evoke release of (^{3}H) noradrenaline from day 3-15 cultures suggested that exogenously applied ACh was hydrolyzed by the nascent AChE synthesized by the cells, part of which is probably located on the plasma membranes of the chromaffin cells. This conclusion was supported by our finding that nicotine ($5x10^{-6}$M) exhibited a constant agonist activity on the cells throughout the 15 day period and that pre-incubation of 3-15 day old cultures with eserine ($4x10^{-6}$M) or with the non-permeant AChE inhibitor BW284C51 ($5x10^{-7}$M) completely restored the efficacy of ACh as an agonist.

1. Mizobe, F, Kozousek, V, Dean, D.M. and Livett, B.G. (1979) Brain Res. 178, 555-566.

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218.5 THE ROLE OF GLUCOCORTICOID STEROIDS IN THE EXPRESSION OF THE ADRENERGIC PHENOTYPE IN THE RAT EMBRYONIC ADRENAL GLAND. M.C. Bohn, M. Goldstein and I.B. Black. Dept. of Neurology, Cornell Univ. Med. Coll., New York, N.Y. 10021 and Dept. Psychiatry, New York Univ. Med. Ctr., New York, N.Y. 10016.

Differentiation of the noradrenergic and adrenergic phenotypes was documented in rat embryonic adrenal medullary cells in vivo from 12.5 days of gestation (E 12.5) to term. The initial appearance of biochemical indices of noradrenergic expression (tyrosine hydroxylase, T-OH; dopamine-β-hydroxylase, DBH, and endogenous catecholamines, CA) and adrenergic expression (phenylethanolamine N-methyltransferase, PNMT) was followed by immunocytochemistry and histofluorescence. At E 13.5, cells containing T-OH, DBH and CA were observed in sympathetic ganglion primordia at the level of the adrenal anlage, along the aorta between sympathetic ganglia and the developing adrenal, and within the adrenal itself. While T-OH, DBH and CA were present in adrenal medullary cells from the earliest stages of adrenal morphogenesis, PNMT, in contrast, was undetectable in ganglion primordia, migrating cells or within the adrenal before 17 days. PNMT initially appeared at E 17 in small clusters of cells scattered throughout the adrenal. The number of PNMT containing cells and the intensity of staining increased dramatically from E 17 to term.

A number of experimental manipulations were employed in vivo to investigate the role of glucocorticoids in differentiation of the adrenergic phenotype. Chronic or acute treatment of mothers and/or embryos with various glucocorticoids, adrenocorticotrophic hormone or s-adenosylmethionine did not result in precocious appearance of PNMT. Moreover, the initial expression of PNMT was not prevented or delayed by embryonic hypophysectomy or by treatment with inhibitors of adrenocortical function. After initial expression of PNMT had occurred, cellular levels of PNMT as indicated by the intensity of immunofluorescence, were augmented by glucocorticoid treatment and diminished by embryonic hypophysectomy.

Consequently, the initial expression of PNMT on E 17.0 (1) is not dependent on normal glucocorticoid levels, (2) cannot be induced prematurely by glucocorticoids, and (3) is independent of the pituitary-adrenal axis. However, the ontogenetic increase in PNMT levels after initial expression has occurred does require intact pituitary-adrenal function. Our observations indicate that different mechanisms regulate initial expression and subsequent modulation of neurotransmitter phenotype.

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218.6 Ca^{2+}-INDUCED FUSION OF ISOLATED BOVINE ADRENAL MEDULLARY CHROMAFFIN GRANULES. S.J. Morris[1], D.H. Haynes*[2] and J.D. Robertson[3]. (1) Dept. of Neurochemistry, Max Planck Institute for Biophysical Chemistry, D-3400 Göttingen, FRG, (2) Dept. of Pharmacology, Univ. of Miami Medical School, Miami, FL 33103, and (3) Dept. of Anatomy, Duke Univ. Medical School, Durham, NC 27710.

Chromaffin granules can be demonstrated by thin section TEM techniques to aggregate and fuse in the presence of 1 - 5 mM CaCl. The reaction proceeds by granules aggregating to form pentalaminar double membranes which eventually fuse into a single, continuous trilaminar structure.

The kinetics of Ca^{2+}-promoted aggregation have been investigated in detail (Morris et al., Membrane Biochem. 2:163-202 (1979); Haynes et al., J. Theoret. Biol. 81:713-743 (1979)). Aggregation is initiated by protein-protein contact. Lateral segregation of protein out of the contact region and lateral phase separation of bilayer lipids lead to protein-poor, destabilized bilayer regions in which membrane fusion eventually takes place.

Synexin, a protein which lowers the K_D of Ca^{2+}-aggregation from ∿ 4 mM to ∿ 200 μM, also aggregates other natural and artificial membranes, and therefore probably has no specific role in aggregation in vitro or exocytosis in vivo (Morris and Hughes, Biochem. Biophys. Res. Comm. 91:345-350 (1979)).

218.7 MUSCARINIC REGULATION OF CYCLIC GMP LEVELS AND CATECHOLAMINE SECRETION IN ISOLATED BOVINE ADRENAL CHROMAFFIN CELLS. S. Lemaire, G. Derome, R. Tseng, P. Mercier and I. Lemaire. (SPON. K. Livingston). Laboratory of Molecular Pharmacology, Dept. of Pharmacology, Centre Hospitalier Universitaire, Sherbrooke, Canada J1H 5N4.

Catecholamine secretion and cyclic GMP levels were measured in chromaffin cells isolated from bovine adrenal medulla. ACh and nicotine but not muscarine induced a 7- to 11- fold increase in catecholamine secretion with respective ED_{50} of 10 μM and 2 μM. Cyclic GMP levels were also increased from 3- to 5- fold in the presence of ACh and this stimulation was mimicked by muscarine but not nicotine. Half-maximal stimulations of cyclic GMP levels with ACh and muscarine were respectively observed at 0.1 μM and 0.3 μM. The order of potency of various cholinergic drugs for cyclic GMP stimulation was as follows: ACh > oxotremorine > metacholine > muscarine > furthretonium > arecholine > bethanechol. Pilocarpine, McN-A-343 and AHR-602 were inactive at concentrations between 10^{-8}M and 10^{-3}M. In the presence of isobutylmethylxantine (0.5 mM), ACh stimulation of cyclic GMP accumulation was potentiated 3- fold whereas the secretory effect of nicotine was inhibited by 20%. Further inhibitions of nicotine-induced catecholamine secretion were also obtained in the presence of oxotremorine plus isobutylmethylxanthine or exogenous dibutyryl cyclic GMP. Our results indicate that the nicotinic stimulation of catecholamine secretion from bovine adrenal chromaffin cells may be regulated by cyclic GMP via the stimulation of a muscarinic receptor.

218.8 CENTRAL PATHWAYS REGULATING ADRENAL TYROSINE HYDROXYLASE. Jean-Pierre Gagner*, Serge Gauthier* and Theodore L. Sourkes. Depts. of Biochemistry, Neurology and Neurosurgery, and Psychiatry, McGill University, Montreal, Quebec, Canada.

Investigations of central mechanisms involved in the neural long-term control of adrenal medullary tyrosine hydroxylase activity (ATHA) have implicated inhibitory serotonergic systems (partially localized in the medial raphe nucleus) and excitatory dopaminergic and cholinergic systems, whose effects would be ultimately funnelled through the splanchnic nerves (Quik et al., Brain Res, 122:183, 1977; Lewander et al., JPET, 200:523, 1977).

To assess the role of spinal pathways in ATHA regulation, sympatho-adrenal preganglionic neurons were isolated from their supraspinal afferents by complete cord section at level T2-T3 in rats. This treatment led after 3d to a progressive reduction in ATHA until at least day 10. Vmax for 6,7-dimethyltetrahydropterin in dialyzed homogenates decreased starting at day 3, whereas enzyme activities remained additive in mixing experiments, suggesting reduction in the amount of enzyme protein. Abolition of impulse flow to the adrenal by hemisplanchnicotomy (which itself does not affect ATHA) prevented the decrease of ATHA elicited by subsequent cord section. This result favors a primary neurogenic origin for these effects, which may involve segmental inhibition in the lower part of the cord. This mechanism, possibly mediated by spinal sympatho-inhibitory interneurons (McCall et al., Am J Physiol, 232:H657, 1977), would be unmasked by the disappearance of some transmitters below the level of section.

All centrally active agonists administered after sham-surgery caused distinct increases of ATHA. In spinalized animals quipazine, 5-hydroxytryptophan (with carbidopa) or oxotremorine (with methylatropine) produced decreases of enzyme activity, whereas apomorphine and piribedil had no significant effect. These data suggest that a) 5HT and ACh (muscarinic) receptors with inhibitory actions on ATHA are present in the lower part of the cord, and b) the DA-mediated increase of ATHA originates from area(s) rostral to the thoracic cord.

We reported that cord hemisection at C6-T7 alters a) the resting values of ATHA bilaterally, and b) the inducibility of this enzyme by immobilization stress and by apomorphine (Gauthier et al., Exp Neurol, 66:42, 1979). DA pathway(s) may not mediate predominantly the response of ATHA to immobilization because in addition a) L-dopa alone blocked ATHA increase induced in that way but potentiated that produced by apomorphine (Quik & Sourkes, Biochem Pharmacol, 25:1157, 1976), and b) DA blockers (pimozide, haloperidol) or 6-hydroxydopamine (with protriptyline) did not significantly mitigate the effect of immobilization on ATHA. (Supported by MRC and CRSQ).

219.1 **A SIMPLE METHOD FOR TESTING HUMAN VISUAL PROCESSING.** **W. Riss and M. Cheikin*. Dept. of Anatomy and Cell Biology, SUNY Downstate Med. Ctr., Brooklyn, NY 11203.**

A simple, inexpensive method of testing visual processing in a variety of experimental and clinical settings has been developed. The method relies on the use of a portable movie projector which can run film forward or backward over a range of 3 to 54 frames per second.

In our experimental super 8 mm. films, we have devised tests for measuring thresholds of visual processing and various reaction times to visual displays. There are tests for psychological simultaneity, psychological successiveness, eye movement in horizontal or vertical directions, memory, decision-making latencies, left-versus-right competition and selective attention. For example, eye-movement reaction times can be measured as follows: an initial fixation point is provided and then replaced by a numerical stimulus; at the moment the numerical stimulus disappears, a second stimulus is presented elsewhere for a defined period of time (number of frames); correct reports of the two successive numbers provide a measure of eye-movement reaction time. In another type of test of decision-making time, the number seen first is a logical clue to which of nine fixation points (arranged in a 3x3 array) will be the location of the second stimulus. If a decision is made in time to correctly shift the direction of gaze, the second, briefly presented numeral will be seen at the expected location. Successive trials are systematically arranged to be easier or more difficult for the subject. From similarly constructed films, the additionally mentioned tests are given.

219.2 CONSTRUCT VALIDATION OF VERBAL INFORMATION-PROCESSING SYSTEMS. R. Edward Geiselman,* J. Arthur Woodward,* and Jackson Beatty. (Spon: F. N. Jones). Department of Psychology, UCLA, Los Angeles, CA 90024.

Several physiological and verbal-report measures were recorded as indicants of a small number of hypothetical verbal information processing constructs. The four latent constructs that were studied are: (a) "type of processing" measured in terms of eye movements during study plus a questionnaire, (b) "processing intensity" (activation) measured in terms of heart-rate and GSR variability during study plus a questionnaire, (c) "recall from short-term store" (STS), and (d) "recall from long-term store" (LTS). The recall constructs were measured in terms of six standard verbal-recall performance measures. Alternative individual-differences models specifying the nature of these four constructs and their functional interrelations were evaluated such that a number of predictions made by contemporary theories of verbal learning were assessed. The mathematical identification of the models, the statistical estimation of the parameters, and testing of goodness of fit were all based upon the standard full information maximum likelihood approach.

The analyses showed that a uniprocess conception of memory recall, where STS and LTS are viewed as two general points on a single memory continuum, was as plausible as the dual-process conception. Given the basic uniprocess model, a second experiment confirmed the hypothesis that the "intensity" with which a subject conducts his or her study is associated with an enhancement of memory recall performance that is independent of how the subject studied (meaningful interassociation versus rote learning). However, this enhancement was confined to recall from the portion of the memory continuum that is analogous to LTS. These data bear critically upon the question of process dualism in memory and upon the long-standing question of the effects of "effort" on memory.

Several additional analyses of the eye-movement records suggested that "processing intensity" is not manifested in either the rate of information processing or in the overall number of different bits of information processed. A physiological explanation of the "intensity" construct in terms of neuronal excitability (D. O. Hebb, A textbook of psychology, 1958) was also considered.

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219.3 PARKINSON'S DISEASE: DEMENTIA AND AKINESIA. J.A. Mortimer, F.J. Pirozzolo, K. Lawson-Kerr* and D.D. Webster*. Geriat. Res. Educ. & Clin. Ctr., V.A. Medical Center, Minneapolis, MN 55417, and Department of Neurology, University of Minnesota, Minneapolis, MN 55455.

The dementia of Parkinson's disease is increasingly recognized as a characteristic and important feature of the syndrome. Clinical observations have suggested that dementia is more likely to occur in patients with substantial rigidity and akinesia. We have investigated this relationship in Parkinson patients utilizing batteries of motor and neuropsychological tests.

Fifty-seven patients with idiopathic Parkinson's disease of 1 to 37 years duration were administered a series of automated motor tests, enabling quantification of akinesia, rigidity and tremor. In addition, each patient was given a battery of neuropsychological tests, which included measures of language abilities, perception, verbal and non-verbal memory, concept set shifting, oculomotor scanning, fund of information, rapid graphomotor transcoding and depression. Patients with a history of alcoholism, mental illness or other neurologic disease were excluded from the study.

Neuropsychologic measures could be grouped into four categories on the basis of test performance: 1) visual-spatial reasoning, 2) memory, 3) psychomotor speed, and 4) fund of information. Of these, visual-spatial reasoning, psychomotor speed and a specific subset of memory measures showed a significant negative correlation with three different measures of akinesia. The deficits in memory seen with the akinesia were of an attentional nature. Little relationship was evident between the measures of rigidity and tremor, and neuropsychological performance.

Disease duration, age at symptom onset, and the duration of L-dopa therapy were found to have little association with neuropsychologic function. Age at the time of the test was correlated with performance on a small subset of tests, mostly those involving some component of speed. Within this particular sample of patients, age and education level were not correlated significantly.

The findings indicate that the major motor symptom associated with dementia in Parkinson's disease is akinesia, rather than rigidity. That this dementia may not be continuously progressive is suggested by the lack of correlation of neuropsychological measures with disease duration.

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219.4 COMPARATIVE NEUROPSYCHOLOGY AND KORSAKOFF'S SYNDROME: SPATIAL AND VISUAL REVERSAL LEARNING. S.M. Zola-Morgan* and M. Oscar-Berman. Boston VA Medical Center, Boston, MA 02130.

The single most obvious symptom of alcoholic Korsakoff's disease is a severe anterograde amnesia. However, there has been increasing evidence that other sensory, cognitive and motivational deficits are an integral part of the disease process.

Damage to diencephalic areas (mammillary bodies and/or dorsomedial nucleus of the thalamus) has been most often linked to the memory impairment. However, Korsakoff patients are known to have diffuse damage outside these areas and recent computerized tomography techniques applied to brains of chronic alcoholics has drawn attention to damage in other areas such as pulvinar, other thalamic nuclei and various neocortical regions.

The effects of neuropathology in humans has rarely been examined in the context of experimental paradigms which are known to be valid and reliable tests of nonhuman functional breakdown following brain damage. This comparative neuropsychological approach might be especially promising in investigating the defects in alcoholic Korsakoff patients. First, a number of tests have been developed which allow us to evaluate several aspects of the Korsakoff syndrome in addition to the memory defect. Second, some of these tests are especially sensitive to damage to specific brain areas in animals and might help us identify more accurately the structures involved in the behavioral defects of the Korsakoff patients.

In the present report we used spatial and visual learning paradigms to evaluate several aspects of the alcoholic Korsakoff syndrome including the ability to acquire new stimulus-reinforcement associations with spatial or visual cues, the development of learning-set strategy, and the manner in which stimuli become invested with motivational significance. Alcoholic Korsakoff patients were compared to a non-alcoholic control group and other brain damaged groups.

The Korsakoff patients were impaired relative to the alcoholics and aphasic groups on some spatial and visual problems. In addition, the patterns of errors exhibited on the reversal tasks by the Korsakoff patients suggested that they have difficulty in forming new associations subsequent to unlearning a related old response. Data from the present investigation along with results from additional experiments in this series suggest that it is unlikely that a single lesion is responsible for the behavioral effects observed; a combination of damage to cortical and/or subcortical structures is implicated.

219.5 INTELLECTUAL FUNCTION AND CEREBRAL BLOOD FLOW IN NORMAL AGING AND DEMENTIA. W. A. Dickinson, R. W. Butler*, and J. H. Halsey*. Department of Neurology, School of Medicine, University of Alabama in Birmingham, Birmingham, AL 35294.

The normal aging process has long been associated with a gradual decline in intellectual abilities. A premature, or unusually rapid progressive loss of intellectual abilities is generally considered diagnostic of a dementing process. Changes in cerebral vasculature and decline in cerebral blood flow rates (rCBF) have been associated with both the normal aging process and dementing processes. Relationships between rCBF and age, rCBF and measured intelligence, and measured intelligence and age were examined in this study.

The non-invasive 133-Xenon inhalation method of measuring regional cerebral blood flow was used in the following study to measure regional rates of cortical gray matter blood flow in groups of young normal subjects (19-32 years), aged normal subjects (55-83 years), and patients diagnosed as suffering from a dementing process (39-75 years). The level of intellectual functioning of all subjects and patients was measured by the Weschler Adult Intelligence Scale (WAIS).

Significant negative correlations were found between age and rCBF ($r = -0.66$, $p < .001$) and between WAIS scores and rCBF ($r = -0.67$, $p < .001$) across all subjects and patients. Young normals, aged normals, and dementia groups were found to significantly differ from each other ($p < .05$) on rCBF and on WAIS scores.

Results of this study support the contentions that intellectual functioning and cerebral blood flow gradually decline with age, that intellectual and cerebral blood flow losses are greater in demented patients than their age matched normal controls, and that losses in intellectual functions and decreases in cerebral blood flow are related to one another in both normal and pathologic conditions. In addition, the results of this study suggest that the noninvasive 133-Xenon inhalation method of measuring regional cerebral blood flow may prove useful as a diagnostic tool in dementia.

219.6 DISCRIMINATION OF SCHIZOPHRENICS WITH AND WITHOUT BRAIN DAMAGE USING THE LURIA-NEBRASKA NEUROPSYCHOLOGICAL BATTERY. A. E. Puente, Dept. of Psychology, Northeast Fl. State Hosp., Macclenny, Fl., 32063, C. Sanders* and N. Lund*, Dept. of Psychology, Univ. of North Fl., Jacksonville, Fl.

Examined the differentiation of schizophrenics with and without brain damage using the Luria-Nebraska Neuropsychology Battery (Purisch, A.D., Golden, C.J., & Hammeke, T.A., J. Consult. Clinical Psychology, 46: 1266, 1978).

Subjects included 40 inpatients at a state hospital. Of these, 17 were diagnosed by a psychiatrist and psychologist as and had a history of schizophrenia. Subjects for this group were excluded if there was a history of seizures, alcoholism, substance abuse, head trauma, or other signs of organicity. The remaining 23 patients were similarly diagnosed as schizophrenics and had a confirmed neurological diagnosis on the basis of a medical examination by a physician and/or had a history of brain damage as documented by such tests as CAT scan, EEG, or X-ray. Since patients were tested exclusively for this investigation and were not part of a clinical referral, consent forms were obtained by the test administrator who was blind to their condition.

Chi squares computed on the scores of the 282 items revealed that schizophrenics without brain damage performed significantly ($p \leq .05$) better on 106 of the measures. Differences between groups on the 14 summary scales were determined using t tests and analyses of variance. Schizophrenics without brain damage performed significantly ($p \leq .01$) on all the scales.

These results indicate that the Luria-Nebraska Battery reliably discriminates between schizophrenics with and without brain damage using 38% of the individual items and the 14 summary scales. Preliminary results also suggest the possibility of developing an abbreviated version of this standardized test.

220.1 LONG-TERM RETENTION OF FOREIGN NEUROMUSCULAR SYNAPSES IN FROGS. Alison Longley, Alan D. Grinnell, and Albert A. Herrera. Jerry Lewis Neuromuscular Research Center, UCLA School of Medicine, Los Angeles, CA 90024, U.S.A.

Suppression of foreign neuromuscular synapses by regenerating original nerves has been reported in axolotls and newts. Mutual suppression between two foreign nerves implanted into a frog skeletal muscle has also been reported. In this study, possible competition between foreign and regenerating original nerves in Rana pipiens is being examined.

Cut ulnar (forelimb flexor) nerves were sutured at the border of the thin cutaneous pectoris (c.p.) muscle on the chest wall, and the cutaneous pectoris nerve was cut or crushed. Control frogs were operated on in the same manner, except that the ulnar nerve was left in the armpit to prevent its reinnervating the c.p. Large, adult frogs were used for all experiments.

Foreign nerve input was still present up to 343 days later in some animals. Both foreign and regenerated original nerves were capable of eliciting muscle twitches, though fewer fibers were innervated by the foreign nerve. Twitch tension was measured in normal (1.8 mM) and low (1.0 mM) calcium Ringer as a measure of synaptic safety factor; junctions which become subthreshold to nerve stimulation in lowered calcium are presumed to be of lower quantal content than those which remain suprathreshold. Twitch tension in 1.0 mM calcium for normal c.p. muscles is 98% of the tension obtained in normal Ringer, but the ratio for the regenerated c.p. nerve was 62% (range: 51-72%), and was 40% (range; 29-60%) for the foreign nerve.

Most fibers were singly innervated, with c.p. innervations predominant, but intracellular recording indicated the presence of some fibers doubly innervated by foreign and original nerves. The double inputs have been found both at the same and at widely separated sites.

Tracing methods for distinguishing the innervation patterns of the two nerves will be discussed, including the use of intracellular staining with the fluorescent dye, lucifer yellow.
This work was supported by research grants from the USPHS (NS06232) and the Muscular Dystrophy Association, and by NRSA postdoctoral fellowships to A.L. and A.A.H.

220.2 MOTONEURON TARGET SELECTIVITY IN CHICKS WITH DELETED, DUPLICATED OR REVERSED LIMB SEGMENTS. Virginia Whitelaw* and Margaret Hollyday. Depts. of Biophysics and Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL, 60637.

In the normal chick, limb muscles derived from neighboring positions within the same embryonic muscle mass are innervated by adjacent motor pools. This finding suggests that a normal pattern of limb innervation could be achieved by mechanisms which simply maintain neighbor relations among motor axons. Alternatively, motoneurons might be specified in a position dependent way to exhibit target selectivity for particular limb muscles. The relative importance of these two postulated developmental mechanisms was tested using surgical manipulations which disrupt the normal neighbor relations of embryonic leg muscles. Animals were produced having the following abnormalities: 1) deletion of the calf and foot, 2) deletion of the thigh, 3) serial duplications of the calf, and 4) proximo-distal reversals of the calf and thigh. The motor pools supplying both individual muscles and groups of muscles in the manipulated limbs were mapped using intramuscular injections of HRP. From this we determined whether axons maintained neighbor relations and in some cases innervated abnormal targets, or whether they exhibited target selectivity.

Chick embryos were operated on between stages 18-22, which is before axons have grown into the limb bud. HRP injections were made when the embryos were at stage 37-38, which is after the period of naturally occurring cell death and the establishment of the adult pattern of limb innervation. The morphology of the operated legs was determined after the legs were stained for cartilage. Interesting cases were sectioned and reconstructed to identify the muscles.

The results from all cases of partial limb deletions were consistent: muscles received innervation from their normal motor pools. Selective motoneuron death was observed in motor pools where those limb muscles were absent. There was no apparent compression of the motoneuron projections into the partial limb. Results from proximo-distal reversals and limb segment duplications indicate that a muscle receives innervation from its normal motor pool despite its aberrant position or double occurrence. These findings show that motoneurons exhibit target selectivity under experimental conditions which disrupt the normal neighbor relations of those targets. This suggests that target selectivity is a more important developmental mechanism underlying limb innervation than is maintenance of neighbor relations.
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220.3 DISTRIBUTION OF IPSILATERALLY AND CONTRALATERALLY PROJECTING RETINAL GANGLION CELLS IN XENOPUS LAEVIS AND ITS RE-ESTABLISHMENT FOLLOWING OPTIC NERVE SECTION. S. Hoskins* and P. Grobstein, Dept. Pharm. Physiol. Sci., Univ. of Chicago, Chicago, IL, 60637.

We have used retrograde HRP transport to assay the retinal distribution of ipsilaterally and contralaterally projecting retinothalamic ganglion cells in post-metamorphic Xenopus laevis and to determine whether the normal distribution is re-established during optic nerve regeneration.

Rostral thalamus (a region including the nucleus of Bellonci, the corpus geniculatum thalamicum, and the rostral visual nucleus) was exposed to HRP by multiple pressure injections of a concentrated solution of HRP in distilled water. After several days survival time, the animals were killed. The retinas were removed, fixed briefly, reacted with diaminobenzidine, and prepared as whole mounts. The brains were also processed to evaluate injection location. Typically, in retinas contralateral to the injection, labelled ganglion cells were distributed throughout the entire retina. In retinas ipsilateral to the injection, labelled cells were widely distributed but largely or completely excluded from a nasodorsal region. The results indicate that a large area of the retina gives rise to both an ipsilateral and a contralateral projection; a dorso-nasal region, however, projects largely or exclusively contralaterally.

In a further series of animals we cut or crushed one optic nerve and four to six weeks later exposed rostral thalamus ipsilateral to that optic nerve to HRP. The retinas and brains from these animals were processed as above. In three animals, a typical pattern of extensive labelling through most of the ipsilateral retina with an absence of significant labelling in the nasodorsal region was observed. In four animals, labelled cells were absent or sparse, perhaps due to incomplete regeneration. However, consistent with the finding in the three animals described, there was in these animals no significant labelling in the nasodorsal retina. These results indicate that the normal retinal distribution of ganglion cells projecting to the ipsilateral thalamus can be re-established during optic nerve regeneration.

It has long been known that mechanisms operating during optic nerve regeneration in the frog are adequate to re-establish normal retinotopy in the contralateral optic tectum. Our studies suggest that such mechanisms are also capable of re-establishing the normal bilaterality of the retino-thalamic projection. In addition, they show that large areas of the retina project bilaterally in Xenopus. There is no line of decussation separating one region projecting strictly contralaterally from another projecting strictly ipsilaterally.
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220.4 FORMATION AND LOSS OF A NORMAL IPSILATERAL RETINOFUGAL PROJECTION IN DEVELOPING CHICK. S. C. McLoon and R. D. Lund. Dept. of Anatomy, Medical Univ. of South Carolina, Charleston, S. C. 29403.

Unilateral enucleations performed on chick embryos during their early stages of development resulted in the presence of an ipsilateral retinofugal projection just prior to the time of hatching. Since in rats a similar ipsilateral pathway appears to result from the retention of an embryonic projection, we wondered whether a similar developmental event occurs in the chick, even though in the adult there is normally no ipsilateral projection. Embryos on successive days between days 7 to 16 of incubation received an injection of horseradish peroxidase into the right eye. After 12 hours survival, the embryos were sacrificed and fixed. Sections of the brains were reacted with tetramethyl benzidine and hydrogen peroxide. The left eye was also reacted to be sure that the HRP injection had not involved that eye.

From days 7 to 10 of incubation, HRP label was clearly visible along the optic tract ipsilateral and contralateral to the injected eye. From the chiasm labeled fibers entered the ipsilateral tract along the borders of the alternating laminae of right and left optic axons. The ipsilateral fibers that entered the tract at any one lamina tended to stay together through their entire course. By ten days of incubation, ipsilateral optic fibers could be identified along the border of the lateral geniculate and ectomammillary nuclei and in the superficial layer of the tectum. From days 11 to 14 of incubation an ipsilateral projection cannot be distinguished by the HRP technique. During this time period a large percentage of the retinal ganglion cells normally degenerate, and there is a considerable amount of endogenous peroxidase in the visual pathway which masks the HRP tracer. On days 15 and 16 of incubation, the high endogenous peroxidase level has subsided; no retinal projection can be identified in the ipsilateral optic tract.

It appears, therefore, that in the chick, like the rat, the ipsilateral retinofugal projection resulting from an embryonic enucleation may be due to retention of a normal ipsilateral projection. The loss of the ipsilateral projection in normal development coincides with a period of synap togenesis and cell death in the visual system. The causal interrelation of these events deserves further attention.
(Supported by NIH EY 03314 and EY 03414.)

220.5 ORDER AND DISORDER IN THE ABERRANT RETINOTHALAMIC PROJECTION IN HAMSTERS WITH EARLY TECTAL ABLATIONS. Roberto Lent* and Gerald E. Schneider. Department of Psychology, M.I.T., Cambridge, MA 02139.

A result of a deep ablation of the superior and inferior colliculi in newborn hamsters is the formation of aberrant terminal fields of retinofugal axons in the lateral posterior (LP) and medial geniculate (MG) thalamic nuclei. We were interested in studying the retinotopic pattern within these axonal populations because no locus specificity for optic tract axons is expected in the MG, and little if any is expected in the LP.

We have aspirated the right superior and inferior colliculi of 10 newborn hamsters within the first 30 hours after birth. After the animals were fully grown (12 weeks), they received left intravitreal injections of tritiated proline and fucose, in addition to focal electrolytic lesions placed in different retinal quadrants. After adequate survival times the animals were sacrificed and perfused, and the brains were cut and processed by the autoradiographic and Fink-Heimer techniques.

Results for all the brains reveal a heavy terminal field in the superficial part of the right LP adjacent to the optic tract, frequently extending over most of the surface of the nucleus. Also, all but 1 hamster have a small terminal field in the MG. Six of the animals have only one restricted focus of degeneration in the LP terminal field, and the others have 2 foci. Projection of these foci to the surface of the diencephalon in order to generate a surface reconstruction reveals a consistent retinotopic distribution of the patches of degeneration. This map has an orientation similar to that displayed in the dorsal and ventral nuclei of the lateral geniculate body (LG) — upper retina represented caudally, nasal retina represented closest to the chiasm. In the MG, although restricted parts of the retina do project to restricted parts of the nucleus, no consistent topographic order has been discerned.

The fact that the nasotemporal axis of the map in LP has a polarity similar to that of the LG suggests that the developing retinothalamic axons follow similar ordering rules in each of these structures. Also, the finding of restricted patches of degeneration within the MG, although without a consistent topography, suggests that retinothalamic axons may attain some orderly distribution even in the absence of adequate polarity cues in the target tissue.

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220.6 PREFERENTIAL GROWTH PATTERNS OF CENTRAL VS PERIPHERAL CA NEURITES TO CO-CULTURED IRIS TISSUES. C.F. Dreyfus and S.M. Crain, Dept. of Anatomy, Columbia University, College of P&S and Dept. of Neuroscience, Einstein Medical College, New York, NY.

We have reported previously that catecholaminergic (CA) neurons in fetal mouse brainstem explants from the region of the locus ceruleus grow preferentially toward cells emerging from co-cultured iris explants. This effect does not appear to be mediated by nerve growth factor (NGF) nor by an iris-induced increase in CA neurites (Dreyfus and Crain, Anat. Rec. 196, '80). We have now compared the neuritic growth patterns from central and peripheral CA neurons toward co-cultured iris explants. Portions of brainstem from the region of the locus ceruleus or whole superior cervical ganglia (SCG) were co-cultured with fetal iris (ca. 0.5 mm apart). All tissues were explanted from 18 day fetal mice and maintained up to 2-3 weeks in vitro. The SCG-iris co-cultures were grown in nutrient media (containing human placental serum and chick embryo extract) ± NGF. The brainstem-iris co-cultures were grown in control nutrient media, since these central CA neurites do not appear to be sensitive to NGF (Dreyfus et al., Brain Res., in press). Within 2 days in culture cells grew out in all directions from the iris and contacted the brainstem or the SCG; by 4-7 days these iris cells partially surrounded the co-cultured neural explants. Glyoxylic acid induced histofluorescence revealed that by 7 days, varicose brainstem CA neurites projected heavily toward the iris, arborizing in a network fashion on cells in the iris outgrowth. A few brainstem CA neurites also grew in directions away from the iris. The neuritic branching of these central CA neurons on iris cells resembled patterns seen on neuroglial arrays around co-cultured hippocampal explants, except that no such preferential dense neuritic projections were seen during invasion of the CNS target tissues (Dreyfus et al., Brain Res., 161, '78). In contrast, varicose CA neurites grew out radially from SCG explants as straight projections in all directions. When NGF (30-50U/ml) was reduced or omitted from our nutrient media, these peripheral CA neurites were more abundant and longer in the direction of the iris. In contrast to the brainstem CA neurites, the SCG neurites appeared to grow over the iris cells with fewer arborizations or terminations on them and, in fact, many projected directly into the muscle tissue of the iris explant. Thus, although both central and peripheral CA neurites appear to grow preferentially toward co-cultured iris, the brainstem neurites arborize in a network pattern restricted primarily to the outgrowing iris cells whereas SCG neurites tend to project straight toward the muscle within the iris explant. Supported by grants NS14990 (NIH) and BNS75-03728 (NSF) to SMC, NS12969 (to M.D. Gershon), NS-08770 (to E.R. Peterson) and BRSG grant SO7-RR05395-18 (NIH) to CFD.

221.1 *THE ORIGIN OF BRAINSTEM PROJECTIONS TO THE THORACIC CORD AT DIFFERENT STAGES OF DEVELOPMENT IN THE NORTH AMERICAN OPOSSUM.* T. Cabana* and G.F. Martin (SPON: B. Stokes). Dept. Anat., Sch. Med., The Ohio State Univ., Columbus, Ohio, 43210.

The opossum is born 12 to 13 days after conception and is then available in an external pouch for behavioral observation and experimental manipulation. Previous studies have documented the early growth of brainstem neurites into the spinal cord of the opossum (Martin et al., 1978). Such axons are present in the marginal zone as far caudally as the lumbosacral cord prior to the development of hindlimb motility. In order to identify the suprasegmental origin of spinal axons at different stages of development, horseradish peroxidase (HRP) was injected iontophoretically into the thoracic cord in a developmental series of 24 opossums. Prior to the appearance of spontaneous hindlimb movements (stage I of Martin et al., 1978) and before there is evidence that brainstem axons grow into the intermediate (mantle) zone, HRP injections labelled neurons in the nucleus reticularis medulla oblongata ventralis, the nucleus lateralis reticularis, the medullary raphe nuclei, the nuclei reticularis gigantocellularis and gigantocellularis pars ventralis, the inferior, medial and lateral vestibular nuclei, the pontine reticular formation and the presumptive coeruleus complex. Some of these areas contain neurons which synthesize monoamines at this stage and monoaminergic varicosities are present in the marginal zone of the cord (Humbertson and Martin, 1979). At subsequent stages of development neurons can also be labelled in the spinal trigeminal nuclei, the solitary complex, the dorsal column nuclei, the midbrain tegmentum, the red nucleus, the interstitial tegmental area, the interstitial nucleus, the nucleus of Darkschewitsch and the paraventricular hypothalamic region. Each of these areas has been shown to project to thoracic levels in the adult opossum (Crutcher et al., 1978). These data indicate: 1) that reticular, raphe and vestibular nuclei of the pons and medulla project spinalward very early in development and 2) that sensory relay nuclei as well as cell groups in the mesencephalon and diencephalon innervate spinal levels somewhat later. (Supported by U.S.P.H.S. Grant NS-07410.)

221.2 *GROWTH OF THE CORTICOSPINAL TRACT IN NEONATAL RATS.* D.J. Schreyer*, and E.G. Jones. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The rat corticospinal projection was investigated using the anterograde and retrograde transport of horseradish peroxidase as a marker for growing axons and their cells of origin. At birth, corticospinal fibers arise exclusively from large pyramidal cells of an already distinct layer V and form a massive bundle extending just past the pyramidal decussation into the posterior white columns of the upper cervical segments. The decussation is often disorderly at this stage and additional small fiber bundles can be seen both descending through the lateral gray matter of the posterior horn and deflecting rostrally toward the dorsal column nuclei. Over the next few days, the corticospinal tract extends the length of the cord, always led by a few fibers a segment or two ahead of the major bundle. Although terminal ramifications are seen in the pons at birth, outgrowth into the gray matter of the spinal cord segments past the decussation is delayed a few days after the tract has passed by, forming a rostrocaudal, temporal gradient. Complete innervation of the gray matter of the cervical enlargement is not seen until about the time when callosal and thalamic afferents in the cortex change from a diffuse to a more orderly pattern of distribution and when pyramidal cells establish their definitive dendritic configuration (Wise and Jones, J. comp. Neur. 175, 129; 178, 187; Wise, et al. Neuroscience 4, 1275). Older animals display a more orderly decussation, though the lateral fibers persist and extend to lower cervical segments. Terminal ramifications in the gray matter of the lumbosacral enlargement appear only after the first week.

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221.3 CHANGES IN TIBIALIS ANTERIOR MUSCLE OF SPINAL CAT WITH LONG-TERM ELECTRICAL STIMULATION. J.T. Mortimer and U. Roessmann*, Applied Neural Control Lab, Dept Biomedical Engineering and Division of Neuropathology, Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106.

The affects of electrically induced exercise have been studied in the tibialis anterior muscle of cats that have been paralyzed for more than 7 months. Unstimulated muscles of the spinal animal showed changes that are consistent with disuse. The cross sectional area of all muscle fiber types was uniformly reduced by approximately 30% when compared to a similar group of unspinalized control muscles. The composition of the unstimulated paralyzed muscle in the superficial region was largely FG (65% compared to 37% for the normal). The FO population had decreased from 56% found in the normal to 34%, and the SO group decreased from 7% as found in the normal to 2%. Continuous stimulation of the paralyzed muscle at 10 Hz yielded a fatigue resistant muscle producing a lower peak force than the unstimulated paralyzed control. The muscle was composed almost entirely of SO fibers without significant change in cross sectional area when compared to its unstimulated control. *Employing an interrupted stimulation* pattern produced a fatigue resistant muscle without compromising the peak force production. The cross sectional area of FO fibers in the muscle was unchanged, whereas the SO fibers showed significant hypertrophy compared to the unstimulated paralyzed control. Stimulation for 8 hours at 10 Hz, 2.5 sec on 2.5 sec off (average 5 Hz) resulted in SO fibers with a cross sectional area of 83% of the normal control. Stimulation for 8 hours with a 30 Hz burst pattern, with interrupting rest periods (average frequency 5 Hz), resulted in SO fibers with an average cross sectional area 113% of the normal control SO group. These results are consistent with those found in human spinal cord injury patients, where increased fatigue resistance and increased muscle bulk have been observed with electrically induced excercise (Peckham et al, Clinic Ortho and Relat Res, 1976, 326). They were not found in the stimulated normal cat.
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221.4 **MOTOR CORTEX ORGANIZATION AFTER FOCAL INJURY IN THE RAT** **M. G. Boyeson, D. M. Feeney, R. T. Linn, W. G. Dail and H. M. Murray Departments of Psychology and Anatomy, University of New Mexico, Albuquerque, NM 87131**

After injury to a portion of motor cortex there is a severe loss of some motor functions but there is a dramatic recovery within weeks. The hypothesis that neighboring cortical areas take over some functions of the damaged area has a long history as a mechanism of recovery. We directly tested this hypothesis by conducting detailed mapping of the movements evoked by microstimulation of the cortex at varying times after injury. Experiments were conducted in the rat since the motor cortex is readily accessible and shows a reliable organization. A unilateral lesion of the hindlimb (HL) cortical area was performed by undercut laceration, suction ablation or contusion. The hemispheres were exposed under Nembutal anesthesia at 1, 4, 9, 15, 30 days *and 6 mo. - 1 yr after injury. The effects of forelimb (FL) area* lesions were also studied but less extensively. *The cortex was* stimulated with 300 cps train of .5 msec pulses at currents from 5 - 300 μA in a grid pattern of approximately 125 points spaced *.5 mm on each hemisphere. The areas giving rise to FL and HL* movement were studied in detail as was the cortex between these areas we denote as synergistic trunk region (ST). This ST area gave synergistic movement of FL and HL as well as some trunk movement. The type of FL movement evoked from the ST area was stereotyped and qualitatively different from the movements evoked from the FL area. The closer to the FL area within ST the stronger the forelimb component and the closer to the HL area within ST the stronger the HL component. After destruction of HL cortex no HL movements could be evoked by stimulation at any point on the cortex at any time postinjury. The HL component of the response evoked from the ST area also disappeared and did not return. However, the FL component of the response evoked by stimulation in the ST region did recover to its normal extent by 9-15 days. Similar results were seen following lesions of the FL area. The data indicate that after motor cortex injury neighboring intact cortex does not reorganize to take over the function of the damaged area. Additionally the data indicate two remote effects of cortical injury. Within the intact ST area following lesions of the HL area, HL components can no longer be evoked and the ability to evoke FL components is depressed for 9-15 days.

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221.5 RECOVERY OF LOCOMOTION FOLLOWING SYMMETRICAL AND ASYMMETRICAL SERIAL LESIONS IN RATS. A. Gentile, J. Held*, R. Muzii*. Teachers College, Columbia University, New York, N.Y. 10027.

In prior study of locomotor deficits resulting from damage to motor cortex in rats, two-stage removals produced a more marked initial impairment followed by faster recovery in comparison to bilateral one-stage removals (Gentile, et.al., Beh. Biol., 1978, 22, 417-455). As serial lesions had been placed asymmetrically (unilateral followed by contralateral cortical ablation), it was proposed that unilateral damage may have stimulated increased synaptic activity in the contralateral homolog which, in turn, may have activated alternative pathways mediating recovery. Symmetrical damage involving simultaneous bilateral removals may have resulted in a different mode of functional reorganization.

The present study examined locomotor performance in rats following first-stage damage of motor cortex placed bilaterally or unilaterally and following second-stage damage extended to equal size bilaterally. Rats were trained preoperatively to traverse a narrow, elevated runway. First-stage lesions were of 4 types: bilateral ($4mm^2$ each side) of either trunk or hindlimb areas (Hall & Lindholm, Brain Res., 1974, 66, 23-38.), bilateral combined trunk and hindlimb areas, or unilateral motor cortex ($18mm^2$). Testing was initiated 14 days postoperatively and continued until preoperative criterion levels were attained. Subsequently, all lesions were expanded to uniform bilateral size ($18mm^2$ each side). After second-stage damage (14 days), testing resumed until criterion levels were re-attained.

Following first-stage removals, the unilateral lesion produced most impairment; the combined trunk and hindlimb lesion resulted in moderate deficit; whereas, trunk or hindlimb lesions produced no impairment in comparison to controls. Movement analysis from high-speed film data indicated a contralateral hindlimb deficit in unilaterally damaged rats.

Following second-stage removals, the expansion of bilateral lesions produced marked lobomotor deficits, with the expanded trunk-hindlimb lesion leading to faster recovery. Contralateral removal following unilateral damage resulted in less impairment than the symmetrical serial lesions. Movement analysis of locomotion following asymmetrical serial lesions now indicated a bilateral hindlimb deficit. These results demonstrate that deficits following second-stage lesions were an inverse function of impairment produced by first-stage damage. Secondly, asymmetrical rather than symmetrical serial lesions resulted in substantial sparing.

221.6 BEHAVIORAL EXAMINATION OF HETEROTOPIC NEURAL TRANSPLANTS IN THE RAT CEREBELLUM Robert B. Wallace, Gopal D. Das*, Michael Molyn*, Charlotte Coren*, and Craig Hawkins*, Developmental Psychobiology Laboratories, University of Hartford, West Hartford, CT 06117.

Recently successful transplantation of embryonic neural tissue into the brains of neonatal and adult host animals has been achieved in several investigations (Das and Hallas, Experientia, 34: 1304, 1978; Stenevi, Björklund and Svengaard, Brain Res., 114: 1, 1976). This transplanted tissue becomes fully integrated into the host brain and, one may conceive, may also be functionally integrated as well. In an effort to test this hypothesis, three groups of male Long-Evans hooded rats were subjected to extended behavioral testing. One group received midline surgical lesions of the cerebellum at 10 days of age; a second group received midline cerebellar transplants of cortical tissue from 17 day embryos at 10 days of age and the third group represented the control condition. All animals were evaluated behaviorally by a battery of tests that included the following: King Emotionality Scale, Neurological Examination, Open Field, Activity Wheel, Elevated Walkway and Rope Climb. Following behavioral testing the animals were sacrificed by transcardial perfusion, the brains cut at 7μ in the coronal plane, and stained with H&E. The results indicated compression lesions of approximately 80% of the host cerebellum and the transplants appeared to be fully integrated with the parenchyma of the host brain. Behavioral results indicated no differences between groups on the King Emotionality Scale; however, on all other tests both control and transplant animals were significantly different from the lesioned animals and not significantly different from each other. It was not possible to run the lesioned animals on the last test (Rope Climb) so impaired were they, but no substantial differences were observed between the control and transplant animals on this task. These data may be taken as suggestive of functional plasticity within this model. Further behavioral testing with this model is intended using other motor systems and also examining the effects of heterotopic tissue transplantation into sensory structures.

221.7 DEVELOPMENT OF THE PRIMARY EVOKED POTENTIAL AND THE STRYCHNINE SPIKE IN MOTOR-SENSORY CEREBRAL CORTEX OF KITTENS. M. D. Mann and K. A. Follett*. Dept. of Physiol. Biophys., Univ. of Nebraska Medical Center, Omaha, NE 68105.

The development of the surface primary evoked potential recorded from the postcruciate forepaw focus and of the corticofugal reflex discharge recorded from the ipsilateral medullary pyramids were studied in kittens at 1,4,7, and 14 days of age, anesthetized with α-chloralose, paralyzed with decamethonium, and artificially respired. The evoked potential and corticofugal reflex discharge were induced by stimulation of each of the 4 paws at frequencies of 1/sec, 1/2sec, 1/5sec, and 1/10sec, using pulses of 25 mA and 0.1 msec. The corticofugal reflex discharge was absent in newborn kittens, making its first appearance at about 14 days. The surface potential evoked by stimulating the contralateral forepaw (CF) was primarily a purely surface-negative event with a latency of 50 msec at 1 day. By 4 days, the initial positivity of the potential had developed, the negativity was reduced in amplitude, and the latency shortened to 37 msec. At 7 days, the positivity was larger but shorter, the negativity was smaller but longer, and the latency dropped to 30 msec. At 14 days, the latency decreased to 18-20 msec and the potential had a larger positivity. Responses to stimulation of the other 3 paws were similar at all ages, usually small, purely negative waves or small negative waves preceded by a smaller positive wave. These were seldom larger than 500 μV peak-to-peak.

Topical application of strychnine sulfate at the recording site produced a strychnine spike in nearly every animal. As in adults, the strychnine spike is an enhancement of both positive and negative phases of the primary evoked potential for CF stimulation, but no change was seen in responses to stimulation of any other paw. At 1 day, the primarily negative response was replaced, following strychnine, with a positive-negative response and, once the positivity developed, it did not disappear for the duration of the experiment--up to 8 hrs. No enhancement of the corticofugal reflex discharge evoked by CF stimulation at weak intensity was seen, as is always seen in adults. The strychnine enhancement seldom lasts longer than 1 hr in adults, but it lasts from 2.5 to more than 4 hrs in kittens.

Towe and Mann (Exp. Neurol. 39:395-413, 1973) concluded that topical strychnine has a direct effect primarily on superficial sa neurons and only an indirect effect on m neurons and that the enhanced discharge of sa neurons accounts for the strychnine spike. On embryological criteria, Mann (Brain Res. Rev. 1:3-45, 1979) deduced that sa neurons were probably still migratory and therefore inactive in newborn somatic cerebral cortex. If this is true, then topical strychnine effects m neurons in newborn cortex.

221.8 REGENERATION OF TROCHLEAR NERVE IN GOLDFISH: MULTIPLE SPROUTS AND ECTOPIC MUSCLE CELLS. S. S. Scherer*, S. S. Easter, Jr., and L. J. Fisher. Departments of Zoology and Anatomy, Univ. Michigan, Ann Arbor, MI 48109.

Cajal reported that peripheral nerve fibers, after being cut or crushed, sprout multiply around the lesion. The number of fibers in the distal stump exceeds the number centrally; this excess is very great at first, diminishes with time, but persists after regeneration and myelination (Cajal, Degeneration and Regeneration in the Nervous System, 1928). These conclusions were based on observations made with the light microscope, which can not resolve small non-myelinated fibers. We have checked these observations electron microscopically.

One trochlear nerve per goldfish (8-10 cm, N=16) was cut or crushed intraorbitally, about 0.5 mm from the superior oblique muscle. Animals were killed 2, 4, 8, 13, and 107 days after surgery. The entire intraorbital portion of the nerve and the attached muscle were prepared for electron microscopy. Cross sections of the nerve were taken both central and peripheral to the lesion. Contralateral (control) nerves were prepared similarly. Electron microscopic photomosaics (6400X) were constructed and the number of fibers counted. Questionable regions on the photomosaics were reexamined on the section at higher magnifications.

The peripheral and central parts of the control nerves and the central stumps of lesioned nerves had about 80 fibers, mostly myelinated. Unmyelinated fibers were first seen in the distal segment at 2 d after surgery, reached a peak of more than 300 at 8 d, and dropped thereafter. The first myelinated fibers in the distal segment were seen at 8 d, and their numbers increased to a mean of 130 by 107 d. Thus, the original light microscopic observations were confirmed electron microscopically.

Ectopic muscle cells were found in and around the regenerated trochlear nerve, hundreds of μm from the superior oblique muscle. They resembled the small red fibers found in normal muscle: they were a few μm in diameter and hundreds of μm long; they had normal sarcomeric structures (Z bands, hexagonally arranged thin and thick filaments, and transverse tubules) and in some cases, synapses. The origin of the presynaptic process is unknown. These mature ectopic muscle cells were present only at 107 d, but fibroblast-like cells with cytoplasmic islands of organized myofilaments were seen at 13 d; they are probably the precursors. It is possible that the regenerating nerve fibers induced the development of the ectopic muscle cells.

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222.1 PROGRESSIVE TOPOGRAPHIC REORGANIZATION OF REPRESENTATIONS OF THE HAND WITHIN AREAS 3b AND 1 OF MONKEYS FOLLOWING MEDIAN NERVE SECTION. M. M. Merzenich*, J. H. Kaas, R. J. Nelson, J. Wall, M. Sur, and D. J. Felleman. (Spon. W. Jenkins). Coleman Lab., Univ. of Calif. at San Francisco, San Francisco, CA 94143; and Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Microelectrode mapping studies within "SI" in primates have revealed that there are two complete, highly ordered representations of the hand surfaces within cytoarchitectonic Areas 3b and 1. In this study, we ask the question: Is there an occupation of cortex "silenced" by a partial deafferentation of these topographic skin surface representations? To answer this question, the representations of the hand were mapped in detail (receptive fields were defined for neurons, in each of about 200 to 600 penetrations within the hand representation) in normal adult owl and squirrel monkeys and/or: a) Again defined in detail immediately following median nerve section; b) mapped 2-11 months after the median nerve was section and tied (to prevent regeneration); or c) remapped several times during the weeks immediately following nerve section.

The "silenced" area of cortex normally representing the skin innervated by the median nerve was progressively occupied by input from bordering ulnar and radial nerve skin fields. Occupation of "silenced" cortex was complete within about 3 weeks after nerve section. In Area 3b of squirrel monkeys (but not owl monkeys) there was a small, fragmentary representation of the finger dorsi immediately "unmasked" within the former area of representation of the volar surfaces of the fingers innervated by the median nerve. In both species, a field of representation of the finger dorsi expanded progressively in Area 3b. When reorganization was complete, this new representation of the finger dorsi was spectacularly topographic, larger than the former representation of glabrous digits in this cortical region, many times larger than the still existent dorsal finger representations elsewhere in the map, and represented the dorsal surfaces of the fingers corresponding with the anesthetic ventral median nerve digital surfaces. The radial nerve representation of the hand dorsum expanded similarly and topographically, especially in Area 1,into the cortical region formerly representing the radial aspect of the glabrous palm. There was a great expansion of the representation of skin surfaces innervated by the ulnar nerve along the median-ulnar border into the former median nerve cortical territory representing its more medial palmar surfaces. During the observed reorganization process, sites of representation of given skin loci were inconstant, re cortical surface vasculature. Finally, no peripheral sprouting into the median skin field was evident. Considered with studies of sensation in patients with such lesions, these studies indicate: a) That there can be no fixed "perceptual maps" within these cortical fields; and b) that there is likely normal use-dependent competition for cortical territory within these fields in adult owl and squirrel monkeys.

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222.2 ORGANIZATION OF THE REPRESENTATIONS OF THE HAND IN AREAS 3b AND 1 OF POSTCENTRAL SOMATOSENSORY CORTEX OF MONKEYS AFTER SECTION AND REGENERATION OF THE MEDIAN NERVE. J. T. Wall, M. M. Merzenich*, M. Sur*, R. J. Nelson, D. J. Felleman*, and J. H. Kaas. Depts. of Psychology and Anatomy, Vanderbilt Univ., Nashville, TN 37240, and The Coleman Memorial Laboratory, Depts. of Otolaryngology and Physiology, Univ. of California at San Francisco, CA 94143.

Previous microelectrode mapping studies have shown that two separate and orderly cutaneous representations of the hand exist in Areas 3b and 1 of monkeys. A second set of investigations revealed that these representations are modified when the median nerve, which innervates the glabrous skin of the radial hand, is transected and prevented from regenerating. Several weeks following the median nerve transection, cortex previously activated by the median nerve is completely responsive in a somatotopically organized manner to stimuli on skin innervated by the ulnar and radial nerves. The present study asks the question of what happens to this reorganized cortex when the median nerve regenerates? To answer this question and obtain information relevant to the issue of recovery of function, the median nerve was sectioned and reconnected by suture of the epineurium in squirrel and owl monkeys. After regeneration periods of 3-12 months, maps of the hand representations in Areas 3b and 1 were derived from multiunit responses recorded in systematic grids with 250-400 recording sites approximately 150-300 μ apart in each animal. Cortical organization following regeneration was abnormal in several ways. 1) Parts of cortex normally activated by the median nerve were activated instead from skin innervated by the radial or ulnar nerves. Thus, regeneration of the median nerve was either incomplete so that some of the cortex taken over by the ulnar and radial nerves after median nerve section remained altered, or regenerated median nerve inputs were unable to displace ulnar and radial inputs and reestablish priority for activating this cortex. 2) Parts of cortex normally activated by the median nerve became reactivated by skin areas innervated by the median nerve. Thus, part of the median nerve was able to regenerate, "compete," and "recapture" parts of its original cortical field. 3) Recording sites in this "recaptured" cortex were typically characterized by multiple and non-overlapping receptive fields, indicating that many regenerated axons grew back to inappropriate skin locations. 4) Cortex driven by the regenerated median nerve exhibited no simple somatotopic organization. Thus, the reoccupation of cortex by the regenerated median nerve did not depend on a normal somatotopic pattern of peripheral connections.

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222.3 VARIABILITY IN THE PROPORTIONAL REPRESENTATIONS OF THE HAND IN SOMATOSENSORY CORTEX OF PRIMATES. R. J. Nelson, M. M. Merzenich*, J. Wall, M. Sur, D. J. Felleman and J. H. Kaas, Coleman Mem. Lab., Univ. of Calif. at San Francisco, San Francisco CA, 94143 and Dept. of Psychology, Vanderbilt Univ., Nashville TN, 37240.

The representations of the hand in somatosensory cortical areas 3b and 1 were mapped in normal squirrel monkeys (Saimiri sciureus). At an inter-penetration spacing of 100-300 microns, minimal receptive fields were determined for neurons within each penetration by stimulating the skin surface with hand held probes. Cortical areas of representation of hand surfaces were measured planimetrically. These measurements led to the following conclusions: 1) The total representational area of the hand varies significantly in different individual adult squirrel monkeys. 2) There were large differences in the proportional areas of representation of different skin surfaces of the hand in Areas 3b and 1 in any given individual monkey. 3) The topography of Area 3b, as well as the real and proportional areas of the representation of different hand surfaces is relatively constant. 4) In general, there was a common basic pattern of topographic organization of Area 1 in all squirrel monkeys studied. There was however, significant variability in both the real and proportional areas, and the detailed internal topography of its representation of the hand. For example: a) There was a several-fold variation in the actual or proportional areas of the representation of glabrous digital surfaces within Area 1. b) Significant individual variations in the relative sizes of representations of specific digits in Area 1 were seen. c) The glabrous surface of the first digit may be represented adjacent to the second digit or in a cortical region far removed from the second digit's representation. d) Although the pattern of representation of the hairy surfaces of the hand was similar in different monkeys, there were clear individual differences in the topographic relationships of these skin surfaces to the representations of glabrous digits and palmar pads. e) In some monkeys, the representations of the glabrous digital surfaces were continuous across the 3b-1 border, while in others, a representation of the volar pads (sometimes large in area) intervened.

The fact that proportional representation of the receptor surface in a given cortical area varied in these ways in different individuals, suggests that there are strong "use-dependent" or other mechanisms directing or modifying the representations of different sectors of the skin surface in primates.

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222.4 EVIDENCE FOR MODULAR SEGREGATION OF "SLOWLY ADAPTING" AND "RAPIDLY ADAPTING" NEURONS IN AREA 3b OF SOMATOSENSORY CORTEX OF MONKEYS. Mriganka Sur*, John T. Wall, and Jon H. Kaas (SPON: Sandra M. Wall). Depts. of Psychology and Anatomy, Vanderbilt University, Nashville, TN 37240.

Single and multiunit recordings were used to determine the spatial distribution of "slowly adapting" (SA) and "rapidly adapting" (RA) neurons in the representation of the glabrous hand in Area 3b of six owl monkeys anesthetized with ketamine HCL. Responses of neurons to repeated one second step indentations of the skin were recorded and displayed in post-stimulus time histograms. All studied neurons were characterized by transient responses at stimulus onset and often at offset, and by brief periods of reduced activity after each transient burst. However, SA neurons also discharged at higher than spontaneous rates during the maintained indentation. SA neurons were recorded commonly in the middle cortical layers, and most had somewhat higher stimulus thresholds than RA neurons. Of 127 single cells recorded in four intensively studied monkeys, 38 were SA and 89 were RA. Electrode penetrations perpendicular to the cortical surface could be classified as encountering only RA neurons or largely SA neurons. In closely spaced grids of such penetrations placed 100-200 μ apart to span the entire digit 3 or digit 4 representation in Area 3b, it was apparent that SA and RA neurons were not randomly distributed throughout cortex but were grouped separately in predominantly rostrocaudal bands. Electrode penetrations angled mediolaterally relative to the brain surface confirmed these observations. The exact positions of the SA and RA bands varied in the representations of particular digits. Among patterns observed were one SA band flanked by RA bands within a digit representation and bifurcation of bands. Bands also appeared to be continuous across the representations of adjacent digits. SA bands were most narrow caudally, in the representation of the proximal phalanx, and broader rostrally where the distal phalanx is represented. Band widths varied from 200 μ to 600 μ. Receptive fields for neurons in RA and SA bands were seen to overlap so that the entire digital skin was represented by both RA and SA cells separately. These experiments, along with similar data on cynomologus monkeys, suggest a functional segregation of receptor types within Area 3b of postcentral somatic cortex in monkeys. SA cortical bands must receive some of their input from cutaneous slowly adapting peripheral receptors while RA cortical bands can be most easily related to rapidly adapting cutaneous receptors.

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222.5 SOMATOTOPIC ORGANIZATION IN THE ANTERIOR ECTOSYLVIAN SULCUS IN THE CAT. H.R. Clemo* and B.E. Stein (SPON: R.F. Spencer). Dept. of Physiology, Medical College of Virginia, Richmond, VA 23298.

It had traditionally been assumed that somatosensory, auditory, and visual cortices each have three distinct topographic representations. Recent observations in the cat showing numerous additional topographically organized areas of visual cortex have fundamentally changed our concept of the organization of sensory cortex. The presence of projections from somatic thalamus to regions outside the traditional somatic cortical areas such as the anterior ectosylvian sulcus (AES) in the cat, raises the possibility that there are more than three somatic cortical representations in this species. The present study examines the somatic representation in the AES.

Receptive fields (RFs) of single and multi-units were recorded in the walls of the AES and along the anterior ectosylvian gyrus (AEG) in 12 cats. The animals were initially anesthetized with ketamine hydrochloride, immobilized with gallamine triethiode and artificially respired with a mixture of 25% O_2 and 75% N_2O.

A complete topographic representation of the body was found on the AEG corresponding to the well known second somatic (SII) area. However, the representation in the AEG was found to extend well beyond the previously described boundaries of SII, reaching the lip of the AES. At the lip of the AES, additional representations of the contralateral forepaw and hindpaw were found which appear to belong to a separate map of the body.

This "new" (SIV) map was a mirror image of that seen in SII: forepaw was rostral on the lip of the AES with forelimb ventral to it (on the medial wall of the AES), and hindlimb was caudal on the lip with the hindlimb ventral. Trunk was represented ventral to the limbs and facial RFs were located in the most rostral portion of the AES.

In addition to a progression of RFs across the body surface in the SIV representation, there was a marked tendency for RFs to increase in size with depth in the AES. Thus the sequence of RFs was often: paw; paw and distal limb; paw and entire limb; paw, limb and trunk. The incidence of bilateral RFs (some covering the entire body) increased in the deep, ventral part of the AES, especially in the fundus and the caudal regions.

Thus, it appears that there are at least four somatotopically organized cortical areas in the cat. The functional roles that these separate sensory regions perform remains to be explained.
(Supported by grant NS 15912.)

222.6 COMMON FEATURES IN ORGANIZATION OF SENSORIMOTOR CORTEX IN PROSIMIAN PRIMATES, GALAGO CRASSICAUDATUS AND NYCTICEBUS COUCANG. Mary Carlson, Carol Welt and Kathleen FitzPatrick, Children's Hospital Medical Center, Boston, MA 02115 and Central Wisconsin Center, Madison, WI 53704.

Our studies of prosimian primates originate from an interest in the evolution of the hand and somatic sensory cortex (SmI) in Old World primates. Our intent is to determine the common features in organization of SmI and adjacent regions in several Lorisidae (two African and one Asian species, each showing unique sulcal patterns and behavioral adaptations) as suggestive of cortical organization in ancestral prosimians.

We have examined the classes of submodal input, topographic organization and cytoarchitectural correlates in SmI in both awake behaving and anethetized Nycticebus, as we have done previously in Galago. Those features in Nycticebus closely resembling Galago are: (1) one precisely organized projection from glabrous palm and digits responding to low threshold (LT) cutaneous stimulation in the rostral region of SmI, with fields on the dorsal glabrous tips at the rostral border of this area; (2) one projection pattern from the dorsal hairy digits located caudally in the hand area with fields on the dorsal hairy digits adjacent to more rostral ventral palm fields; and (3) the correspondence of the LT cutaneous projection area with the dense granular field in sensorimotor cortex.

The differences noted in Nycticebus have been: (1) the consistent finding of a small (less than $1mm^2$) area responding to LT stimulation of glabrous palm and digits, medial to the dorsal hand region and caudal to the major glabrous region in anesthetized animals; (2) more neurons responding to passive joint movement in the dorsal hand region than in the glabrous region in awake animals, which is more consistent with the distribution of submodal input in the Old World simian, Macaca.

We are continuing our study of these novel features in Nycticebus, re-examining these details in Galago, and beginning studies on SmI organization in Perodicticus potto. We believe that intensive analysis of all classes of submodal input, precise topographical details, and morphological and cytoarchitectonic correlates on a variety of prosimian primates is necessary prior to the assessment of homologous areas in the sensorimotor cortex of Old and New World primate species.
(Supported by NSF grant BNS 79-14103)

222.7 NEURAL CODING OF KINESTHETIC INFORMATION IN SI CORTEX OF MONKEYS. E.P. Gardner and R.M. Costanzo, Dept. of Physiology & Biophysics, NYU Medical Center, 550 First Avenue., New York, N.Y. 10016.

The sense of kinesthesis involves appreciation of joint position or velocity of movement. To study neural mechanisms used to encode kinesthetic information in somatosensory cortex, we recorded in awake monkeys from 228 single neurons responsive to joint movement or specific postures of the forelimb or hand. 37 neurons were identified as joint neurons and 26 as muscle neurons based on responses to joint capsule and muscle belly palpation; the remainder were characterized functionally.

Kinesthetic neurons were divided into 3 groups depending on their firing patterns. Rapidly-adapting neurons (44%) responded only to limb movements, with no tonic responses to limb posture over the entire range of joint excursion. Movements in the preferred direction (on-direction) produced a burst of impulses proportional both to the velocity of movement and to initial limb posture. The remaining 56% showed tonic discharges related to static joint angle over at least part of the range of joint movement. These neurons increased their firing rates with increasing degrees of flexion or extension, showing maximum excitation at the extremes of joint position in the on-direction.

Two populations of tonically active neurons were observed. These were distinguished by their sensitivity to the velocity of movement, the size of their responsive angle, and the phase relation of their responses to sinusoidal joint movement. Slowly-adapting neurons (43%) showed overshooting responses during on-direction movements proportional to the velocity of motion, and low-frequency, irregular discharges during maintained postures. They were excited by movements over most of the range of joint angles, but fired tonically to postures comprising less than 60 deg of joint excursion. Sinusoidal joint movement modulated unit firing rates sinusoidally with maximum activity in phase with joint velocity. We propose that such neurons provide information mainly about velocity and direction of movement, and contribute postural information only at the extremes of joint excursion.

Postural neurons (13%) are relatively insensitive to limb velocity; their firing rates reflect only static joint angle. They show no overshoot in activity during on-direction movements, but simply increase firing to a higher level proportional to the new posture. They are tonically active over the entire range of joint excursion, showing higher and more regular firing rates than slowly-adapting neurons. Sinusoidal limb movements produce maximum firing in phase with limb position. These response features make postural neurons optimally suited for coding joint position. (Supported by NIH Grants NS11862 and RCDA NS00142 and an award from the Irma T. Hirschl Trust).

222.8 MULTIPLE-JOINT NEURONS IN S-I CORTEX. R.M. Costanzo and E.P. Gardner, Dept. of Physiology, NYU Medical Ctr. New York, NY 10016

Kinesthesis involves not only an appreciation of static position or velocity of movement of individual joints, but perception of the angles of adjacent joints relative to one another. This involves synthesis of a picture of the entire body in space, and could be provided by the activity of higher-order feature-detecting neurons which integrate information from several joints. We here report on 54 such multiple-joint neurons (MJNs) in somatosensory cortex of alert monkeys. MJNs receive convergent information from two or more adjacent joints, and use their firing patterns to encode the posture of an entire limb.

Most MJNs in our sample were related to postures of the hand, particularly those involved in grasping. For hand receptive fields, we found an equal number of MJNs as single-joint neurons (SJNs), whereas for elbow and shoulder receptive fields, the ratio of MJNs to SJNs was 1:3. MJNs with hand receptive fields were excited by flexed postures of the digits and wrist, or by extended postures, but not by complex arrangements of individual fingers, nor by extended fingers and flexed wrist or vice versa. MJNs related to proximal joints showed a much greater variety of joint combinations.

Two basic types of joint interactions were observed. The simpler neurons responded to postures of several different joints such as wrist flexion or elbow extension. However, combination of the two preferred postures produced no further increase in firing compared to assumption of either one of them. The more complex cells show facilitation or inhibition between the angles of adjacent joints. For these neurons, responses were graded with joint angle, and there was an optimum or preferred position for both joints which gave the strongest response.

Three-fourths of the MJNs showed tonic discharges proportional to static joint angles, and 1/3 of these cells were insensitive to stimulus velocity (postural kinesthetic neurons). This suggests that MJNs might be important for encoding limb postures rather than the dynamics of movement.

We conclude that MJNs provide a neuronal substrate for extracting postural information from several different populations of simple kinesthetic neurons, and that this activity could be used to depict particular postures of the arm. Such integrated information may be important for motor behavior where one has to orient an entire limb in a specific posture to accomplish the desired goal.
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222.9 AFFERENTS TO IMMATURE SOMATOSENSORY CORTEX IDENTIFIED WITH HORSERADISH PEROXIDASE. D. Kristt and J. Silverman*, Dept. Pathol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Horseradish peroxidase (HRP) was administered to somatosensory cortex of newborn to 6-day-old rats as implanted pellets or as a pressure-injected aqueous solution at low and high doses. Tissue sections were histochemically processed using the tetramethyl benzidine reaction. Implantation of a single pellet was superior to a single injection for delivering low doses to a specific part of somatosensory cortex (predominently SmI). High-dose administrations of HRP as either multiple pellets or injections uncovered many more nuclear groups; some of these have not been previously noted either in infant or adult brain. Both modes of high dose administration revealed the same cell groups, although more neurons/group were labeled after implants, and cells were more densely labeled. The single pellet implants (low dose) demonstrated afferents, ipsilaterally, from the ventral basal complex (VBC), central lateral, ventral medial, and posterior nucleus of the thalamus, the locus coeruleus and scattered somata in the caudal basis pontis (A5). High-dose administration showed, in addition, labeled neurons in all of the intralaminar thalamic nuclei, contralateral locus coeruleus, contralateral somatosensory cortex, contralateral pyriform and the dorsal raphe region of the midbrain. In ipsilateral neocortex, SmII contained labeled neurons. Moderate numbers of labeled neurons were scattered throughout brainstem reticular formation, often in clusters. Other forebrain groups were occasionally labeled. Since it was possible that cortical or subcortical regions adjacent to SmI were actually taking up the HRP after high doses, two further sets of experiments were done. First, multiple pellets were implanted at superficial depths. The only difference in the findings was the absence of labeled cell bodies in contralateral forebrain. In the second series of experiments, cortical or subcortical regions contiguous with SmI were ablated, but this did not alter the findings. Also, deep coronal, hemispheric knife cuts anterior to SmI eliminated labeling of brainstem neurons, predominently in locus coeruleus, but did not affect A5 labeling. These results suggest that terminal uptake is primarily in SmI. Consequently, by the end of the first postnatal week, most of the identified extrinsic inputs to adult SmI are probably already represented. VBC and locus coeruleus appear to provide a relatively dense innervation to rat somatosensory cortex, while few terminals from contralateral SmI are present in the supragranular layers. It also appears that projections from the thalamus and the brainstem reticular formation may be more extensive than was previously recognized, at least during the newborn period.

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222.10 THE TERMINAL ARBORS OF VENTROBASAL CELLS PROJECTING TO AREA SI IN CATS. P. Landry* and M. Deschênes* (SPON: R. De Estable-Puig) Lab. of Neurophysiology, Laval Univ. Fac. of Med., Québec, Canada G1K 7P4.

Thalamocortical axons originating in the ventrobasal complex were recorded intraaxonally in the white matter below area SI. These fibers were identified electrophysiologically by their direct response to ventrobasal stimulation and by their transsynaptic responses to stimulation of the medial lemniscus. The receptive field for each fiber was determined and subsequently the fiber was injected intraaxonally with horseradish peroxidase (HRP). The method of perfusion and histochemical visualization of the HRP is described elsewhere (Deschênes, M., Landry, P. and Labelle, A., Neurosci. Letters, 12: 9-15, 1979). Thirteen fibers were injected sufficiently to permit a visualization of most of the terminal arbors. Seven of these fibers were activated by hair displacement; three responded tonically to skin indentation, three were driven by joint movement, and one by stimulation of deep tissue. The cortical territory covered by the main terminal arbor of ventrobasal afferents is around 0.8 to 1.5 mm. All fibers have a common laminar distribution. Most terminals are located in layers VI, IV and the lower part of lamina III. Very few terminals were seen in layer V and none in layers I and II. All hair-driven fibers terminated in area 3b or in the posterior part of area 3a and had very dense terminal plexi. They also sent one or two fine collateral branches in areas 1, 3a, and possibly 2. The terminal arbors that issued from these few collaterals were much less elaborated. Furthermore they reached the same layers as the main terminal arbor. Axons that responded tonically to skin indentation, deep stimuli, or joint movements were mostly located in area 3a. The terminal arbors of all these fibers were much less dense than those driven by hair movement. Within this group, skin driven fibers had a more intricate arbor than units driven by deep tissue distortion or joint movement. Most of the fibers in this group sent collaterals to areas 3b and area 4. Although hair-driven units have a distinctively dense terminal arbor, the morphological criteria separating the units remains to be determined. To answer these topological problems, computer-assisted topographical analysis, which is currently underway, is expected to provide the desired criteria.

222.11 THALAMIC PROJECTION TO AREAS 3a AND 2 IN MONKEYS. D.P. Friedman and E.G. Jones. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The thalamic projection to areas 3a and 2 of the macaque somatic sensory cortex has been determined using combined electrophysiological and neuroanatomical techniques. Initially, the ventrobasal complex (VB: the VPLc and VPM nuclei) and the adjacent VPLo nucleus were mapped for somatic responses using standard microelectrode recording techniques in Nembutal anesthetized monkeys. Electrode penetrations entering this region horizontally, from behind, first encountered units which responded to light cutaneous stimulation. Anteriorly, near the border of VPLc and VPLo and still within the zone of lemniscal fiber terminations, as determined in concurrent anatomical experiments, a shift to deep receptive fields (e.g., joint rotation, pulling on tendons or manipulation of muscle bellies) occurred. When the location of this shift was marked with microlesions, it was seen to lie within the anterior 500 µm of VB. Tracks entering VB vertically, from above, often passed through a similarly narrow zone (about 500 µm), within which units also responded to deep stimuli. These vertical tracks then entered the larger "cutaneous core" zone of VB, within which units responded to light tactile stimuli as above. Tracks placed anteriorly, passing through VPLo, but not VPLc, encountered units with deep receptive fields only.

Small injections of tritiated amino acids placed into the anterior portion of VPLc, where responses to deep stimuli were recorded, labelled thalamocortical terminal ramifications in area 3a. Injections placed in the dorsally situated deep zone of VB, irrespective of their anterior-posterior position, labelled areas 3a and 2. Injections confined to VPLo or its extension, VLc, labelled only area 4.

The thalamic projection to area 3a thus arises from a "deep" shell zone of VB which occupies its anterior and dorsal surfaces. The projection to area 2 appears to arise primarily from the dorsal portion of the shell. The larger cutaneous core zone underlying this shell, projects to areas 3b and 1.

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222.12 QUANTITATIVE ANALYSIS OF GOLGI-IMPREGNATED STELLATE CELL AXONS AND THEIR PROXIMITY TO DENDRITES IN MOUSE BARREL CORTEX. R.M. Harris and T.A. Woolsey. Dept. Anat. & Neurobiol., Washington U. Med Sch., St. Louis, MO. 63110.

Our previous analyses of neuronal morphology as revealed by Golgi-impregnations in mouse and rat barrel cortex have focused on the dendritic patterns of the stellate cells. From their dendritic morphology these cells can be classified into two types: Class I cells with spiny dendrites and Class II cells with smooth beaded dendrites. These classes can be subdivided further according to their somal position and spacial distribution of dendrites with respect to barrel boundaries. In this study the axons of these cells were examined and the locations of close appositions to dendrites of other impregnated neurons mapped. All data are taken from Golgi-Cox preparations, cut parallel to layer IV at 140µm, counterstained with Nissl to reveal the barrels, and measured with a computer-microscope. Axons which had extensive branching within the section (10% of all cells) were chosen for measurement.

The analysis of the axons revealed: 1) Class I axons are thin and directed to the white matter, with recurrent collaterals in the barrels, while Class II axons are thick, frequently beaded, and directed towards the pia before cascading down into the barrels. 2) The axons tended to be as restricted to a barrel as the dendrites of the same cell were (i.e. most axons were confined to one barrel). 3) The Class I axons had a total length about three times that of Class II axons, and about four times as many branch points. Analysis of the appositions of these axons to impregnated dendrites of other cells revealed: 1) A greater number of appositions occurred near the distal end of dendritic segments. 2) No striking patterns were found, such as an obvious propensity for axons of one cell type to prefer or avoid another cell type, such as might be expected if very specific cell-cell "connections" were being observed.

These results show that the axons of barrel cells of each class are as consistent and distinctive as their dendritic trees. The cells can thus be distinguished by their axonal patterns on purely numerical bases. The new computer programs which were developed to systematically search for sites of apposition between axons and dendrites - a necessary prerequisite for the existence of synaptic contacts - greatly facilitated the collection of these data. The data may be of value for defining "probable" sites of interaction between particular cell types. In particular, the observations of the distribution of appositions along dendritic segments is comparable to White and Rock's ('79) EM mapping of synapses on barrel neurons.

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222.13 FEATURES OF S-I CORTICAL ORGANIZATION IN THE CAT DEMONSTRATED BY ANTEROGRADE DEGENERATION AND METABOLIC LABELLING. E. Kosar, P. Hand and T. McKenna* (SPON:L. Ritz). Dept. Animal Biology, Sch. Veterinary Med. and Inst. of Neurological Sci., U. Pennsylvania, Philadelphia, Pa. 19104 and Dept. Physiology, UNC, Chapel Hill, N.C. 27514. Relations between the distribution of thalamocortical connections and of stimulus-related metabolic activity were examined. The Fink-Heimer I technique was employed to demonstrate degenerating thalamocortical connections and the C-14-2-deoxyglucose (2-DG) metabolic labelling method was used to demonstrate regions activated by natural stimulation. Electrolytic microlesions (30-275μ) placed in VPLm led to degenerating fibers terminating within laminae III and IV of areas 3b, 1, and 2. Individual patches of degenerating fibers seen in single coronal sections had well defined radial boundaries, mediolateral dimensions of from 80-120μ, but extended for much longer distances in the A-P dimension of S-I (2500-3000μ). The total pattern of anterograde degeneration resulting from a single thalamic lesion revealed that the patches are arranged in bands that merge and diverge in a zebra-like pattern. C-14-2-DG labelling studies were carried out in unanesthetized cats under neuromuscular block with gallamine. Unilateral brush strokes were applied to the digits of the antebrachium prior to, during, and following intravenous pulse-injection of 150-250 μcuries of 2-DG. After continuous stimulation (interstimulus interval = 3 sec.) for 45 minutes, the animal was sacrificed by i.v. injection of pentobarbital followed by intracardiac perfusion. All subsequent procedures were identical to those described by Sokoloff, et. al. (J. Neurochem. 28: 1977). Preliminary results indicate that 2-DG labelling in the contralateral S-I cortex exists in the form of radially oriented spindle shaped columns, 180-500 microns in mediolateral width, extending through lamina II to VI. The greatest density of labelling was in lamina III and IV. Labelled columns were mainly located in the medial bank of the coronal sulcus, in all cytoarchitectonic areas of S-I. In general, the columnar structures labelled by C-14-2-DG also form sinuous A-P bands. Differences were observed in the pattern of metabolic labelling in S-I and S-II. In S-I, laminae III and IV were densely labelled, whereas in S-II the labelling more uniformly involved laminae II-VI. Reconstructions were prepared to enable comparison of the pattern of A-P bands revealed by the degenerating thalamocortical afferents with the cortical bands exhibiting enhanced metabolic activity in response to continuous tactile stimulation.

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222.14 METABOLIC MODULAR ORGANIZATION IN THE SOMATOSENSORY CORTICES OF THE MONKEY. S.Juliano,P.Hand,B.Whitsel. Dept. of Animal Biology, School of Veterinary Medicine,and Institute of Neurological Sciences, Univ. of Pennsylvania, Phila.,PA 19104;Dept. of Physiology, School of Medicine, Univ. of North Carolina, Chapel Hill,NC 27514

Previous (14C)2-deoxyglucose (2DG) investigations by this laboratory, (Anat. Rec., Neurosci. Abs.,1979) have shown a complex pattern of organization of functional columns or modules in the primary somatosensory(SI) and associated cortices of the Cynomolgous monkey,in response to a light cutaneous brush stroke (BrS). Modules ranged in mean diameter from 434 to 651um, and when viewed on unfolded surface reconstructions, formed medio-laterally oriented strips or modules. These results have been extended by using different somatic stimulation on 2 additional paralyzed, unanesthetized monkeys. Each animal received a pulse injection of 2DG and either (a) unilateral flutter-vibration stimulation (FV) at 15cps,from a point source on the distal joint of the index finger, or (b) servo-motor controlled, unilateral, flexion/extension of the elbow joint at 22°/sec. The animal receiving unilateral FV showed functional columns in cortical areas similar to the BrS animals. The sizes of these columns, in mean width, ranged from 486 to 679um. Noticeable differences from the BrS animals were in areas 1,5 and 7 where the mean column widths for the FV animal were larger than those of the BrS animals. When a surface reconstruction for the FV animal was compared to that of a BrS animal stimulated over the same body part, the foci of label in SI were found to be similar in both animals. The FV animal also showed labeling in the form of medio-laterally oriented strips, however, the strips appear wider in a rostral-caudal dimension, and show an additional focus of label in areas 1 and 2. Also, the FV animal has somewhat fewer modules throughout SI and the medio-lateral strips often appear shorter than those of the BrS animal. Preliminary results from the animal with elbow joint movement show columns of similar size to those seen in the other animals, as well medio-lateral strips of functional label in areas 3a and 3b, plus a focus of columnar labeling in areas 1 and 2 at the elbow region of SI.
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222.15 SELECTIVE UPTAKE OF [^{3}H] GABA BY INTERNEURONS IN MONKEY SENSORY-MOTOR CORTEX. S.H.C. Hendry and E.G. Jones Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

[^{3}H] GABA (20 or 50 μCi. per μL) was injected into areas 4, 3, 1 and 2 of the motor and somatic sensory (SI) cortex in Macaca fascicularis, following intravenous administration of the GABA-transaminase inhibitor, aminooxyacetic acid. After 30-40 minutes, blocks from the cortex were fixed by immersion in 3.5% glutaraldehyde and frozen or plastic-embedded sections were processed by standard autoradiographic methods. Neurons which had accumulated [^{3}H] GABA, and were thus presumed to be GABAergic, were identified by heavy concentrations of silver grains overlying their somata. In all areas no pyramidal cells are labelled above background. All labelled neurons are thus considered to have axons that are intrinsic to the cortex. The labelled somata are found in each of the cellular layers of the cortex but their numbers vary with depth, particularly in SI. There, large numbers are present in layers II, IIIB, IV and in the upper part of layer VI; fewer are present in layers IIIA, V or in the deeper part of layer VI. By comparison, the labelled cells in area 4 are more homogeneously distributed throughout layers II, III and V and almost completely absent from layer VI. Labelled somata in SI fall into two size categories: small (7-14 μm) and large (18-25 μm). Two similar groups are found in area 4 but both are somewhat larger. The smaller size group is found in all layers containing labelled cells, but the larger size group is restricted to layers IIIB, IV and V. The larger type in both areas is interpreted as the type 1 (basket) cell of Jones (J. Comp. Neurol. 160:205, 1975). The smaller type probably includes several of the other non-pyramidal cell types identified in Golgi preparations.

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222.16 A POSSIBLE CORTICAL PATHWAY FOR SOMATOSENSORY PROCESSING IN MONKEYS. Elisabeth A. Murray, Richard K. Nakamura, and Mortimer Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205.

Anatomical and electrophysiological evidence indicates that both area 5 of the posterior parietal cortex and the second somatosensory area (SII) are possible relays in a processing pathway linking the primary somatosensory area (SI) with the motor and limbic systems. To evaluate these possibilities behaviorally, rhesus monkeys with bilateral lesions of either area 5 or SII were compared with normal controls on a battery of six somatosensory discriminations which required them to differentiate objects on the basis of texture, shape or size. Whereas animals with removals of area 5 showed no impairment relative to the controls in learning any of the tactual discriminations, monkeys with lesions of SII were markedly impaired relative to both of the other groups in learning all tasks except a gross size discrimination. Furthermore, despite learning the size discrimination normally, the SII group was impaired relative to the other groups in subsequent testing of size discrimination thresholds. Although tactual deficits following lesions of area 5 have been reported previously, deficits after such damage were found in the present study only when area 5 lesions were extended slightly rostrally, in a third operated group, to include the dorsomedial part of the hand representation of area 2. The combined results indicate that SII, and not area 5, is a critical station in a somatosensory processing pathway, and suggest that cortical areas to which SII projects may be further links in the pathway; this possibility was examined next.

Anatomical investigations have indicated a projection from SII to part of the insular cortex, and unit recording data show that neurons in the insular and retroinsular regions are activated by somatosensory stimuli. Therefore, a fourth operated group was prepared with bilateral lesions of the insular and retroinsular cortex, made accessible by removal of the supratemporal plane. These animals were impaired in learning four of the tasks, including a roughness discrimination, but were unimpaired in size and roughness discrimination thresholds. Comparison of this profile of deficits with that following SII lesions suggests that the insular/retroinsular cortex is a higher order somatosensory processing area than SII.

The known projection of parts of the insular cortex to the amygdala suggests a sensory-limbic pathway for touch analogous to the one in vision. Just as visual sensory information is relayed to the limbic system through successive links of striate, prestriate, and inferior temporal cortex, a sensory-limbic pathway for touch may proceed from SI to the medial temporal lobe through serial links in SII and the insular/retroinsular cortex.

222.17 CORTICAL BARREL FIELDS OF ADULT MICE AS ANALYZED WITH CYTOCHROME OXIDASE CYTOCHEMISTRY. Margaret Wong-Riley. Dept. Anat., Univ. Calif., San Francisco, CA 94143.

In a previous histochemical study (Wong-Riley and Welt, Proc. Natl. Acad. Sci., in press), the barrel centers (hollows) in layer IV of SmI face cortex of normal mice were found to have a high level of cytochrome oxidase activity, indicating a high degree of oxidative metabolism there. In order to determine the distribution of such enzyme reactivity at the EM level, vibratome-cut sections of normal barrel fields were reacted for cytochrome oxidase activity, and the individual reactive barrels were dissected out under a stereozoom microscope and processed for EM. Thus far, a total of 5000 mitochondria were counted from five large montages, totalling 17,940um^2 in cross-sectional area of barrel centers. Approximately 30% of the mitochondria resided in non-synaptic dendritic profiles (ie., profiles not showing synaptic junctions in the field), 11% in postsynaptic dendrites, 16% in axon terminals making asymmetrical synapses, 10% in processes making symmetrical synapses, 12% in myelinated axons, 12% in perikarya of neurons, and the remaining percentage in non-synaptic axonal profiles, glia and unclassifiable profiles. Mitochondria were classified as darkly-reactive (D), moderately-reactive (M), and lightly-reactive (L). Of the axonal population making asymmetrical synapses, approximately 20% of their mitochondria were D's, 52% M's, and 28% L's. Of the processes making symmetrical synapses, approximately 25% of their mitochondria were D's, 57% M's and 18% L's. Among the non-synaptic dendritic population, however, 67% of their mitochondria were D's, 25% M's and 8% L's. A similar pattern of distribution was found among the postsynaptic dendrites. Thus, dendrites contain the greatest percentage of mitochondria in barrel centers, and the majority of these mitochondria have a high level of cytochrome oxidase activity. In contrast, most of the mitochondria in presynaptic axonal profiles are only moderate to lightly reactive, and the highly reactive ones contribute to only a small percentage of the total mitochondrial population. Studies are under way to determine changes in the distribution pattern of the mitochondrial population in chronically dewhiskered barrel fields of adult mice.

223.1 CONDITIONED CORTICOSTERONE SECRETION TO STIMULI ASSOCIATED WITH STRESS. S. R. Burchfield* (SPON: A. Nonneman). Department of Psychology, University of Kentucky, Lexington, KY 40506.

50 two-month-old male and female Long-Evans' rats were used in a study investigating endocrinological conditioning to cues predictive of stress. A typical classical conditioning paradigm was used. The unconditioned stimulus (USC) was loud noise lasting five minutes. The unconditined response was corticosterone secretion. The conditioned stimulus (CS) was a click which occurred when the automatic timer clicked on to play the noise (gun fire) tape. Control animals were used to determine the basal level of corticosterone secretion, plasma corticosterone levels in response to the click only, and to the noise only. The experimental group was exposed to seven pairings of the CS and UCS at random times, once a day for one week. On the eighth day rats were exposed to the CS only and truncal blood was collected between 3-15 minutes after CS presentation. Results indicated no differences in plasma corticosterone levels ($\bar{X}$ = 3.93 ug/dl) in baseline and click only conditions. Corticosterone was elevated in the females ($\bar{X}$ = 15.47 ug/dl) but not the males ($\bar{X}$ = 4.92 ug/dl) in the noise only condition. Both males and females showed a significant ($P < .05$) elevation of corticosterone response to the click on day eight. Females responded with a higher corticosterone level after the CS ($\bar{X}$ = 36.5 ug/dl) than did males ($\bar{X}$ = 15 ug/dl). A final control group was included to determine if conditioning actually occurred or if the higher corticosterone level represented an altered basal secretion. This group received seven CS-UCS pairings. On day eight, truncal blood was collected in the absence of either the CS or UCS. Males displayed a plasma corticosterone level equal to their basal levels ($\bar{X}$ = 2.0 ug/dl) while females displayed a significantly elevated level, equal to their conditioned response ($\bar{X}$ = 37.6 ug/dl). These data suggest that female rats, after chronic intermittent stress, either alter their basal corticosterone output or experience sensitization (e.g., respond to stimuli not usually considered stressful). Males, however, appear to respond only to the specific stressor and exhibit a normal basal corticosterone level at other times. Possible mechanisms for these results are currently being investigated.

223.2 CHANGES IN PLASMA VASOPRESSIN DURING MOTION SICKNESS IN CATS. R. Fox*, L. Keil, N. Daunton, D. Thomsen*, M. Dictor*, and O. Chee*. NASA Ames Res. Center, Moffett Field, CA 94035 and San Jose State Univ., San Jose, CA 95192.

Changes in levels of plasma vasopressin (AVP) and cortisol (C) have been shown to be correlated with motion sickness and nausea in man. As part of the research aimed at validation of the cat as an appropriate animal model for motion sickness research, levels of these hormones were investigated in the cat during motion sickness elicited by vertical linear acceleration of approximately 0.6 Hz and 1 + 0.6 G.

In Study 1, 15 cats previously screened for susceptibility to motion sickness were prepared with indwelling jugular catheters to permit withdrawal of blood with minimal disruption of the stimulus and minimum stress to the animal. AVP and C were measured in blood samples obtained during exposure to vertical linear acceleration and during control sessions in which the animals were placed in the stationary apparatus. Samples were drawn according to a predetermined time schedule as follows: 10 min and 1 min prior to motion; 1, 5, 10, and 20 min after start of motion. Total duration of exposure to motion was 20 min. The data from this study indicate that both AVP and C are elevated during exposure to motion if emesis occurs. AVP reaches maximum levels during or about the same time as emesis, while C increases gradually throughout the period of vertical acceleration.

In Study 2, four cats were prepared with indwelling catheters and AVP was measured in blood withdrawn during exposure to the vertical linear acceleration. A single pre-motion sample was drawn 5 min prior to motion onset. Two series of samples consisting of three samples drawn at 3-min intervals were obtained during motion. The first series was initiated at emesis, and the second 25 min after emesis. Results show that levels of circulating AVP were elevated (2 to 27 times the control and pre-motion levels) in the samples taken during emesis and decreased, but remained 1 to 6 times above the pre-motion or control levels within 25 min.

The results of these two studies indicate that AVP is elevated during motion-produced emesis in the cat, and that AVP is more closely related to emesis than is C. These findings are in general agreement with those obtained from humans under motion sickness conditions, and indicate that it is appropriate to continue to use the cat in studies of hormone changes during motion sickness.

223.3 A ROLE FOR THE MIDBRAIN CENTRAL GRAY IN ESTROUS BEHAVIOR OF THE CAT. J. D. Rose and L. K. Smucker*. Dept. of Psychol., Univ. of Wyoming, Laramie, WY 82071.

Previous results from single-unit recording and lesion-behavioral experiments have indicated that the lateral caudal medulla plays an essential role in the estrous vocalization and afterreaction responses to genital stimulation in the female cat. Results of neuroanatomical, electrophysiological and estradiol autoradiographic studies suggest that estrogen influences may be transmitted to caudolateral medullary neurons via a projection from the central gray. In the present investigation, the significance of the central gray in estrous behavior was examined by placing lesions in this structure in 5 ovariectomized cats. The lesions produced no marked impairment in estrous behaviors, but instead resulted in the appearance of persistent estrous behavior patterns in the absence of estrogen replacement. These estrous behaviors included lordosis, treading and tail deflection in response to perigenital stimuli and afterreactions following genital stimulation. In all cases, the lesions abolished the typical hostile responses to genital stimulation in the hormonally anestrous condition. Although the lesions eliminated or weakened vocalizations to social or nociceptive stimuli, the estrous cry response to genital stimuli remained largely intact. Subsequent single-unit recording experiments in 10 urethane-sedated estrous and anestrous cats without brain lesions confirmed an earlier observation that most caudolateral medullary neurons show a sustained decline in firing following genital stimulation, whereas the converse, an acceleration is seen in units in anestrous cats. Recordings from medullary units in 3 hormonally anestrous cats which displayed estrous afterreactions to genital stimulation due to central gray lesions revealed a preponderance of units showing the estrous pattern of firing following genital stimulation. In addition, in electrode tracks through the central gray of the animals without brain lesions, fewer spontaneously-active units could be recorded in the estrous animals in the central gray at the A-P level of the trochlear nucleus, but no difference in spontaneous unit activity was seen in more anterior central gray tracks. The region of the central gray showing depressed activity in the estrous animals coincides with that containing the largest number of neurons projecting to the caudolateral medulla (Rose, J. D., Soc. Neurosci. Abstr., 5:457, 1979). The findings from these lesion and recording studies suggest that the central gray normally acts to suppress estrous behaviors in the anestrous cat and that estrogens may promote estrous behaviors through a suppression of the activity of central gray neurons, including those projecting to the caudolateral medulla.

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223.4 SITE-SPECIFIC INDUCTION OF SEXUAL BEHAVIOR BY INTRACRANIAL ESTRADIOL IMPLANTS IN MALE AND FEMALE HAMSTERS. Joseph F. DeBold, Charles W. Malsbury and Douglas E. Arnoult* Dept. Psychol.,Tufts Univ., Medford, MA; Dept. Psychol., Memorial Univ., St. John's Newfoundland;Dept. Psychol., Carnegie-Mellon Univ., Pittsburgh,PA

Estrogen is necessary for the induction of female sexual receptivity in rodents and a number of other species. Estrogen may also be an important component of androgen action in the maintenance of male sexual behavior. These effects of estrogen appear to be mediated by direct actions on the CNS. In female rats and guinea pigs direct estrogenic stimulation of the ventromedial hypothalamus (VMH) is sufficient to induce sexual receptivity. However in female hamsters the only published report on hypothalamic estrogen implants stresses the importance of the anterior hypothalamus. In castrated male rats estrogen implants into the preoptic area (POA) is particularly effective in restoring mounting behavior while there is little comparable information in other male mammals. The present study compared the location of effective intracranial estradiol (E_2) implants for female sexual receptivity in gonadectomized male and female hamsters and for mounting behavior in castrated male hamsters.

In the first experiment, 2 wk after gonadectomy male and female hamsters were stereotaxically implanted with a single 27g cannula containing crystalline estradiol into one of several brain sites. Males and females received implants in the POA, anterior hypothalamus (AH), VMH and cortico-medial amygdala (CMA). In addition, other females received implants into the bed nucleus of the stria terminalis (bnst), the arcuate (arc) and the central grey. Some animals received instead implants containing cholesterol. One wk later the animals were injected with 500µg progesterone and 4-5 h later tested for sexual receptivity. The best response was seen in females with E_2 implants in the VMH, but a few females with implants in the AH, CMA or bnst also were receptive. Only males with VMH E_2 implants were receptive. Except for a few AH females there was little stimulation of uterine weight suggesting minimal E_2 diffusion.

In the second experiment, males were castrated and then 8-10 wk later were implanted bilaterally with 30 g E_2 or cholesterol cannulae into either the bnst, POA, AH or VMH. All animals also received a subthreshold amount of dihydrotestosterone (50µg/d s.c.) and were tested with a sexually receptive female for mounting 3 or 5 d later and then 3 additional time at 5 d intervals. E_2 implants in or near the POA and bnst were most effective in restoring mounting behavior while VMH implants were least effective.

These experiments demonstrate that the VMH is the most sensitive brain area for estrogenic stimulation of sexual receptivity in both male and female hamsters, however, the same hormone restores mounting behavior most effectively in POA and bnst.

223.5 EFFECTS OF CANNABINOIDS AND SEXUAL STIMULATION ON PITUITARY-GONADAL FUNCTION IN MALE MICE. S. Dalterio,* R. Huffman, A. Bartke,* M. Harper,* C. Sweeney.* Dept. Pharm. & Ob-Gyn, UTHSCSA, San Antonio, TX 78284

A single dose of Δ^9-tetrahydrocannabinol (THC, 50 mg/kg), the main psychoactive constituent of marihuana decreased plasma testosterone (T) (2.46±0.6 vs 0.54±0.2 ng/ml, p<.05), LH (38±8 vs 15±3 ng/ml, p<.01) and FSH levels (1219±53 vs 990±44 ng/ml, p<.01) in immature mice but the same dose of the non-psychoactive cannabinol (CBN) had no effect. Repeated exposure to cannabinoids from 30 days of age to adulthood influenced endocrine responses to mating, although there were no differences between groups in sexual activity during a 1 hr test session. Mice were treated with vehicle (oil), or THC, CBN or cannabidiol (CBD) at a dose of 50 mg/kg and half of each group were either exposed to a sexually receptive female or maintained in an all male group. Twenty hours after sexual experience testicular weights were reduced (p<.02) in THC (269±17 vs 247±7 mg), CBN (294±25 vs 266±3 mg) and CBD (283±17 vs 254±7 mg), but not in control males (289±7 vs 287±11 mg) Similar effects were noted for seminal vesicle weights. Plasma FSH levels were elevated (p<.05) in CBN-treated mice compared to oil-controls after sexual stimulation (1167±91 vs 1515±357 ng/ml) as well as after grouped housing (1179±99 vs 1549±250 ng/ml). Sexual experience resulted in a significant (p<.05) increase in plasma T levels in oil (1.5±0.4 vs 5.3±2.4 ng/ml), CBN (1.0±0.4 vs 2.1±1.2 ng/ml), and CBD (1.0±0.2 vs 5.2±2.8 ng/ml), but not in THC-treated males (4.5±3.4 vs 4.2±3.4 ng/ml). In THC exposed mice plasma LH (29±16 vs <3 ng/ml) and FSH levels (1392±51 vs 1134±74 ng/ml) were reduced (p<.05) in sexually stimulated compared to grouped males. Testicular responsiveness to gonadotropin stimulation in vitro was reduced (p<.05) by exposure to mating stimuli in THC (2757±428 vs 1792±71 ng/ml) and CBN-treated mice (2549±201 vs 1997±154 ng/ml). However, in THC and CBN-exposed animals maintained in all male groups, T production in vitro was elevated compared to oil-controls (1894±246 vs 2757±428 and 2549±201 ng/ml, p<.05). Interference with prostaglandin (PG) synthesis may mediate these effects of cannbinoids and sexual experience since in vitro production of PGE and PGF was reduced (p <.05) in testes (0.23±.01 vs 0.18±0.01, and 0.28±0.02 vs 0.10±0.06 ng/mg protein) and pituitaries (9.2±0.8 vs 6.5±0.2 and 6.8±0.3 vs 4.8±0.2 ng/mg protein) in pooled cannabinoid-treated males compared to oil-controls. These results indicate that both psychoactive and non-psychoactive constituents of marihuana are capable of altering the function of the pituitary-gonadal axis and of influencing the endocrine responsivity to sexual stimulation in male mice. Supported by NIDA grant 1 R01 DA 02342-01.

223.6 MATERNAL BEHAVIOR DEFICITS AND ENDOCRINE RESPONSES IN FIMBRIA-FORNIX LESIONED POST-PARTURIENT RATS. M.K.Steele and S.M.McCann, Physiol. Dept, Univ of Tex Hlth Sci Center, Dallas, TX, 75235.

Various components of maternal behavior, ability to cycle normally, as well as basal and suckling-induced changes in prolactin secretion were evaluated in post-parturient female rats that received bilateral electrolytic lesions of the fimbria-fornix fiber bundle at 10 days of gestation. While lesioned females (n=10) gained more weight over the 7 days of behavioral testing than control animals (n=6), they were deficient in pup-care related behaviors: nest ratings, number of pups nipple attached, number of pups with milk, retrieval of pups to the nest or home quadrant, and pup weight gain over the testing period.

In order to compare behavioral measures (esp. nursing) with possible deficits in prolactin secretion, suckling tests were conducted on experimental and control mothers on day 5 post partum. A chronic indwelling jugular cannula was inserted on the day prior to testing. Females were separated from their pups for 5 hours prior to (basal sample) and 15, 30 and 60 minutes after introduction of the pups. Prolactin levels were measured by RIA and the results indicate that prolactin responses were absent or delayed in 8 of 9 lesioned females, while 6 of 8 control mothers displayed the normal dramatic increase in prolactin levels within 15 minutes of pup introduction. Basal levels of prolactin did not differ between groups.

AFter behavioral testing terminated, resumption of estrous cyclicity was monitored and while all 6 control females rapidly resumed normal 4 or 5 day cycles, only 50 per cent of lesioned females displayed estrous cyclicity. Prolactin levels in blood were also measured during this time and values did not differ between lesioned and control animals.

Experiments in progress may delineate whether the aberrant prolactin responses seen in fimbria=fornix lesioned females are due to a primary endocrine deficiency or are secondary to basic behavioral deficits.

224.1 PERIPHERAL SPROUTING OF HIGH THRESHOLD AFFERENTS IN THE SAPHENOUS NERVE AFTER SCIATIC NERVE SECTION IN THE RAT. B. Pomeranz* and H. Markus. Dept. of Zoology, University of Toronto, Toronto, ONT., CANADA M5S 1A1.

Previous studies have shown that following section and ligation of the sciatic nerve, the saphenous sprouts and reinnervates portions of the denervated paw. Moreover high threshold mechanoreceptive fibres show sprouting, while the low threshold fibres do not (Devor, M. et al., J. Comp. Neurol., 185:211, 1979). We have reexamined this phenomenon in a more quantitative manner and found that high threshold mechanoreceptive afferents do sprout from intact neighbouring nerves; in contrast however high threshold thermosensitive afferents seem to show little or no sprouting. In the study we examined the ventral surface of the rat paw using both temperature and mechanical stimuli. Responses were recorded from various sites on the paw (1) Mechanical Stimulus: the skin was pinched using a pair of serrated forceps and the force required to elicit withdrawal of the leg was determined with a Stratham strain gauge. (2) Temperature Stimulus: a heated probe with feedback temperature control was applied to the skin and the time required to elicit leg withdrawal was measured.

(1) Mechanical: In the normal animal the mean required pressure was 89.60 gm (n=7). In the denervated animal the medial 2/3 of the paw and the medial 2 toes were normal (82.33 gm) (n=7). However the lateral 1/3 and the distal 3 toes required a greater force (418.51 gm) (n=7). This suggested that sprouting occurred beyond the normal boundaries of the saphenous nerve.

(2) Temperature: In the normal animal leg withdrawal was elicited on the average after 3.48 sec (n=6). In the denervated animal a normal response (2.98 sec) (n=7) was elicited only if the heat probe was applied to the medial 1/3 of the paw. When the heat probe was applied to the rest of the paw there was an increase in the time required to elicit a response (12.20 sec) (n=7). One possible conclusion is that the temperature afferents showed little or no sprouting. Hence high intensity mechanoreceptive fibres of the saphenous nerve showed more extensive sprouting than the high intensity thermal-sensitive fibres. Results from electrophysiological recordings will be presented and correlations with the behavioural results will be discussed.

224.2 RELATIONSHIP BETWEEN AXONAL SPROUTING AND NEUROTRANSMITTER ENZYMES IN SUPERIOR CERVICAL GANGLION IN VITRO DURING THE RETROGRADE REACTION. R.A. Ross, T. H. Joh, and D.J Reis, Laboratory of Neurobiology, Cornell Univ. Med. College, NY, NY 10021.

In the superior cervical ganglion (SCG), axonal injury initiates, as part of the retrograde reaction, a decrease in the activities of tyrosine hydroxylase (TH) and dopamine-B-hydroxylase (DBH), enzymes synthesizing norepinephrine. This response is often reversible. A temporal relationship has been suggested between this reversible decrease in enzyme activity and the growth of regenerative sprouts from the injured neurons. The present study was undertaken to test this hypothesis. Specifically, we sought to determine: (a) if, following axonal injury produced by explantation into culture, SCG would show a reversible decrease in TH and DBH activities characteristic of a retrograde reaction and (b) if blockage of axonal sprouting in SCG neurons in vitro would prevent the enzyme changes normally seen.

SCG removed from embryonic rats at 18 days of gestation were cultured on coverslips in media containing 100 ng/ml NGF. Changes in TH and DBH activities were measured in different SCG from 0 to 14 days after explantation. A reversible reduction in TH activity was seen in vitro, reaching a nadir at 20.1% of control (d 0 =1.74±0.23 nmols/h/ganglion) by day 3 and returning to control level by day 12 in culture. The changes in DBH activity were similar to TH in direction, magnitude of decrease (41% of control, d 0 = 0.98±0.09 nmols/h/ganglion), and duration. Immunotitration with a specific antibody to TH showed that the reduction and subsequent increase in TH activity were entirely attributable to changes in the accumulation of enzyme protein.

The SCG undergoes rapid axonal sprouting in vitro. To determine the effect of neurite outgrowth on the retrograde reaction, SCG were grown in suspension culture, a condition which totally inhibits axonal sprouting. Ganglia grown in suspension did not show any decrease in TH or DBH activity for 14 days in culture. SCG grown for 4 days on substrate were transferred into suspension culture. Sprouting ceased and TH activity increased to control level in these ganglia after 2 days (2.10± 0.19 nmols/h/ganglion), a full 5 days before that observed in ganglia grown only on substrate.

We conclude that: (a) removal of SCG into culture initiates a retrograde reaction characterized by a reversible decrease in the activities and amounts of TH and DBH similar to that seen in vivo and (b) arrest of sprouting reverses the reduction in enzyme accumulation. These findings support the hypothesis that the onset and time course of the retrograde reaction is intimately and inversely correlated to the production of axonal sprouts.

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224.3 MOSSY AND CLIMBING SPROUTING IN THE CEREBELLAR HEMISPHERE DENERVATED BY PEDUNCULOTOMY IN NEWBORN RATS. P. Angaut ★, R.M. Alvarado-Mallart ★ and C. Sotelo ★ (SPON : M.C. Hepp-Reymond). I.N.S.E.R.M. U-106. 92.150 Suresnes, France.

Most of the developmental changes of the cerebellar cortex, and especially the establishment of synaptic connections, take place after birth in rodents. Newborn to 4-day old rats were subjected to unilateral sections of the middle and inferior cerebellar peduncles. The animals were killed between 2 and 6 months of age and the cerebellar hemisphere ipsilateral to the pedunculotomy was studied. In spite of a total absence of the crossed inferior olivary complex and a partial regression of the crossed pontine gray, the cytology and synaptology of the concerned hemisphere were qualitatively normal, although only scarce climbing terminals were identified. In order to test the hypothesis of a sprouting of mossy and climbing fibers passing through the contralateral peduncle, 2 different approaches were used on rats pedunculotomized at birth : i) the contralateral peduncle was sectioned 2 days before sacrifice and the anterograde degeneration traced at the ultrastructural level; ii) horseradish peroxidase was injected in the hemisphere under concern 2 days before fixation and labeled cells looked for in the remaining ipsilateral inferior olive. These 2 sets of experiments confirmed that the presence of mossy and climbing terminals in this cortical region was due to a sprouting of undamaged afferents ending in the neighboring vermis. The sprouting process is very active for mossy afferents, much less so for climbing fibers. Contrary to what occurs under other conditions of impaired synaptogenesis (e.g. in the agranular cerebellum, Sotelo, C. and Privat, A., Acta Neuropathol., 43, 19-34, 1978), the present results demonstrate that the sprouting consecutive to early suppression of extracerebellar afferents does not induce the formation of heterologous synapses but maintains the specificity of the synaptic connections formed.

224.4 AFFERENT AND EFFERENT CONNECTIONS OF NEOCORTICAL TRANSPLANTS IN THE CEREBELLUM OF ADULT RAT HOSTS. Monica M. Oblinger* and Gopal D. Das* (Spon: C. K. Henrikson). Dept. Biol. Sci., Purdue University, W. Lafayette, Ind. 47907.

Previously reported studies have demonstrated that neocortical transplants in the cerebellum of neonate as well as adult rat hosts survive, grow and differentiate normally. In the case of neonate host animals, such transplants were seen to receive characteristic afferent connections from the host brain and, reciprocally, provide efferent axonal projections to the host brain. Since it was not known whether neocortical transplants placed into the cerebellum of adult host animals would also establish afferent and efferent connections with the host brain, the present investigation was conducted.

Neocortical tissue (3-4mm^3) obtained from 17-day-old rat embryos was transplanted into the right cerebellar hemisphere of 90-day-old Long-Evans rats. After three months the afferent and efferent connections of these transplants were analyzed by means of the Fink-Heimer and HRP procedures.

To study the sprouting of afferent fibers from the host brain electrolytic lesions were made in either the contralateral pontine nuclei, contralateral inferior olivary nuclei or ipsilateral cervical spinal cord. Subsequent degenerating fibers were traced through cerebellar medullary areas, across the interface of cerebellum and transplant, and into areas of the neocortical transplants. In addition, following localized HRP injections into neocortical transplants, labelled neurons were observed in the contralateral inferior olive, contralateral pons and ipsilateral spinal cord. The growth of efferent axons from the transplants into the host brain was studied with the Fink-Heimer procedure following electrolytic lesions in the transplants. Degenerating fibers emerging from the transplants were observed to terminate in the deep cerebellar nuclei as well as in the nearby normal host cerebellar cortex. Numerous intratransplant bundles were also evident.

These results indicate that axons present in the cerebellum of adult animals have a considerable capacity to sprout and innervate a neocortical transplant in the cerebellar hemisphere. The afferent connections of such transplants are characteristic of those of the normal cerebellar hemisphere. Likewise, such transplants provide efferent axons to the deep cerebellar nuclei, an innervation pattern that was characteristic of the normal cerebellar hemisphere which the transplant had replaced.

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224.5 NORADRENERGIC SPROUTING IN THE CEREBELLUM OF PCD (PURKINJE CELL DEGENERATION) MUTANT MICE. C. T. Harston and R. M. Kostrzewa. Dept. of Pharmacology, College of Medicine, East Tennessee State University, Johnson City, TN 37601.

In the autosomal recessive mouse mutation known as Purkinje cell degeneration (pcd), the Purkinje cells of the cerebellum spontaneously degenerate in large numbers from 22 to 28 days postnatally, and in adulthood less than 1% of these cells survive (Mullen, R.J., et al., Proc. Nat. Acad. Sci. USA, 73:208, 1976). These Purkinje target cells for noradrenergic efferent fibers of the locus coeruleus (Bloom, F.E., et al., Brain Res., 25:501, 1971) were considered to be of importance in the regulation of noradrenergic input to the cerebellum. Therefore, mice were sacrificed at 35 days postbirth or later, and cryostat sections of the cerebellum were stained by the glyoxylic acid method of de la Torre and Surgeon (Histochem. 49:81, 1976), in order to observe the histofluorescent noradrenergic fibers. It was found that the density of green fluorescent-stained fibers was markedly increased in both the granular and molecular layers of the cerebellum. Accompanying this change was a dramatic increase in the number of intensely-fluorescent particles in both the cerebellar cortex and hippocampus---analogous to the catecholamine-containing bodies found in aging mice (Masuoka, D.T., et al., Brain Res., 169:335, 1969). These alterations in noradrenergic innervation of the cerebellum indicate that the Purkinje target cells normally play an inhibiting role on noradrenergic fiber growth in the adolescent mouse. Moreover, the effects of Purkinje cell loss on the noradrenergic system appears to be of consequence even in regions outside the cerebellum. (Supported by Biomedical Development Research Grant 1-S08-RR 09171-01 and PSH Grant NEUA 5 R01 NS14797-03).

224.6 COMPENSATORY INNERVATION IN THE ANOPTHALMIC MUTANT MOUSE: AN ONTOGENETIC BUFFER MECHANISM THAT OFFERS EVOLUTIONARY POTENTIAL. M.J. Katz*, R.J. Lasek, and I.R. Kaiserman-Abramof. Department of Anatomy, Case Western Reserve University, Cleveland OH 44106.

Anopthalmic mutant animals have evolved and successfully colonized caves throughout the world. Thus, anopthalmic mutations can give rise to evolutionarily viable species. How are anopthalmic mutations integrated into functioning nervous systems? We have studied this question in the ZRDCT-An mouse, an anopthalmic mutant that differs from its sighted counterpart by only a single genetic change. In the ZRDCT-An, the retinal ganglion cells all degenerate before they have sent out axons. The anopthalmic dorsal lateral geniculate nucleus (DLGN) has fewer neurons and fewer glia than normal, and its synapses are restructured. A new class of axon terminals forms the large synaptic complexes normally formed by axons of the retinal ganglion cells (Cullen & Kaiserman-Abramof, J. Neurocytol. 5: 407-424, 1976). Interestingly, the projections from the anopthalmic DLGN form a normal pattern on the visual cortex and maintain a normal density and a normal distribution of spines on the cortical pyramidal cells (Kaiserman-Abramof, Brain Res. 179: 385-389, 1979; Kaiserman-Abramof et al., Neuroscience 5: 41-52, 1980). The synaptic changes in the anopthalmic DLGN are an example of compensatory innervation. Many other examples of compensatory innervation have been described, such as collateral sprouting in the hippocampus, the spinal cord, and the cutaneous sensory system. In the anopthalmic mouse, the DLGN compensatory innervation is secondary to the primary effects of the ZRDCT-An mutation (which disrupts normal interactions between the developing optic vesicle and the lens primordium ectoderm). Furthermore, bilateral surgical enucleation of young normal mice produces almost identical compensatory innervation. Thus, the DLGN compensatory innervation has not been directly programmed by the genetic change but rather appears to be a local adjustment in response to conditions in the immediate environment of the developing terminals. The ZRDCT-An mutation is a potential evolutionary change, and the anopthalmic mouse illustrates how such genetic mutations may be integrated into an evolutionarily viable organism. Specifically, compensatory innervation has insured that the anopthalmic DLGN remains part of the intact and well developed thalamocortical circuitry. Compensatory innervation is an exampleof the ontogenetic buffer mechanisms which normally integrate the many varied products of different genes into a harmonious organism. We have proposed that ontogenetic buffer mechanisms also offer evolutionary potential because thay can insure that complex systems willstill function after genetic changes. Ontogenetic buffer mechanisms such as compensatory innervation may be responsible for the viability of the anopthalmic mutants that populate caves today.

224.7 COMPETITION BETWEEN EMBRYONIC SEPTAL IMPLANTS AND ANOMALOUS SYMPATHETIC INGROWTH FOR REINNERVATION OF DEAFFERENTED HIPPOCAMPUS Rebekah Loy and Lawrence F. Kromer. University of California at San Diego, La Jolla, CA 92093

Transection of the fimbria in adult rats permits the anomalous ingrowth into the hippocampus of sympathetic axons which normally innervate the extracerebral vasculature. The stimulus for this axonal sprouting is not known, however several studies suggest that damage to the medial septum is essential to elicit the response. In addition, the anomalous ganglionic axons collateralize in a pattern which closely resembles the distribution of septal afferent axons within the area dentata and CA3. In order to determine more closely the relationship of the sympathetic axonal sprouting to septal deafferentation we have removed the septum from adult female rats while aspirating a cavity in the fimbria. Into this cavity we implanted a 1 mm^3 piece of embryonic septum dissected from 17-day rat fetuses. Additional rats received no implant into the cavity as controls. Animals were allowed to survive from 3 weeks to 3 months. Alternate cryostat sections were treated with glyoxylic acid for the visualization of catecholamines or stained for acetylcholinesterase (AChE). At the earliest survival times the implant had fused to the cut surface of the anterior hippocampal formation and contained many neurons staining positively for AChE. AChE-containing axons also penetrated approximately 1mm caudally within the area dentata and CA3. Alternate sections showed a vigorous sympathetic axonal plexus within and around the granule and CA3 pyramidal cell layers and deep within the hilus. These sympathetic axons were confined, however, to the more caudal levels of the hippocampal formation. At 2 months postoperatively the AChE-containing axons of the implant had extended to midseptotemporal levels and alternate sections again showed very little overlap with the more caudally located sympathetic plexus. By contrast, in rats with no septal implant in the fimbria cavity, the sympathetic axons extended throughout all levels of the hippocampal formation. This, together with our earlier studies, suggest that the sympathetic axons grow into the partially deafferented hippocampal formation beginning at 9-10 days then collateralize throughout the structure by 30 days. The septal implant axons grow in somewhat more slowly, and as they advance caudally appear to cause retraction of the anomalous sympathetic axons. Whether this is due to competition between the two systems for a common postsynaptic target or to competition for some trophic factor in the environment remains to be demonstrated. Supported by NINCDS Grants #NS-14372 (RL) and NS-15207 (LFK).

225.1 DEVELOPMENT AND DEPLOYMENT OF SCHWANN CELLS IN PERIPHERAL NERVES OF TREMBLER ↔ NORMAL MOUSE CHIMAERAS. H.B. Rayburn*, A.C. Peterson and A. Aguayo. Montreal General Hospital Research Institute, Montreal, Quebec, Canada.

The mouse chimaera preparation has been used extensively to investigate cell lineage, clonal deployment and cellular interactions during development. Numerous investigations have successfully addressed these issues in the central nervous system, but peripheral nerves have not received the same attention due to lack of suitable genotype markers for axons and Schwann cells. Previous transplantation studies in mature animals have provided evidence that the myelin deficiency in Schwann cells of Trembler mice results from an intrinsic Schwann cell deficiency (Ann. N.Y. Acad. Sci. 317: pp 512-533, 1979). Therefore, we produced Trembler ↔ normal chimaeras in the hope that myelin phenotypes would permit discrimination of two genotypically distinct classes of Schwann cells. If expression of the appropriate phenotypes did occur, it would provide a unique opportunity to explore the deployment and possible interactions of Schwann cells during primary development.

Trembler ↔ normal chimaeras do demonstrate variable proportions of typically myelinated Schwann cells and Schwann cells revealing the mutant hypomyelinated phenotype; a result predicted from an intrinsic deficiency in the Trembler Schwann cell. In some chimaeras, cross-sections of spinal roots reveal large, relatively coherent patches of Schwann cells demonstrating either normal or Trembler phenotypes. In addition, we have observed both myelinated and hypomyelinated Schwann cells on individual axons. Thus, the Trembler phenotype appears to be expressed in mutant Schwann cells which have undergone their entire primary development in proximity to both genotypically normal Schwann cells and presumably, to axons of both genotypes.

It is also anticipated that analysis of the size and shape of Trembler and normal Schwann cell territories will provide a further understanding of the clonal deployment of Schwann cells during primary development of peripheral nerves.

225.2 IONIC MECHANISMS FOR IMPULSE PROPAGATION IN GROWING NONMYELINATED AXONS: SAXITOXIN BINDING AND ELECTROPHYSIOLOGY. G. Strichartz*, R. Small, C. Nicholson, K. H. Pfenninger, and R. Llinás (SPON: M. E. Hatten). Dept. Physiol. Biophys., SUNY at Stony Brook, NY 11794; Dept. Anatomy, Columbia Univ. P&S, New York; Dept. Phys. & Biophysics, New York Univ. Medical Ctr., New York.

Transection of olfactory nerve in bullfrogs produces a total degeneration of the nerve bundle and its parent neurons. Five to six weeks postoperatively a new fiber bundle is seen in the olfactory canal as the fibers grow synchronously toward the olfactory bulb. Action potentials in this growing nerve may be obtained by direct electrical stimulation in a recording chamber. These compound action potentials have conduction velocities lower than mature nerve and seem to be resistant to tetrodotoxin (10^{-5} M). However, they may be blocked with application of 10^{-3} M cadmium chloride, indicating the presence of calcium-dependent spikes at or near the growth cones. These results are consistent with a reduced density of Na^+ channels shown by (a) the lower density of saxitoxin binding sites (40% of mature), (b) the small depolarization of membrane potentials produced by Na^+-channel activators, such as veratridine and bactrachotoxin, compared with normal olfactory nerve, and (c) the reduced density of intramembranous particles detected by freeze-fracture of the axonal membranes (Small and Pfenninger, this volume). In addition, action potentials in mature nerves are not blocked by 30 nM saxitoxin, consistent with the low saxitoxin affinity (K_D = 30-50 nM) measured in the binding studies; thus, these nerves differ from other nonmyelinated nerves which have a high sensitivity and low K_D values. The present results provide evidence for the participation of Ca^{++}-channels in growing neurites as previously observed in developing Purkinje cells (Llinás and Sugimori, Soc. Neurosci. Abst. 4: 66, 1978) and in regenerating cockroach giant axon (Meiri et al., Soc. Neurosci. Abst. 5: 680, 1979).

225.3 POSTNATAL DEVELOPMENT OF RETINO-GENICULATE AXON ARBORS IN THE KITTEN. C.A. Mason, Dept. of Pharmacology, New York University Medical Center, New York 10016.

The morphology of retino-geniculate axon terminal arbors at different postnatal ages was studied in normal kittens. The maturation of branching patterns and of shape and synaptic relations of terminal swellings was examined. In kittens of 1-8 weeks of age, axons were filled with horseradish peroxidase (HRP) by injections into the optic tract. After 3-8 hours survival and routine aldehyde fixation, brains were processed for light and electron microscopy as previously described (Mason & Robson, Robson & Mason, 1979, Neurosci. 4: 79 and 99).

At 1 week of age, light microscopic observations at low power reveal that axons resemble adult arbors in their "cypress tree" shape and general restriction to intralaminar zones. At higher power, however, there appear to be more divisions of fine terminal branches, some of which bear no swellings. The most striking feature of axons at this age is that many leading tips on a single arbor are enlarged into structures similar to growth cones seen in vitro and in Golgi preparations. These structures consist of broad flanges up to 10 μm long that give rise to two or three filodopia. Other terminal forms not seen in mature axons are ovoid swellings, V-shaped spikes, and small club-shaped endings. At 2 weeks, arbors are less spray-like. Branches still commonly end in growth cones, but also end in more slender terminal swellings having a slightly indented edge. By 4-6 weeks, terminals are grouped in clusters and have fuller shapes with either round or crenulated forms. By 8 weeks, branching patterns of terminal arbors look virtually mature but branches seem to be more heavily laden with terminals than in the adult. Occasionally, a fine branch terminates in a growth cone.

The ultrastructure of axons at 1 week indicates that identified growth cones labeled with HRP contain large vesicles, mitochondria, and microtubules. Synaptic junctions are made by the main flank of growth cones and round clear vesicles are often clustered near the density. By 3 weeks, crenulated terminals contact dendritic and other profiles. Although the retinal terminal is central in this arrangement, glial sheaths are absent and do not appear until after 4 weeks, agreeing with Kalil & Scott (Soc. Neurosci. Abstr. 5: 2660).

These observations indicate that, although retinal axons have reached their targets days before birth and have made synapses by birth (Kalil & Scott), the remodeling of their arborizations and terminals continues for some months after birth. (Supported by NIH Grant EY 02374).

225.4 DEVELOPMENTAL STUDIES OF RAT RETINAL CELL SURFACES USING CELL-TYPE SPECIFIC MONOCLONAL ANTIBODIES. Colin J. Barnstable* (SPON: Torsten N. Wiesel). Dept. of Neurobiology, Harvard Med. Sch., Boston 02115.

A series of monoclonal antibodies have been produced following immunization of BALB/c mice with a membrane preparation isolated from retinas of adult CD rats (Barnstable, Nature, 1980). The specificity of these antibodies has been determined by immunofluorescent labeling of fixed tissue sections.

Using a number of antibodies that specifically label discrete areas of the photoreceptor cell surface, the times at which the corresponding antigens appeared during the postnatal development of the rat retina was studied. RET-P1 is an antibody that labeled the cell bodies, inner segments and outer segments of adult rat photoreceptors. In Tiger Salamander retinas, this antibody labeled rods but not cones. In newborn rats it labeled a small number (< 1%) of cells, many of which had two processes, one ending at the ventricular surface and the other in the middle of the retina. The number of labeled cells increased with time so that by day 6-8 a broad band of cells in the outer portion of the retina showed fluorescence. From the antibody specificity, these were inferred to be photoreceptor precursors. At day 8, when the outer plexiform layer was easily recognizable, a small number of displaced photoreceptor precursors could be seen in the inner nuclear layer although those disappeared later. RET-P2, which, in the adult, labeled only photoreceptor outer segments, labeled small projections at the ventricular surface at day 5--the time of beginning of outer segment formation. An anti-rhodopsin antibody also labeled some of these projections at day 5. RET-P3, which labeled only photoreceptor cell bodies, gave labeling only at day 12-14, the time of eye opening.

A similar analysis of Muller cell development was carried out using five specific antibodies. Only one, RET-G1, labeled cells in newborn rats. This was also the only one of the five to label glia elsewhere in the adult CNS. Another, RET-G2, labeled 7 day old retinas--by which time the cells have undergone significant morphological differentiation. The antigens detected by the other three antibodies did not appear until day 12-14 when the retina was fully formed.

Monolayer cultures of cells dissociated from retinas of different ages have been set up and characterized using the above, and other, monoclonal antibodies. Although the morphology of the cells did not always follow the in vivo pattern, e.g. photoreceptor precursors detected by RET-P1 were spherical and had only one process containing large varicosities. With time these cultures reacted with some of the antibodies detecting antigens characteristic of the later stages of retina development. (Supported by NIH grant EY00606 and the Damon Runyon-Walter Winchell Cancer Fund)

225.5 PROPERTIES AND MATURATION OF AXOLEMMA IN GROWING NEURONS. Rochelle K. Small and Karl H. Pfenninger, Department of Anatomy, Columbia University, College of Physicians and Surgeons, New York, NY 10032.

Intramembranous structure, density of Na^+-channels and electrical excitability were compared in mature and growing axons in order to characterize the properties of growing axolemma. Freeze-fracture analysis of the olfactory nerve of the bullfrog, Rana catesbeiana, reveals the following differences. Axolemma from mature nerve contains an equal density (1100/μm^2) of intramembranous particles (IMPs) throughout and shows a consistent profile of IMPs ranging in diameter from 4.5 to 16 nm; higher IMP densities (2000/μm^2) are observed only at the perikarya. In contrast, rapid addition of new plasma membrane to the growing axon results in heterogeneous membrane composition along the axis of the neurite. Axolemmal IMPs are distributed along the neurite in the form of a distally decreasing, exponential density gradient ($y = 1485e^{-0.49x}$; $r^2 = 0.99$). IMP densities near the perikarya are comparable to levels seen in the mature nerve, but are reduced 10-fold at the growth cones (150/μm^2). Moreover, for individual IMP size classes, density gradients for smaller diameter particles are flatter than those for larger IMPs. In several cases, a narrow zone of increased IMP density was noted in the membrane region immediately proximal to the growth cone. These observations indicate that rapid expansion of the neuritic axolemma results in sequential differentiation of the plasma membrane. Furthermore, they suggest a spatial segregation of the site of addition of membrane matrix (e.g., lipids) needed for elongation, from the site of insertion of those membrane proteins constituting IMPs. New, IMP-free membrane appears primarily at the growth cone, while IMPs may be inserted at the perikaryon and then diffuse within the axolemma into the distal neurite. In addition, functional parameters of excitability (see Strichartz, Small, Nicholson, Pfenninger, Llinás, this volume) indicate a gradual differentiation of the axolemma. The density of saxitoxin binding sites in growing neurites is only 40% of control values, and the impulse has an unusually low conduction velocity and seems to be tetrodotoxin-resistant at the tip. The suggested differences in the number and nature of cation channels are likely to be related to the particular IMP populations that characterize growing and mature axolemma. (Supported by USPHS Grants NS13466 and 07062).

225.6 MECHANISM OF MEMBRANE EXPANSION IN THE GROWING NEURON. Karl H. Pfenninger, Department of Anatomy, Columbia University, College of Physicians and Surgeons, New York, NY 10032.

Neuronal sprouting involves rapid expansion of neuritic plasmalemma. The site and mode of insertion of new membrane components were studied in cultured sprouting neurons of the rat superior cervical ganglion by (i) pulse-chase experiments with ^{3}H-glycerol, followed by biochemical analysis of microsurgically separated perikarya and neurites, and (ii) similar pulse-chase experiments followed by EM radioautography. Control studies show that 99% of bound ^{3}H-glycerol is incorporated into phospholipid (PL), and that 70-80% of labeled PL is retained in the tissue during EM processing. The following results were obtained: Following a 15 min pulse of ^{3}H-glycerol, the specific radioactivity of perikaryal PL reaches a peak and then drops off exponentially (t 1/2 = 90 min) over the next 120 min. In the neurites, by contrast, specific radioactivity of PL increases with a delay of about 60 min. Both the increase in neuritic dpm/μgPL and the rapid decrease of perikaryal dpm/μgPL can be blocked with 2.5 x 10^{-6} M colchicine or 5mM 2,4-dinitrophenol applied after the pulse. Radioautographic analysis of the perikarya of ^{3}H-glycerol-pulsed sprouting neurons reveals, at 15 min, 93% of silver grains over various cytoplasmic organelles, but only 7% over the plasmalemma. At 15 min, growth cones exhibit only a fraction of the labeling seen over perikarya. However, by 30 min, radioactivity in the growth cones has risen several fold, and clusters of large, clear vesicles show the highest number of silver grains per unit area of membrane found in the neurites. At 60 min, the peak of radioactivity has shifted from the vesicles to the plasmalemma of the distal neurite. During these first 60 min, the agranular reticulum of growth cones exhibits fewer silver grain counts than the large vesicles or the plasmalemma. These findings suggest: (i) New PL is synthesized primarily in the perikaryon and then exported by rapid axonal transport to the most distal region of the neurite. (ii) The PL vehicle - and the plasmalemmal precursor - is a large, clear vesicle which is found in subplasmalemmal clusters in the growth cone, where fusion with the cell surface and addition of new membrane components occurs. Thus, newly-synthesized membrane is inserted into plasmalemma predominantly at the tip of the growing neurite. This observation is consistent with earlier freeze-fracture data on the distribution of intramembranous particles (Pfenninger and Bunge, 1974; Small and Pfenninger, this volume) and with the results of lectin pulse-chase experiments which reveal the selective appearance of new surface glycoconjugates in the growth cone region over the clusters of large, clear vesicles. (Supported by NSF grant BNS14071 and NIH grant NS13466).

225.7 GOLGI STUDY OF HIPPOCAMPAL PYRAMIDS IN TWO PRETERM TWIN FETUSES: A COMPUTERIZED MORPHOMETRIC ANALYSIS OF BRANCHING CHARACTERISTICS. A. M. Paldino and D. P. Purpura. Dep't. Neuroscience and the Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, NY 10461.

The computer microscope was utilized to obtain three dimensional information representing neuronal structure and to analyse this information in regards to angles of branching, fiber separations to nodes and terminals, and other statistical parameters reflecting neuronal shape and maturation. Data have been obtained from two twin fetuses of 21 weeks gestation, with no extrauterine survival. Twin A, the first born, succumbed shortly after delivery due to fetal immaturity incompatible with life. This twin had no congenital anomalies and had a body weight of 640 grams. Twin B, a stillborn fetus weighing 600 grams, was delivered shortly after premature rupture of the membranes. Gross and microscopic examination of the brains of these two fetuses revealed no pathological processes.

Golgi impregnated neurons within or near the CA3 zone of the hippocampus were digitized and analysed in regards to statistical parameters considered to be associated with defining dendritic shape and likely to be of major importance in assessing the maturational status of neurons of the immature human cerebral cortex. Preliminary results indicate that the angles of branching (branch and fission angles) at dendritic bifurcations are similar in both infants. For example, in neurons of Twin A, the average dendritic branching angle was computed to be 63^0; for neurons of Twin B, this angle was found to be 64^0. Also, the average dendritic fission angle for neurons of Twin A was 98^0; for neurons of Twin B, this value was 93^0. Distributions concerning the dendritic fiber separation to nodes and terminals for neurons from each infant were also similar; however, some discrepancies were noted at few discrete intervals. Data concerning these parameters for the axonal processes will also be presented.

The emerging data from these twin studies are consistent with recent evidence suggesting that dendritic branch and fission angle values vary indirectly with gestational age, and thus strengthen the hypothesis that these angles are indices which reflect the maturational status of neurons in the human cerebral cortex.

(This study was supported in part by grants NS-13832 and HD-01799 from the National Institutes of Health.)

226.1 POSTNATAL DEVELOPMENT OF VISUAL ACUITY, RETINA, AND FOVEAL STRIATE CORTEX OF THE SQUIRREL MONKEY (Saimiri sciureus). J. M. Ordy, K. R. Brizzee and Pam Medart*. Neurobiology Department, Delta Regional Primate Research Center, Tulane University, Covington, La. 70433

Postnatal visual acuity was examined in relation to maturation of the fovea and foveal projection area of the striate cortex in the diurnal squirrel monkey. Optokinetic and visual discrimination tests of acuity from birth to maturity indicated an increase of minimum separable binocular acuity from 72 to 18 minutes by the end of the 1st month, and 1.1 minutes by 6 months, compared to an adult asymptotic acuity of 0.90 minutes of visual angle, at 4 years of age. Ophthalmoscopic observations of the funduscopic appearance indicated a vascular non-pigmented area centralis at birth, and a progressive demarcation of macula, fovea, with their respective light reflexes by 6 months. A foveal depression was present in the retina at birth, but the bipolar cell layer had not thinned completely. From birth to 90 days, there was a significant increase in foveal cone density, outer segment length, and cone layer thickness in the rod-free, all cone foveola. In the occipital cortex, 6 laminae were present at birth, but foveal striate cortex depth increased significantly from 1406 μm to 1648 μm by 90 days. Also, cell packing density decreased, whereas neuropil density increased. Meynert cells in laminae V-VI of the foveal striate cortex appeared well advanced at birth, compared to their appearance at 90 days. The observation of the study, involving acuity, fundus appearance maturation of fovea, cytoarchitecture of foveal striate cortex, and development of Meynert cells, all indicated a progressive developmental sequence in the retina-geniculo-striate projection of the central visual field. (Supported in part by NIH Grant HD09942 and RR0016418.)

226.2 DEVELOPMENTAL FEATURES OF DORSAL LATERAL GENICULATE NUCLEUS IN MONKEYS. Michael D. Gottlieb*, Tauba Pasik, and Pedro Pasik. Dept. Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

The maximal cross-sectional areas of 4200 neuronal somata and their nuclei were measured in coded specimens of 0, 1, 2, 4, 8, 16 week-old and adult monkeys (M. mulatta). Samples consisted of camera lucida drawings of 600 cells per age obtained from six specified locations within laminae (L) in eight equally spaced Nissl-stained coronal sections. The average area of somata varies as a function of age but differently in the parvocellular and magnocellular laminae. In the former, it increases 40% from birth to 2 weeks, then decreases until 8 weeks, and subsequently remains at about 25% above the level for the newborn. The mean cell size in the magnocellular layers changes little before 8 weeks. From 8 to 16 weeks, it grows approximately 38%, and then declines somewhat stabilizing 27% above the level at birth. As expected, both the mean size and the variability are larger in the magnocellular than in the parvocellular laminae at all ages although the differences become less dramatic between 1 and 4 weeks. The average nuclear area does not follow the same pattern in both types of layers. Surprisingly, the nuclei are similar or larger in the parvocellular laminae during the period between 1 and 8 weeks. The mean nuclear size is approximately the same at birth and after 16 weeks within each type of lamina. The dynamics of development however is different, drastic changes occurring at 2 weeks in parvocellular layers (increases of 40%) and at 4 weeks in the magnocellular layers (decreases of 25%).

Comparisons were also made between cells receiving crossed (L6) vs uncrossed (L5) retinal afferents, cells with on-center (L6) vs off-center (L4) receptive fields, and zones representing central vs peripheral retina. In the adult, significant differences are found only for cell and nuclear sizes of central vs peripheral representation in L1, larger values being obtained from the periphery. This difference also occurs during development but the sign is not consistent. A uniform trend is present in parvocellular layers such that the soma and nuclei of peripheral zones are always larger than in central areas at all ages examined, but significant differences are obtained only for nuclear areas at 1, 4, and 8 weeks.

These findings suggest that maturation occurs earlier in parvocellular than in magnocellular laminae as evidenced by the timing of peak cell area (1 to 4 weeks vs > 8 weeks), stabilization of cell size (4 to 8 weeks vs > 8 weeks), and major changes in nuclear size (4 to 8 weeks vs > 16 weeks). Parvocellular layers develop at similar rates regardless of their reported physiological differences. (Aided by NEI Grant EY-01926)

226.3 AN INDIRECT RETINO-HYPOTHALAMIC TRACT IN THE NEWBORN FERRET (MUSTELA PUTORIUS FURO). Josephine B. Cucchiaro* and R.W. Guillery. Neurobiology Program, Department of Pharmacol. & Physiol. Sci., The University of Chicago, Chicago, IL, 60637.

A small bundle of retinofugal axons which takes an indirect, circuitous course around the lateral and dorsal aspects of the thalamus to terminate in the hypothalamus, has been identified in the newborn, but not in the adult ferret. Concentrated (50%-70%) horseradish peroxidase (HRP) was injected into the vitreous of one eye of young ferrets. The animals survived 18 hours before they were perfused through the heart with 4% glutaraldehyde in phosphate buffer (pH 7.4). Frozen sections of the brains were cut and the sections were reacted with 3,3',5,5' tetramethyl benzedine (TMB). Another developmental sequence of ferret material, using the tritiated proline autoradiographic demonstration of retinofugal pathways, and several paraffin embedded Nissl series of ferret brains were also available.

The newborn ferret is relatively immature. Compared to the cat, which is born on the 63rd day of gestation with a fully laminated lateral geniculate nucleus, the ferret is born after 42 days and develops geniculate laminae 15-20 days later. Using the HRP method we have observed the retino-hypothalamic tract in ferrets sacrificed 1, 3 and 5 days after birth. We have not seen clear evidence for this tract in our autoradiographs nor have we been able to demonstrate it by any method in animals 8 days or older. The crossed tract is well defined in 1 day old animals. Its fibers leave the optic tract at the rostrolateral border of the ventral lateral geniculate nucleus, coursing anteromedially around the anterior and dorsal aspect of the thalamus. Near the anterior thalamic nuclei the tract turns in a ventrolateral direction running caudal to the anterior commissure and lateral to the fornix. Finally, the tract terminates dorsomedial to the optic tract in the lateral hypothalamic area. The crossed tract is present at 3 and 5 days, but is small and difficult to trace in the 5 day old animals. A small uncrossed component is present in some of the youngest animals, but could never be traced beyond the anterior thalamic nucleus. The fate of the retino-hypothalamic tract during the development of the visual system remains unclear. It may degenerate, cease transporting HRP, or become so scattered that it can no longer be identified.

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226.4 THE DEVELOPMENT OF THE RETINO-GENICULO-CORTICAL PROJECTION IN THE TREE SHREW, TUPAIA BELANGERI G. Rager, R.S. Nowakowski, S. Lausmann+, M. Tanaka+ and A. Schwaier+. Dept. of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Postfach 968, D-3400 Göttingen, West Germany.

The two eyes of the tree shrew are represented in cortical area 17 not in ocular dominance columns but in ocular dominance sublaminae. We investigated how this projection develops during the postnatal period using neuroanatomical and electrophysiological techniques. Anterograde transport of horseradish peroxidase injected into one eye reveals that three terminal fields can be recognized in the contralateral and two in the ipsilateral lateral geniculate nucleus (CGL) at postnatal day 1 (P1). The contralateral as well as ipsilateral fields seem to be broader and more diffusely labelled than in older animals. Two of the three fields in the contralateral CGL are heavily stained. Between them a diffuse network of labelled fibres and terminals of much lower density can be seen. By P10, a fourth clearly definable field emerges in the position of the diffuse network giving a four field pattern similar to the adult, although the fields are still broader and less well-defined than in the adult.

To study the geniculo-cortical pathway we injected tritiated proline and fucose into the eyes of three animals at PØ, P14 and P44. After 14 days of survival the animals were sacrificed and the brains were processed for autoradiography. Transneuronal transport can be seen in all three animals. In all cases labelled terminals are confined to lamina IV of area 17. The animal injected at PØ does not show any laminar subdivision, while in both of the older animals the ipsilateral projection begins to spare sublamina IVβ (nomenclature according to Hubel, 1975). No clear increase of silver grain density could be found in the contralateral lamina III. These results indicate that the retino-geniculo-cortical projection is first diffuse in the CGL and area 17. The laminar pattern of the adult can be seen only later in development. Thus, although the binocular representation in tree shrews differs from that in cats and rhesus monkeys, the development of the projection seems to be based on similar mechanisms, i.e. the segregation of terminals from a diffuse to a restricted pattern.

Our preliminary electrophysiological results show that electrical stimulation of the optic nerve elicits an evoked potential shortly after birth. A visual evoked response can be obtained a few days before eye opening.

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226.5 THE DEVELOPMENT OF EFFERENT PROJECTIONS FROM AREA 17 OF THE TREE SHREW, TUPAIA BELANGERI. R.S. Nowakowski and G. Rager. Department of Neurobiology, Max Planck Institute for Biophysical Chemistry, Postfach 968, D-3400 Göttingen, West Germany.

As part of an analysis of the structural and functional development of the visual system of the tree shrew, Tupaia belangeri, the development of the efferent projections from the primary visual cortex has been studied by examining the retrograde and anterograde transport of horseradish peroxidase (HRP; Boehringer Grade I) following small iontophoretic injections. The anterograde transport of HRP from area 17, visualized by using the tetramethylbenzidine technique of Mesulam (1978), indicates that the projection of the cortex to the lateral geniculate is present as early as the day of birth. This is before the individual cell laminae are identifiable in Nissl-stained material. The cortico-thalamic terminals are intermingled with the cells labeled by retrograde transport. Both the labeled terminals and the labeled cells are distributed across the entire width of the lateral geniculate, forming a small cylinder which varies in size with the size of the injection site.

The cortical projection to the tectum develops later than the cortico-thalamic projection. In fact, both anterograde and retrograde transport of HRP indicate that the projection of the cortex to the superior colliculus is not present at birth. At postnatal day 9, however, the cortico-tectal projection can be demonstrated by anterograde transport of HRP. Thus, the development of the cortical projection to the tectum seems to lag behind the development of the cortical projection to the thalamus. This sequence of events may reflect the fact that the cells of origin of the cortico-thalamic pathway (layer VI) are generated earlier than the cells of origin of the cortico-tectal pathway (layer V).

The cortico-cortical fibers also seem to develop after the cortico-geniculate fibers, and we are presently exploring whether they appear before or after the cortico-tectal pathway.

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226.6 ORDERED ANOMALOUS RETINAL PROJECTIONS TO THE MEDIAL GENICULATE, VENTROBASAL AND LATERAL POSTERIOR NUCLEI. D.O. Frost. Inst. of Anat., U. of Lausanne, Switzerland.

In newborn Syrian hamsters, two of the retina's principle targets, the superior colliculus (SC) and dorsal lateral geniculate (LGd) nucleus were destroyed, respectively, by a direct lesion and by retrograde degeneration following a lesion of posterior neocortex. Simultaneously, alternative terminal space was made available in the lateral posterior (LP) nucleus by the SC lesion and in the medial geniculate (MG) and/or ventrobasal (VB) nuclei by removing the auditory and/or the somatosensory lemniscal inputs. The SC lesions were always bilateral; the lesions of the auditory and somatosensory lemniscal pathways were either bilateral or ipsilateral to the (always) unilateral cortical lesions. When the operated animals were adults, the projections of the eye contralateral to the cortical lesion and degenerated LGd were traced by making 1 or 2 small peripheral retinal lesions and intraocular injections of 50 μCi of ^{3}H-proline, 5- and 1- days, respectively, prior to sacrifice. Adjacent series of sections from each brain were processed by the Fink-Heimer and autoradiographic techniques. A computer was used to reconstruct surface views of the thalamic nuclei and the patterns of autoradiographic labeling (ARL) and degeneration (D) within their borders. An additional neonatally operated animal with auditory and somatosensory lemniscal lesions survived 14 days following intraocular injection of 400 μCi of ^{3}H-proline.

The neonatal surgical procedures reliably produced in the partially denervated LP, MG and VB, anomalous crossed retinal projections that terminate preferentially near the surfaces of those nuclei. Label was transported transsynaptically to the "auditory" and "somatosensory" cortices in the case with the large injection and long survival time.

The anomalous retinal projections show several signs of order: i) When a nucleus contains both ARL and D only part of the total projection field so revealed contains D. Thus a restricted retinal region gives rise to a restricted part of the projection. ii) Small retinal regions appear to be represented along "lines of projection" in LP, MG and VB, as they normally are in LGd and SC. iii) In most instances, ARL and D are completely or partially segregated. Therefore, different parts of the retina project to different parts of each nucleus. iv) In a given nucleus, lesions in different retinal quadrants produce degeneration at different poles of the anomalous projection. Thus, in each nucleus there is a consistent polarity in the abnormal projection. This is clearest in MG, but also appears to hold for LP and VB.

In some animals, one or more nuclei contain only AR or only D, while the others may contain both ARL and D. Thus each nucleus may contain only a partial representation of the retina and the projections to different nuclei can originate from overlapping or non-overlapping retinal territories.

The anomalous retinal projections to nuclei that normally receive little (LP) or no (MG, VB) retinal input show that the preference of retinal axons for their normal targets is relative, not absolute. If there are positional or polarity cues in LP, MG or VB that contribute to the ordering of an abnormal retinal projection, they can not be specific for the normal afferents of those nuclei. Individual variations in the degree of segregation of ARL and D within a given nucleus suggest variations in the precision of retinotopic ordering of the anomalous projections.

227.1 CHANGES IN SPEECH AND UPPER LIMB MOVEMENTS IN CEREBRAL PALSY PATIENTS AFTER FOCAL CEREBELLAR STIMULATION. L. Robertson. Neurological Sci., Inst., Portland, OR 97209.

Focal electrical stimulation of the cerebellar cortex of patients with various movement disorders has been reported to reduce spasticity and athetosis and improve activities of daily living for some patients. However, the clinical results are very controversial and there is considerable variability in the type and degree of changes observed. A quantitative study of the effects of focal cerebellar stimulation was made on oral-motor control, vocal characteristics, and upper limb skill and reaction time movements in 10 patients with cerebral palsy. The patients were evaluated prior to surgery and after approximately 2 and 6 months of cerebellar stimulation. Four patients were further tested with and without stimulation in a series of evaluations of upper limb movements.

Eight of the ten patients showed a small degree of speech improvement, the other two cases were not affected by the stimulation. Seven of the patients significantly increased their duration of vowel phonation; four cases (all with moderate dysarthria) improved their articulation, particularly for consonants S, Sh, and Th; and two patients had better oral-motor control. Most of the changes in sound production and speech intelligibility appear related to improved intraoral breath control. Thus, cerebellar stimulation may be modifying the coordination of pharyngeal and laryngeal muscles.

Five cases showed some improvement on a motor accuracy test after two months of stimulation. In general, the patients made more straight lines and decreased the frequency of squiggles and loops that are associated with uncontrolled movements. Four of these patients were tested with a reaction time procedure with and without stimulation. During the initial sessions without stimulation, all cases showed a decrement of extension of the nondominate arm. Cerebellar stimulation reduced the reaction time for 3 of the 4 cases. However, after two sessions during which cerebellar stimulation was used, the reaction time for the non-stimulation trials also decreased to the level obtained during stimulation. Subsequently, the presence or absence of stimulation did not influence the reaction. Similar results were obtained for a sequential movement. Initially cerebellar stimulation improved the performance but continued stimulation was not necessary to maintain the new performance level. Thus, these preliminary results suggest that cerebellar stimulation may be helpful during the acquisition of a motor skill, but have little or no influence once the movement has been learned.

227.2 IMMUNOLOGICAL STATUS OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS--- A LONGITUDINAL STUDY. F.C. Westall and Charles Jablecki*. Autoimmune and Neoplastic Disease Laboratory, Salk Institute for Biological Studies and Department of Neurosciences, University of California, San Diego, CA 92037.

The immunological status of 30 Amyotrophic Lateral Sclerosis (ALS) patients has been assessed over a two year period. Total IgG, IgM, and IgA for the patients were not significantly different from controls. However, patients whose disease process were progressing more rapidly as a group showed lower IgG and IgM values and higher IgA than those who were progressing more slowly. Eighty percent of the patients showed increases in IgA with time. Two patients had IgA values greatly exceeding normal, and in one case the IgA was monoclonal.

The percentages of various subclasses of T and B lymphocytes were estimated. The ALS patients showed a slight increase in the percentage of T cells compared to normal. There was a significant ($p<.001$) reduction of $T\mu$ cells within the ALS population (ALS $T\mu$ = 24% compared to normal $T\mu$ = 47%). No significant differences were found in the $T\gamma$, $B\mu$ and $B\gamma$ classes. We conclude that there are substantial immunological abnormalities in patients with ALS. It may be that these abnormalities reflect an altered host response to an infectious agent.

227.3 EFFECT OF CHRONIC PHENYTOIN ON ULTRASTRUCTURAL MORPHOLOGY AND BENZODIAZEPINE RECEPTOR DENSITY IN RAT BRAIN. P.P. Deshmukh*, T. Mimaki* and H.I. Yamamura (Spon: L. Stern) Dept. of Pharmacology and Psychiatry, University of Arizona HSC, Tucson, AZ 85724.

Phenytoin (DPH-Dilantin®) is the most widely used anticonvulsant in the treatment of epilepsy. Despite the forty years of its use in clinical medicine, the mechanism(s) of action of this compound is not fully understood. The effect of chronic or toxic administration of DPH is still less clearly defined. The clinical evidence relating DPH to possible cerebellar dysfunction was recognized by Merritt and Putnam (1939). They included cerebellar ataxia, tremor, diplopia and nystagmus as part of the symptom complex associated with DPH therapy. Neuropathological changes have been found in the cerebellum of epileptic patients, but whether these changes are associated with the chronic use of DPH in epileptics or with seizure activity or both remains unclear.

Interestingly, DPH and Diazepam- a muscle relaxant- exhibit similarities in their molecular structure. These similarities suggest a steric basis for their anticonvulsant activity. The present investigation was undertaken to correlate ultrastructural changes with the biochemical alterations seen in the neurotransmitter and drug receptors after chronic DPH therapy. Brain benzodiazepine receptors were measured in the cerebellar and cerebral cortex following 14 days and 28 days of DPH administration (200 mg/kg/day i.p.) Control rats were injected with the vehicle alone for same duration of time. Our data show there was no change in the K_d values between controls and the experimental group, but the Bmax was significantly lower in the DPH treated cortex and cerebellum indicating a decreased receptor density. Two areas of vehicle and DPH treated rat brain were processed for electron microscopy. In the cerebellar vermis from DPH treated rat, numerous degenerated Purkinje cells were seen at both time periods. The degree of degeneration varied from one region of the cerebellum to another as well as from one Purkinje neuron to the next. At the electron microscopic level the degenerated Purkinje cells had hyperchromatic nuclei with infolded nuclear envelopes. Clumping of the Nissl substance and hypertrophy of the Golgi apparatus was also evident in the perikaryon. Concommitant with the Purkinje cell degeneration the Bergmann astrocytes showed edematous changes indicative of metabolic alterations. Other cerebellar neurons were unaffected by this treatment. Currently, the cerebral cortex and the hippocampus from the treated rats are being examined for morphological alterations. It is evident from these data that high doses of chronic DPH administration produces degenerative changes in the Purkinje cells and a decrease in the density of the benzodiazepine receptors in the cerebellum.

227.4 GALLOPING FORWARD LOCOMOTION PRODUCED BY LOCALIZED VENTRAL PONTINE TEGMENTUM DAMAGE IN THE RAT: A POSSIBLE ANIMAL ANALOG OF PARKINSONIAN FESTINATION. Jung-Tung Cheng*, Timothy Schallert, Marc De Ryck*, and Philip Teitelbaum. Psychology Department, University of Illinois, Champaign, IL 61820.

Akinesia is a major symptom of parkinsonism. However, some patients, although having difficulty in initiating locomotion, may then rapidly accelerate to the point where they are unable to stop (festination). Rats (n=11) with bilateral electrolytic lesions in the ventral pontine tegmentum (VPT) accelerate their forward locomotion (they walk, then run, then gallop) headlong into an obstacle or over the edge of a high table. They lack lateral head scanning and orienting. Because some parkinsonian patients have damage, not only in the nigrostriatal dopaminergic system, but also in the VPT (Greenfield, et al., J. Neurol., Neurosurg., Psychiat., 1953, 16, 213-226), we suggest that the festination shown by our animals may be analogous to human parkinsonian festination. If so, such festination should coexist with akinesia produced by catecholamine blockade.

Normal rats become completely akinetic when treated with haloperidol (5 mg/kg, i.p., n=5). Such haloperidol treatment does not prevent or even slow down the accelerating forward locomotion seen in rats with ventral pontine tegmental damage (n=8). Indeed, later in their recovery when they have begun to inhibit their forward locomotion (they no longer run into the wall), haloperidol (up to 10 mg/kg) reinstates (i.e., increases) their acceleration making it once again uncontrollable.

We suggest that the festination shown here may represent the action of a forward locomotion subsystem. Such isolated locomotion, though not inhibited by haloperidol, can be abolished by bilateral pressure on the snout. Supported by NIH Grant RO1 NS 11671.

227.5 METHYSERGIDE RELEASES FORWARD LOCOMOTION WITHOUT SUPPORT IN OTHERWISE AKINETIC LATERAL HYPOTHALAMIC RATS. Rebecca M. Chesire* and Philip Teitelbaum. Psychology Department, University of Illinois, Champaign, IL. 61820.

Akinesia, a prominent symptom of parkinsonism, can be produced by damage to dopamine systems in the brain. Animal models suggest that the akinesia results, in part, from excessive inhibition of the remaining neural subsystems involved in locomotion. Thus, anticholinergics (atropine or scopolamine) can release walking in otherwise severely akinetic, catecholamine-depleted rats (6-hydroxydopamine intraventricularly; Schallert et al., Science, 199, 1978). However, atropine is ineffective in rats made akinetic by bilateral electrolytic lateral hypothalamic damage (1 mA for 20-32 sec, anodal), a treatment that also damages the dopaminergic nigrostriatal system. We show here that even on the first day postoperatively, when the akinesia is most profound, methysergide maleate (a serotonin receptor blocker; 45 mg/kg, i.p.) can release vigorous forward locomotion in lateral hypothalamic rats for as long as an hour and a half.

The locomotion is abnormal. On Day 1, when postural support is largely absent in the akinetic animal, such locomotion occurs without support so that the animal appears to be crawling. The animal is propelled straight forward by its hindlegs with its chin dragging along the ground and its forelegs lagging in their associated stepping. Head scanning is completely absent (n=8). Later in recovery (5 days; n=6), when postural support and head scanning have recovered, methysergide-elicited locomotion is integrated with these movement subsystems into a more normal form of locomotion.

These findings indicate that at least two independent inhibitory neural systems, one cholinergic and the other serotonergic, exaggerate the akinesia produced by damage to the nigrostriatal dopaminergic system. They also reveal the independent movement subsystems isolated earlier by us (Golani et al., Brain Research, 164, 1979). Supported by NIH Grant RO1 NS 11671.

227.6 RED NUCLEUS CHANGES IN THE MOUSE MUTANT DYSTONIA MUSCULORUM Edith M. Stanley*, Anne Messer, and Norman L. Strominger; New York State Department of Health and Albany Medical College, Albany, New York 12201.

The mouse mutant dystonia musculorum is an autosomal recessive, progressive neurological disease. Symptoms are first observed in animals of 2 weeks of age and worsen steadily for the next 2 weeks. Little further deterioration occurs after 4 weeks of age. The first descriptions of these animals by Duchen and Janota reported pathology in the sensory afferent systems beginning at birth. We have previously reported a spontaneously occurring allele of this mutation which shows pathology in the red nucleus and the striatum as well as sensory afferents. In this study the effects of the mutation on the red nucleus are examined in both our strain (dt^{Alb}) and the allele carried at the Jackson laboratory (dt^{J}).

The time course and severity of pathology differ in the two strains. The pathology starts earlier and is somewhat more severe in the dt^{J} strain, with the large coarse neurons almost entirely absent in the adult dt^{J} red nucleus. There is a dramatic loss of acetylcholinesterase activity in adult dt^{Alb}, especially after recovery from diisopropyl fluorophosphate (DFP) injection. In animals pretreated for 8-10 hours with 5mg/kg DFP, the dt^{Alb} neurons show little if any staining, while controls show a substantial amount.

The time course of effects shows that the maximum effect of the mutation on the red nucleus at 3-4 weeks matches the time of maximum clinical effect. Clearly the changes in the red nucleus are not solely responsible for the clinical effects, as modest cell changes in young mice show a strain difference which is not reflected in crude clinical analysis of the earliest stages of the disease. The strain differences in mature animals may not be significant, since severely affected cells show greatly reduced cholinesterase, implying a loss of function. There are still many interesting questions to be examined in the red nucleus, as well as in the striatum and afferent sensory pathways of this mutant. With a variety of age and strain differences available, this mutant may offer a possible way of studying both the development and functional effects of progressive hereditary systems degenerations.

Supported by The Hereditary Disease Foundation and The Dystonia Foundation.

228.1 ANTIGEN- AND ACETYLCHOLINE-INDUCED CURRENT NOISE IN THE DENERVATED GUINEA PIG DIAPHRAGM. A.Auerbach* and M.Titmus* (SPON:J.del Castillo). Lab. Neurobiology, UPR Sch. Med., San Juan, PR 00901.

Smooth muscles from animals allergized by injection of an antigen contract when re-exposed to the sensitizing protein (Dale-Schultz reaction). While innervated skeletal muscles do not exhibit antigen sensitivity, the denervated hemidiaphragm of the guinea pig does. Local application of specific antigen has been shown to cause a depolarization and an increase in the conductance of the muscle membrane (de Florida et al, J. gen. Physiol. 51:677,1968). We have used the noise analysis technique to characterize the channels which open across the denervated muscle membrane under the influence of ACh and three antigens: ovalbumin, DNP-BSA (DNP-lysine as hapten), and BSA. Pressure application of antigen to voltage-clamped muscle fibers induces an inward current (up to 120 nA) which is of smooth macroscopic time course. The variance of the current noise increases with the intensity of the inward current. Analysis of the current fluctuations yields a spectrum which can be adequately fit by a single Lorentzian. Channel lifetimes(τ) and conductances (γ) were calculated from the noise spectra; the responses to both ACh and antigens were assumed to reverse at 0 mV. The results (2-10 animals for each ligand) are given below (mean$\pm$sd, number of fibers).

	τ(-100mV) msec	τ(-70mV) msec	γ(all Vm) pS
ACh, 24°	2.6$\pm$0.9 (8)	1.8$\pm$0.5 (11)	63$\pm$17 (29)
Oval., 24°	3.5$\pm$0.9 (3)	2.7$\pm$0.5 (6)	41$\pm$10 (7)
Oval., 16°	8.2$\pm$1.2 (8)	6.0$\pm$1.5 (12)	19$\pm$18 (18)
DNP-lys, 24°	4.2$\pm$0.7 (7)	--	51$\pm$19 (11)

Neither α-bungarotoxin (2.5 ug/ml) nor d-tubocurarine (5 uM) blocks the antigen-induced contraction. Denervated diaphragms also contract in response to bath-applied histamine or norepinepherine (NE) at uM concentrations. We have not been able to elicit current noise of significant variance with pressure application of NE, and we have only infrequently observed histamine-induced current noise. The mechanism of the antigen response is being investigated. Three possibilities will be considered: 1) release of mediators from non-muscle cells, 2) activation of tissue complement, and 3) interaction of antigens with IgG antibodies bound directly to the muscle surface.

We thank P. Specht for help with the computer programming. AA is a MDA Postdoctoral Fellow; supported by P.H.S. Grant NS07464 to J. del Castillo.

228.2 DENERVATION-INDUCED LOSS OF MUSCLE END-PLATE ACETYLCHOLINESTERASE ACTIVITY IS REDUCED BY PROTEASE INHIBITORS. Myron Duell* and Hugo L. Fernandez (SPON: Dr. F. Samson, Jr.). Neuroscience Research Lab, VA Med. Center, Kansas City, MO 64128 and Dept. Physiology, University of Kansas Medical Center.

The protease inhibitors leupeptin and pepstatin, used in combination, partly delay the structural deterioration of avian muscle fibers caused by either muscular dystrophy or denervation. Leupeptin also reduces protein degradation in normal rat skeletal and cardiac muscles, as well as in rat denervated and mouse dystrophic muscles, maintained in vitro. It was of interest to determine whether these effects also extend to any neuromuscular transmission-related protein. We therefore chose to study muscle end-plate acetylcholinesterase (AChE; E.C. 3.1.1.7), because of its important role in transmission, and because its activity rapidly decreases following denervation. Changes in muscle wet weight and protein content were also studied.

Intact and denervated rat anterior gracilis muscles were injected intraarteriallv with the protease inhibitors leupeptin, pepstatin, and aprotinin, either alone or in combination. After 24 hr, 3 mm sections of muscle end-plate regions were weighed and processed for analysis of AChE activity and protein. A combination of the inhibitors partly prevented the early (24 hr) denervation-induced decrease in muscle weight and protein content. Leupeptin and aprotinin, either alone or in combination, markedly reduced the decay of AChE activity in the denervated muscles, whereas pepstatin alone was ineffective. The effects were additive in that the inhibitors in combination were more effective than when they were used separately.

These experiments suggest an important effect of protease inhibitors in partly preventing the denervation-induced decrease in muscle end-plate AChE activity. These effects probably occur through the inactivation of proteolytic enzymes, which otherwise would be increased in denervated muscle. Although the results of the present study are not sufficient to single out the involvement of any particular protease(s), further studies are underway to better define protease participation in end-plate AChE catabolism.

228.3 SHORT-TERM ACTIVATION OF TYROSINE HYDROXYLASE IN RESIDUAL NORADRENERGIC NERVE TERMINALS AFTER INTRAVENTRICULAR 6-HYDROXYDOPAMINE. Ann L. Acheson and Michael J. Zigmond, Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Intracerebroventricular administration of 6-hydroxydopamine (6HDA) produces a rapid and permanent loss of norepinephrine (NE) stores in rat brain due to a selective degeneration of catecholaminergic nerve terminals. We have reported that the decline in NE in noradrenergically innervated areas such as hippocampus is initially accompanied by a proportionate decrease in tyrosine hydroxylase (TH) activity as measured in the presence of saturating cofactor (3 mM 6MPH4). However, this is followed by a gradual increase in the apparent Vmax of TH toward control values, with no change in NE (Acheson et.al., Science 207:537, 1980). We now report a second, more rapid effect of 6HDA lesions on TH activity in residual terminals.

Adult male rats received 6HDA (200 ug free base in 20 ul, ivt.) and were sacrificed 36 hr later. Hippocampal TH activity, assayed at its pH optimum (pH 6.2) in a Tris-acetate buffer in the presence of subsaturating cofactor (0.7 mM), was reduced to 32% of control, a decrease comparable to that of NE levels (27% control). When TH activity from control animals was assayed at pH 6.5, a marked reduction in activity was observed (55% of activity at pH 6.2). In contrast, no decrease in TH activity was observed in samples from lesioned animals (120% of activity at pH 6.2). Thus, at pH 6.5, TH activity in lesioned animals was no longer significantly different from control values. We observe a similar change in the relationship between TH activity and pH when TH is incubated under in vitro phosphorylating conditions (Acheson et.al., Neurosci. Abst. 5:395, 1979). We therefore examined the effect of phosphorylating conditions on TH prepared from hippocampus 36 hr post-6HDA. We observed that while preincubation with ATP, cAMP, and protein kinase increased TH activity from control animals as assayed at pH 6.5 by 150%, there was no effect on TH activity from lesioned animals. The apparent activation of TH in hippocampus is still present 7 days post-lesion but not at 21 days, a time when the increase in the apparent Vmax of TH is present. We conclude that following 6HDA treatment, TH activity in residual terminals is increased by two mechanisms: first, existing TH is rapidly activated, perhaps by a mechanism involving in vivo phosphorylation; second, there is a gradual increase in maximal TH activity. These changes may permit residual neurons to increase synthesis and release of NE, thereby reducing the impact of the lesion.

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228.4 QUANTITATIVE HISTOCHEMICAL ANALYSIS OF SINGLE IDENTIFIED BARRELS IN THE MOUSE SmI CORTEX. W.D. Dietrich*, D. Durham, O.H. Lowry and T.A. Woolsey. Depts. Pharm. and Anat. and Neurobiol., Washington U. Sch. Med., St. Louis, MO. 63110.

Individual barrels in the posteromedial barrel subfield of the somatosensory cortex of mice are the cortical anatomical correlates of the large contralateral mystacial vibrissae. Recent studies have shown that following the removal of vibrissae in adult and neonatal mice, a significant decrease occurs in the histochemical staining of the mitochondrial enzymes cytochrome oxidase and succinic dehydrogenase within the corresponding barrels (Wong-Riley and Welt, '79; Durham, Welt and Woolsey, '78). The present study was undertaken to investigate the feasibility of determining various enzyme activities in individual barrels with quantitative histochemical techniques. The analyses were made on frozen sections cut in a cryostat and freeze-dried at -40°C. It was found that individual barrels could be identified in unstained sections and portions of them subsequently dissected and weighed on a quartz fiber balance. Micro-methods which included the use of oil wells and enzymatic cycling amplification were used. Because only small samples were needed (40 ng), levels of several enzymes in the same barrel, or the distribution of an enzyme within a single barrel could be determined. For instance, the activities of the mitochondrial enzymes, malic dehydrogenase (21.6 ± 0.5 mol/kg (dry)/h) and citrate synthase (5.0 ± 0.2 mol/kg (dry)/h) were measured in the same individual barrels. The basal levels of various glycolytic enzymes, including lactic dehydrogenase, hexokinase and P-fructokinase are being determined.

Using the same protocol the activity of various enzymes in the barrels following removal of selected groups of whiskers are being analyzed. Any changes in enzyme activity in the appropriate barrels consequent to the whisker damage can be followed at selected times after the peripheral damage and can be compared directly to activity in barrels in the same section associated with intact vibrissae. The methods developed are useful for describing the baseline levels of a number of biochemical properties of normal barrels and could provide information about changes in any of these parameters in the cortex, as a consequence of chronic sensory deafferentation occurring at least three synapses away.

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228.5 (14C)DEOXYGLUCOSE STUDY OF EFFECTS OF SENSORY DEPRIVATION ON FUNCTIONAL ORGANIZATION OF A VIBRISSAL COLUMN IN RAT SI. L.Smith*,P.Hand, M. Kossut*Dept.of Animal Biology,School of Vet. Medicine, and Inst. of Neuro. Sci., Univ. of Penn., Phila.,PA.19104; Dept. of Neurophsiology, Nencki Institute, Warsaw, Poland.

A previous deoxyglucose (2DG) study demonstrated a spindle-shaped column in rat SI activated by stimulation of contralateral facial vibrissa #3 of row C (C3) (Hand et al,Neurosci.Abs.,'78).The effect of unilateral neonatal ablation of all vibrissal follicles, except C3, on the development of a single functional column was studied in 3 month old rats; a larger cortical area was labeled by C3 stimulation and the tapering columnar profile was lost, replaced by diffuse labeling in all layers(Kossut et al, Neurosci.Abs.,'79). The present study was designed to determine to what extent chronic sensory deprivation alone contributed to these changes. Repetitive unilateral vibrissal clipping or plucking was performed from birth on either all vibrissae, except C3, or C3 alone (contralateral vibrissae were unaltered and served as controls). After 2-3 months, either the C3 vibrissa or the surrounding vibrissae B3, C2, C4, and D3 were stroked bilaterally as previously described by Hand et al., following injection of 2DG. The column activated by vibrissa C3 in the former C3 "spared" group retained the characteristic spindle shape and dense labeling in laminae IV but the area labeled was enlarged in all laminae, its limits less sharply labeled. Column diameter was increased by a mean 28% in lamina IV. The local cerebral metabolic rate of glucose (LCMRG) was elevated 15% in lower lamina V. In the latter group (C3 clipped) surrounding columns showed similar spread of label (an 8% increase in LCMRG over control) into the region of the deprived C3 column. Light microscopy showed no alteration in dimension or morphology of layer IV barrels in either group, in agreement with previous reports. In conclusion, a deprivation-induced functional plasticity was detected in the "barrel" column. Metabolic changes seen in this study, however, were less pronounced than those reported after neonatal vibrissal receptor ablation; deprivation alone undoubtedly has a less disruptive effect on developing thalamocortical and corticocortical connectivity. (Supported by grants NS-14935:01,Sloan-76109,& Grass Foundations)

228.6 MORPHOLOGY OF LAMINAE IX NEURONS ROSTRAL AND CAUDAL TO THORACIC SPINAL CORD TRANSECTION IN WEANLING RATS. J. P. Cummings* and D. J. Stelzner, Department of Anatomy, SUNY, Upstate Medical Center, Syracuse, NY 13210.

A recent study in our laboratory has correlated the morphology of laminae IX neurons after thoracic spinal cord transection in neonatal rats with the morphology of laminae IX neurons during the normal developmental sequence. From 5 days to 9 months following transection the laminae IX neurons bordering the lesion retained both their normal size, distribution of their dendritic tree, and other morphological characteristics when compared with tissue taken from control animals of the same age. However, there was a slight increase in the number of varicosities on 2°, 3° and 4° dendrites. These somewhat swollen varicosities with irregular surfaces did not resemble the elongated, smooth varicosities found on the dendrites of the normal adult laminae IX neurons.

The current study was undertaken to correlate the morphology of laminae IX neurons after thoracic spinal cord transection in weanling rats with the morphology of laminae IX neurons following thoracic spinal cord transection in neonatal rats and with developing laminae IX neurons of control animals. A minimum of three weanling transected (24 days of age) animals were studied at 5, 10, 20, 30, 60, 90 and 180 days after transection. Camera-lucida drawings of representative laminae IX neurons from 0-9 mm rostral and 0-9 mm caudal to the stumps of the transected cord were made from coded slides.

Beginning as early as 10 days following transection, the laminae IX neurons within 0-4 mm (rostral and caudal) of the lesion had decreased dendritic fields, loss of dendritic branches and an increase in number of swollen, irregular varicosities along the entire length of their remaining dendrites. There was also a reappearance and an increase in both dendritic and somatic spines at all postoperative survival times. Laminae IX neurons located more than 4 mm from the lesion retained their normal adult morphology.

The laminae IX neurons bordering the lesion site in the current study did take on more characteristics of immature cells than were observed in laminae IX neurons bordering the lesion site in neonatally transected rats. This is probably related to the increased denervation caused by the spinal transection in the weanling rat since synaptogenesis is still ongoing at birth but is complete in the spinal cord by the weanling period. (Supported by Grants NS-14096 and NS-06353)

229.1 PEPTIDE HORMONE INITIATION OF PROGRAMMED CELL DEATH OF THE INTERSEGMENTAL MUSCLES OF THE SILKMOTH ANTHERAEA POLYPHEMUS. L.M.Schwartz* and J.W. Truman (SPON: K.Graubard). Dept. of Zool. NJ-15, Univ. of Washington, Seattle, Wa. 98195

The intersegmental muscles(ISM) are a group of embryonically derived muscles in the abdomens of developing adult Lepidoptera. These muscles provide the force necessary for adult eclosion and then degenerate during the subsequent 36 hrs. Previous work (Lockshin, R.A. and Williams, C.M., J.Insect Physiol. 11:601-610, 1965) has suggested that the trigger for this programmed cell death is the cessation of electrical stimulation to the muscles from the CNS. Working with the silkmoth Antheraea polyphemus, we have determined that ISM degeneration is initiated by a direct action of the eclosion hormone (EH) on the muscles themselves. EH is an 8,500 dalton peptide which is released from the brain prior to adult eclosion and acts directly on the CNS to release the stereotyped eclosion behavior (Truman, J.W., J.Exp. Biol., 74:151-174, 1978).

When animals were ligated between the thorax and abdomen prior to EH release, ISM breakdown was prevented. This muscle retention was reversed by hormone injection. The action of EH is not on the CNS to shut off motor activity, since denervated isolated abdomens did not breakdown their ISM. However, these denervated animals do show normal degeneration when injected with EH. To control for the potential action of EH on the distal stumps of the axons, animals were denervated three weeks earlier, prior to the initiation of adult development. The behavior of their ISM was comparable to the later manipulated animals. These data suggest a direct action of EH on the ISM.

EH acts on the muscles to stimulate the accumulation of cGMP as demonstrated by both biochemical and immunocytochemical techniques. This response is quite dramatic in both its time course and amplitude. Sixty minutes after hormone stimulation, cGMP levels are twenty-two fold over basal levels and are still ten fold higher three and a half hours after hormone exposure. No significant changes were observed in cAMP titers during this time in response to EH injection. Further work is focusing on other biochemical steps involved in this muscle degeneration.

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229.2 PROGRAMMED CELL DEATH IN THE NERVOUS SYSTEM OF AN INSECT: HISTOLOGICAL AND PHYSIOLOGICAL ASPECTS. J. W. Truman and R. B. Levine. Dept. of Zoology, Univ. of Washington, Seattle, WA 98195

In the moth Manduca sexta, the emergence of the adult from the pupal cuticle is followed by the death of neurons in the segmental ganglia. The fourth andominal ganglion (A_4) was selected for detailed study. At eclosion this ganglion contains about 70 large motorneurons and approximately 600 interneurons. By 72 hours after eclosion the total number of cells declines to about 350 neurons, a number that then remains stable through the remainder of adult life.

The time course of neuron death was followed by examining serial sectioned material from animals of various ages. Interneuron death began at emergence, peaked at 12 to 14 hours, and was substantially complete by 28 hours. Interneuron loss was more extreme in the anterior half of the ganglion. Among the motorneurons, each cell had its own characteristic time of onset of breakdown. Degeneration onsets ranged from 8 hours in the case of the earliest cells to 30 hours for the latest.

Electrophysiological recordings from identified motorneurons that were destined to die showed that identified inputs into the cells as well as outputs of the cells remained functional well into the early histological stages of degeneration. The first electrical changes seen in the cells were the appearance of excitability in the dendrites. This was followed by an increase in input resistance, the spread of excitability to the soma, and then the breakdown of conduction between the soma and the axon. Thus, in the case of the motorneurons, death does not appear to be due to the withdrawal of inputs to the cell but rather to an intrinsic response of the cell itself.

229.3 **TIME OF CELL ORIGIN AND CELL DEATH IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS.** **L. Wright. Neuroembryology lab., N.C. Found. Mtl. Hlth. Res., Inc., Raleigh, NC 27611.**

In the attempt to understand the mechanisms of cell death, it would be helpful to know more about the range of neural regions in which cell death occurs, and the diversity of characteristics these regions display, including pattern of cell proliferation, and number and type of target regions. The dorsal motor nucleus of the vagus (DMV) was chosen for study because, while it is a motor nucleus, i.e., the source of the preganglionic parasympathetic axons, it does not synapse directly upon muscle, but rather upon parasympathetic ganglia. Thus, this nucleus provides the opportunity to study the patterns of cell proliferation and cell death in a motor nucleus that innervates multiple, non-muscle targets.

The time of origin of cells in the DMV was studied autoradiographically. Chick embryos were injected with (3H)-thymidine at various ages, then sacrificed at day 18, and their brains were processed for autoradiography. Cells in every tenth section throughout the nucleus were evaluated according to the density of label present. Embryos injected with (3H)-thymidine on day 2 of incubation had label in all cells of the DMV, indicating that all the cells were still dividing at this time, and also demonstrating that the label was maintained in the cells from the earliest time of injection to the time of sacrifice at day 18. The peak of heavy label occured on day 2, and the first unlabelled appeared on day 3. Thus the first cells withdraw from the cell cycle prior to day 3. By day 4, 94% of the cells were unlabelled. A caudo-rostral gradient in the cessation of proliferation was indicated, with the caudalmost cells withdrawing from the cell cycle slightly in advance of the middle and rostral cells.

Cell counts of the DMV were made in embryos aged 6 days to hatching, and in one rooster. On day 8, when the DMV could be reliably distinguished from the nucleus intermedius, there were an average of 7464 cells present after Abercrombie's correction. By day twelve, cell death had resulted in a 21% loss of cells. By hatching, 37% of the cells had died, and counts made in the rooster indicated a posthatching loss, producing a total cell loss of 49%. A caudo-rostral gradient in cell death was noted, with the caudal cells undergoing more cell death between days 8-10.

The caudo-rostral gradient and the protracted period of cell death observed are discussed in relation to the competition hypothesis of cell death.

229.4 ROLE OF ACETYLCHOLINE RECEPTOR IN NEURON SURVIVAL DURING DEVELOPMENT. William R. Boydston* and G.S. Sohal (SPON: R.K. Holt). Dept. of Anat., Sch. of Med., Medical College of Georgia, Augusta, GA 30912.

Spontaneous death of motor neurons during the course of normal development has been observed in numerous parts of the nervous system. The reasons for cell loss are unknown, but recently a role for muscle activity in this process has been demonstrated. Continuous paralysis of embryonic neuromuscular transmission with α-bungarotoxin in white Peking duck embryos prevents the death of a significant number of trochlear neurons. Accompanying the increased cell survival is an increase in extrajunctional acetylcholine (ACH) sensitivity in the paralyzed superior oblique muscle and an increased number of nerve terminals per endplate. It is suggested that the increased cell survival may be due to the altered muscle membrane properties which permit the paralyzed muscle cells to maintain additional nerve terminals. Hence, a study was made to determine whether the acetylcholine receptor plays a role in neuron survival.

3.0 ug/g body wt. of the anticholinesterase drug, neostigmine methylsulfate, were applied to the chorioallantoic membrane of white Peking duck embryos on day 11 and continuing daily until sacrifice. Neostigmine has been shown to reduce the number of ACH receptors in adult mammalian muscle. ACH receptor quantification in the developing superior oblique was performed by ^{125}I-α bungarotoxin binding with corresponding cell counts of trochlear neurons made. In addition, the effect of cholinesterase inhibition on muscle differentiation was studied by electron microscopy.

Chronic treatment with neostigmine reduced the number of ACH receptors in the superior oblique muscle by 43% on day 19 (^{125}I cpm/ug protein: control-137.5, treated 78.3). No effect on muscle differentiation was seen. Coinciding with the decreased receptor number is an enhanced loss of trochlear neurons. Cell counts of the trochlear nucleus from neostigmine-treated embryos on day 19 was 830 as compared to 1603 in controls. This represents an additional 48% reduction in trochlear neurons. The size of the initial neuronal population was unaffected as shown by cell counts before the onset of cell death nor was any direct, toxic effect of the drug on trochlear neurons observed. Thus, it is suggested that the ACH receptor number or a signal released by the receptor may play a role in neuron survival during development.

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229.5 THE POSSIBLE ROLE OF EXTRAJUNCTIONAL ACETYLCHOLINE RECEPTORS IN EMBRYONIC DEATH OF MOTOR NEURONS. T.L. Creazzo* and G.S. Sohal. Dept. of Anat., Sch. of Med., Medical College of Georgia, Augusta, GA 30912.

Recent reports from this laboratory have demonstrated that pharmacologic blockade of nerve-evoked muscle activity reduces the magnitude of normally occuring embryonic death of motor neurons probably because the target muscle can accept additional axons terminals while rejecting none. In adult paralyzed muscle the ability to accept forgein innervation appears related to the increase in the density of extrajunctional acetylcholine receptors (AchR). Since initially embryonic muscle has a high AchR content, we investigated the content and distribution of AchRs in the normal and paralyzed superior oblique muscle (SOM) of the white Peking duck embryo. The trochlear nerve reaches the SOM on day 10 and cell death begins in the trochlear nucleus on day 13. Interestingly, there is a 4.5 fold decline in the content of I^{125} α-bungarotoxin labelled AchR between days 10 and 12 (day 10: 461.4±49 12: 111.6± 10.0 cpm/ug muscle protein). From day 12 through the remainder of incubation the decline is more gradual (e.g. day 25, 44.1 + 3.0). Muscles paralyzed with curare (2 mg/day) or botulinum toxin (15 ug/day) showed less decline and significantly greater AchR content than normal. For example, the content was 30% greater than normal on day 14, 36% on day 18 and 130% on day 25. Autoradiographic analysis indicates that the increased AchR content of paralyzed muscles is due to a higher extrajunctional AchR density. We suggest that embryonic death of motor neurons may result from the decline in extrajunctional AchR density and subsequent rejection of superfluous axon terminals.

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229.6 THE LOCATION OF MOTOR NEURON POOLS RELATED TO SPECIFIC WING AND LEG MUSCLES IN THE CHICK IS NOT ALTERED FOLLOWING THE PREVENTION OF NATURAL CELL DEATH.

R. W. Oppenheim. Neuroembryology Lab., Dept. of Mental Health, Raleigh, N. C. 27611

In previous reports, we have shown that neuromuscular blockade with a variety of pre-and postsynaptic pharmacological agents, during the period of naturally occurring cell death of spinal motor neurons, led to a substantial reduction in the number of such cells which degenerate (i.e. the prevention of cell death) (Pittman, R. & Oppenheim, R. W., Nature, 271:364, 1978; Pittman, R. & Oppenheim, R. W., J. Comp. Neurol., 187:425, 1979). Although wing and leg muscles are somewhat atrophic following such treatment, muscle differentiation appears relatively normal, including the appearance of acetylcholinesterase (ACHE) stained endplates and innervation of these synaptic sites. All muscles examined so far appear hyperinnervated. In some muscles, hyperinnervation is reflected by an increased number of synaptic sites per muscle fiber, whereas in others, the number of sites is relatively unchanged, but the number of axons terminating at each site is increased.

Since cell death has been prevented in these preparations from the earliest stages of limb innervation on days 4 or 5, it was expected that any inappropriate synaptic connections present at that time (or formed later) would be retained as long as the cells and their projections are prevented from regressing. Consequently, I have made small injections of horseradish peroxidase (HRP) into specific wing and leg muscles on embryonic day 10 in order to retrogradely label motor neuron pools in the spinal cord.

The location of HRP labelled motoneurons was found to be the same in control and experimental embryos. The specific muscles and related motor neuron pools that were examined included the gastrocnemius, peroneus, iliofibularis and sartorius in the leg and the biceps, triceps, extensor metacarpi radialis and flexor carpi ulnaris in the wing. In all cases, the rostral-caudal and medio-lateral locations of the specific motor neuron pools were indistinguishable from controls. These results are consistent with previous reports in which lumbar motor neurons of the chick were shown to innervate their appropriate muscles at the onset of innervation, and prior to the beginning of natural cell death (Landmesser, L. & Morris, D., J. Physiol., 249:301, 1975; Landmesser, L., J. Physiol., 284:371, 1978). Thus, the natural cell death of chick spinal motoneurons is not designed to remove inappropriate synaptic connections.

229.7 INTRAUTERINE MOTOR NEURON DEATH IN NORMAL MOUSE AND IN THE WOBBLER MUTANT. P.A. Hanson and N.L. Strominger. Depts. of Neurology, Pediatrics and Anatomy, Albany Medical College, Albany, NY 12208.

Mechanisms of programmed cell death (PCD) have been studied extensively in the chick and amphibian. Alterations in PCD have been produced by limb transplantation or amputation, and by exposure to toxins. Few studies have been concerned with this phenomenon in mammalian ontogenesis. We have studied PCD in the normal mouse in order to generate baseline data to be compared with similar studies on mutant strains.

The mutant mouse wobbler (wr/wr), an autosomal recessive, is a model for infantile spinal muscular atrophy in the human. The wobbler homozygote can be identified by 3 weeks of age; the spinal cord shows a depletion of motor neurons and there is evidence of denervation in the muscle. We have chosen to study this mutant.

Serial transverse sections stained with cresyl violet were prepared throughout the spinal cord. In the normal mouse a population of large motor neurons were counted which were identified after day E12 on the basis of large pale nuclei and prominent nucleoli. Morphometric studies of this population of motor neurons in the entire spinal cord shows a marked reduction in cell numbers from day E12 to E16 with the most prominent cell death on E13-14. Analysis of our data shows a high degree of reproducibility of motor neuron counts in the normal mouse. Thymidine autoradiography of cells generated on days E9-E11 shows their involvement in this PCD.

Preliminary data from runts in the litters of heterozygote wobbler matings, identified at the time of sacrifice by weight and size, show a significant reduction (50%) in the population of large motor neurons on day E18 when compared to their normal litter mates. These findings demonstrate an intrauterine abnormality in the wobbler mouse which may be related to programmed cell death.

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230.1 RECEPTIVE FIELD PROPERTIES OF NEURONS IN THE VISUAL CORTEX OF THE RAT. J.G. Parnavelas, R.A. Burne, C.-S. Lin and D.J. Woodward. Dept. of Cell Biology, Univ. Tx. Health Sci. Ctr., Dallas, TX 75235.

The receptive field properties of single cells were examined in the visual cortex, area 17, of Long Evans pigmented rats maintained under 1% halothane anesthesia. Extracellular spike responses of neurons were recorded to visual stimuli presented manually or with the aid of a computer-controlled system. Post-stimulus time histogram analysis was used to determine the response characteristics following presentation of the visual stimuli. In addition, an attempt was made to correlate structure and function by intracellularly filling with horseradish peroxidase (HRP) of single electrophysiologically identified neurons in the same cortical area. Beveled glass micropipettes (40-75 Megohm) containing 5% HRP in 0.2 M KCl with 0.1 M Tris buffer at pH 7.6 were used in this study.

Visually responsive neurons comprised more than 75% of the recorded cells. The majority of these neurons responded to both stationary and moving stimuli or only to movement. The remaining cells responded only to stationary stimuli. Cells of the former category were either complex (most frequent), simple or hypercomplex (least frequent). The neurons that responded to stationary stimuli were either on-center, off-center or on-off in type. Cells that responded to movement possessed at least an orientation, a direction or a velocity selectivity. These results indicate that, contrary to the widely held view based on previous studies[1, 2], the majority of neurons in the rat visual cortex have well-defined receptive field properties and are similar to those reported for animals with more highly developed visual systems. To date, all complex cells filled with HRP had the morphological characteristics of pyramidal neurons while the successfully filled simple cells had variable morphology with some being of the pyramidal and others of the non-pyramidal varieties. Our findings clearly demonstrate the feasibility of accurate structural-functional correlations in the rat visual system.

Supported by grants from the Biological Humanics Foundation to DJW and by NIH EY02964 to JGP

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230.2 PRINCIPAL COMPONENTS ANALYSIS PRODUCES A UNIFIED SCHEME FOR CLASSIFYING CELLS IN THE VISUAL CORTEX OF THE CAT. C. Hagerty*, F.C. Lees* and H.V.B. Hirsch. Depts. of Biology and Anthropology, SUNY Albany, Albany, N.Y. 12222.

Cortical cells may differ along many dimensions. When the number of these dimensions becomes large and highly interdependent, statistical techniques are needed to study relationships among them. Such techniques have been developed by numerical taxonomists to study variation within populations of organisms. We have applied these techniques to study a population of cells in the visual cortex of the cat. Eleven physiological properties of a sample of cortical cells were measured and the interrelationships among the response properties examined by principal components analysis. This procedure searches out the smallest number of mathematically independent combinations of the 11 variables which can account for most of the variance in the original data set. Each combination of variables represents an axis of the mathematical space within which the cells are distributed. The particular combination of variables used for each of the axes may characterize underlying mechanisms which generate the physiological properties of the cells.

We identified five major axes which together account for about 70% of the overall variability in the data. The contributing variables on these axes were: (1) peak response, layer, cutoff velocity, receptive field length, spontaneous activity, and eccentricity--all reflecting differences in afferent input to cortex, (2) simple/complex, receptive field width, cutoff velocity, and direction selectivity--all reflecting the presence of sideband inhibition and its contribution to response properties, (3) spontaneous activity and eccentricity--both reflecting differences in afferent input as a function of eccentricity, (4) tuning width and distribution of preferred orientation--reflecting an as yet unknown feature of cortical organization, and (5) ocular dominance--reflecting the distribution of geniculate afferents from the two eyes.

These axes were derived in an objective fashion. They show that certain response properties of cortical cells (e.g. cutoff velocity, receptive field size) are determined both by the type of afferent input and by the influence of sideband inhibition, and that these response properties may be generated by different mechanisms in different cell populations. Finally, the axis produced in a principal components analysis can also be used to classify cortical cells. The results obtained show that variables used in the simple/complex and in the W/X/Y classification must be incorporated in a unified scheme for classifying cortical cells. Supported by NIH grant R01EY01268 to H.V.B.H.

230.3 DIRECTIONAL MOVEMENT SELECTIVITY IN CORTICAL COMPLEX CELLS. J. Anthony Movshon, Elizabeth T. Davis* and Edward H. Adelson*. Psychology Department, New York University, New York, NY 10003.

Complex cells in cat striate cortex are often selective for the direction of movement of visual stimuli. We have studied this selectivity with isotropic random dot patterns and with sinusoidal grating and checkerboard stimuli.

The direction selectivity of complex cells for moving dot fields is broader than their selectivity for the direction of a moving grating. At low stimulus velocities, the preferred direction for a dot field always matches the preferred direction for a moving grating. At higher velocities, however, cells often prefer directions different from the preferred direction for a grating, producing a bi-lobed tuning curve. This behavior may be understood by considering that the moving dot field contains components at all orientations, and that the velocity of each component is scaled by the sine of the angle between its orientation and the overall direction of movement. At low velocities, components oriented orthogonally to the direction of movement are most effective, and the direction preference matches the orientation preference. At high velocities, however, only components whose orientations nearly parallel the direction of motion move slowly enough to be effective, resulting in a disparity between orientation and direction preference.

This suggests that a distinction must be drawn between component motion and pattern motion. Each oriented component of a visual pattern may in principle move in a range of directions; seen alone, each component (e.g. a sinusoidal grating) moves in a direction orthogonal to its orientation. In complex stimuli, however, pattern motion is inferred from the unique conjunction of the possible motions of many oriented components. A neuron selective for direction but not orientation would have the property of extracting the invariant pattern motion from a variety of possible component motions. The observed dependence of direction tuning upon stimulus velocity suggests that striate cortical neurons only signal component motion, and are incapable of sensing pattern motion.

This is further supported by experiments in which gratings of different orientation were superimposed to form sine-wave checkerboards. The direction-selectivity of cortical cells for these stimuli is determined by the orientation of the component gratings (and thus component motion) rather than the direction of pattern motion, even when the angle between the two gratings is widely varied.

We thus offer a definition of a pure direction selective neuron as one sensitive to pattern rather than to component motion. No striate cortical cell type appears to have selectivity of this sort, and studies are in progress to see if it may be found in neurons in the superior colliculus or lateral suprasylvian visual cortex. Meanwhile, it appears that all cortical neurons - even those of the "pure direction selective" type - have a selectivity for direction that is dependent on the orientation components of the stimulating pattern, and not on the direction of movement of local pattern elements.

230.4 ANALYSIS OF NEURAL PROCESSES WHICH CONVERT ELECTRICAL STIMULATION OF STRIATE CORTEX INTO "SENSATION". Edgar A. DeYoe and Robert W. Doty. Center for Brain Research, University of Rochester Medical Center, Rochester, New York 14642.

Electrical stimuli applied directly to striate cortex in man elicit only a sensation of punctate, stationary, slightly flickering white light (e.g., Brindley et al., J. Physiol., 1972; Dobelle and Mladejovsky, J. Physiol., 1974). This surprising uniformity of effect suggests that the neural mechanisms subserving this experience might be sufficiently restricted and unique to be identifiable. Observations on macaques over the past 20 yr strongly suggest that they too have a singularly uniform sensation for such stimulation; for if trained to respond to excitation at one locus in striate cortex, it is consistently found that they respond immediately, without further training, to excitation at any other striate locus. Our present goal is to identify, especially with microstimulation, the characteristics and laminar origin of the neurons in striate cortex responsible for this effect. Six M. nemestrina have been trained to grasp a rod or release contact when and only when cathodal, monophasic pulses are applied to striate cortex, via chronically implanted "macroelectrodes" (127 or 200-μ Pt-W or Pt-Ir), or transdurally advanced, glass-insulated Pt-Ir microelectrodes (ME). In the latter instance head movements are restrained by an individually fitted, fiberglass mask, and the ME passes through a 1-mm hole drilled in the skull during general anesthesia. For 44 macroelectrodes the mean threshold for response at 50 Hz, 0.2-msec pulses, was 109 μA, range 50-350 μA. In contrast, for all penetrations with MEs the range is commonly 15-25 μA, and one monkey could respond consistently to 2-4 μA at restricted loci. The transition from 10 to 90% correct responding occurs within 5-10 μA or less for MEs, whereas a 15-20 μA change is required with the macroelectrodes. Studied with the chronically implanted electrodes, each locus has a characteristic profile of increasing threshold with decreasing pulse frequency, and this profile, ranging from a 2 to 10-fold increase in going from 50 to 1 Hz, is stable, at least over a period of weeks. Preliminary evidence suggests that this function will commonly be much steeper for MEs than for macroelectrodes, i.e., that relatively much more intense currents will be required at 1 Hz for MEs. If the chronaxies are the same at the various pulse frequencies, as seems likely, and assuming the "sensation" is qualitatively unaltered by pulse frequency, as the human data affirm, then analysis of the interplay of temporal versus spatial summation (as revealed by the threshold/pulse frequency functions) at discrete loci within striate cortex may indicate which neuronal types are responsible for the peculiarly uniform percept. (Supported by NIH Contract 70-2279).

230.5 POSSIBLE NEUROTRANSMITTER OF CELLS IN LAYER 6 OF THE VISUAL CORTEX. R.W. Baughman and C.D. Gilbert. Dept. of Neurobiology, Harvard Medical School, Boston, Mass.

Cells in layer 6 project heavily to layer 4 and are the source of the recurrent pathway from the cortex to the lateral geniculate nucleus. We have attempted to characterize the neurotransmitter of these cells as a means to understand their role in cortical and geniculate function. To identify possible neurotransmitters, high pressure liquid chromatography was used to measure release of endogenous compounds from a tissue slice preparation of the visual cortex of the rat. When the K+ concentration of the incubation medium was raised (from 5mM to 50mM) to induce synaptic release, of the compounds measured, marked increases in release rate were observed for aspartate, (Asp) (12-fold), glutamate (Glu) (15-fold) and GABA (6-fold). This increased release was blocked in low Ca2+ high Mg2+ medium. Similar increases in release rate were observed when the tissue slices were treated with 100 micromolar veratradine, which is known to open the voltage sensitive sodium channels, and thereby provides an alternative means of exciting the cells. In this case the increased release was prevented by the sodium channel blocker tetrodotoxin.

To determine whether layer 6 cells might use Asp or Glu as their transmitter, 3H D-Asp was injected into the LGN of the cat in an attempt to label the cells by retrograde transport. In other pathways for which the transmitter is known, such retrograde labeling is specific for the transmitter involved (Streit and Cuenod, 1979). Striking labeling was observed in the layer 6 pyramidal cells and in a diffuse band in layer 4. Following an injection of 3H D-Asp in the cortex, no labeled cell bodies were seen in the LGN, confirming the specificity of the retrograde transport. Diffuse labeling was present in the LGN, however, presumably produced by anterograde transport from the layer 6 cells. Since the geniculate cells did not take up 3H D-Asp, we believe that the band in layer 4 represents filling of the collaterals of layer 6 axons. The cortical injection also provided evidence for the specificity of the uptake of 3H D-Asp by the layer 6 cells in that labeled cells were concentrated in this layer. The results are consistent with the observation that high-affinity uptake of 3H D-Asp is reduced in the LGN following ablation of the visual cortex (Lund-Karlsen and Fonnum, 1978). Although further studies are needed to completely test the specificity of the uptake and transport of 3H D-Asp, the present results suggest that the 6th layer cells participating in the corticogeniculate pathway may use the acidic amino acids Asp or Glu as their neurotransmitter. This research was supported by NEI grants EY00606 and EY01995, and by the Medical and Sloan Foundations.

230.6 LOCALIZATION OF GABA IN THE MONKEY STRIATE CORTEX. A. Hendrickson, S. Hunt*, and J-Y Wu. Dept. Ophthalmology, Univ. Washington Seattle WA 98195, Neurochemical Pharmacology Unit, Cambridge England and Dept. Cell Biology, Baylor Col. Med., Houston TX 77030.

The localization of the neuronal transmitter gamma amino butyric acid (GABA) in Macaca monkey primary visual cortex (striate) has been studied by staining its synthesizing enzyme glutamic acid decarboxylase (GAD) using immunocytochemical methods.

Four normal mature monkeys were studied; two received multiple injections of colchicine (10ug/ul) into one striate cortex 1 day before sacrifice. All were perfused with phosphate-buffered 4% paraformaldehyde or modified paraformaldehyde-lysine-periodate fixative. Frozen sections were cut serially at 25u in planes both perpendicular and parallel to the cortical surface. Seetions were incubated overnight in GAD-specific antiserum diluted 1/150 and then were processed for peroxidase-antiperoxidase staining using standard techniques. Alternate sections were stained for cytochrome oxidase (Wong-Riley 1979).

GAD+ terminals were found in all layers of the cortex, but were most numerous in layers I, IVA and IVC, slightly less in lower II, III and VI, and quite sparse in upper II, IVB and V. GAD+ cell bodies were found in all layers except I and ranged in size from very small to very large. They were common in layers II-IVC and VI, but layer V contained only a few, mainly large GAD+ neurons. On GAD+ neurons, the dendritic tree was usually visible for only a short distance so the specific type(s) of cell remains to be identified. GAD+ terminals could be seen to encase both unlabeled and labeled cell bodies. In sections cut parallel to the surface of the cortex, a regular pattern was found above layer IVA of alternating high and low concentrations of GAD, arranged into dots aligned into rows. This same pattern occurred in neighboring cytochrome oxidase stained sections. In both stains the spacing between rows was 450u±50u. This pattern above IVA resembled the deoxyglucose labeling described previously in Macaca striate cortex (Hendrickson and Wilson 1979). In both methods layer IVC was uniformly labeled while IVA showed a dot and dash pattern. A faint repetition of the dot pattern was also found in VI.

These results indicate that GABA is widely distributed in all striate layers, but has a differential laminar distribution. There was no evidence from either GAD or cytochrome oxidase staining for a differential distribution in layer IV correlated with ocular dominance columns, but above IVA there was a regular alteration of GAD concentration. Experiments are underway to determine if this is an extension of the ocular dominance system into supragranular layers, or represents a separate cortical organization. (Supported by Burroughs-Wellcome and EY-01208)

230.7 COMMISSURAL CONTRIBUTIONS TO VISION MEASURED BY THE 2-DEOXYGLUCOSE TECHNIQUE. K. A. Macko, M. Miyaoka*, C. Kennedy, L. Sokoloff and M. Mishkin. Lab. Neuropsychology and Lab. Cerebral Metabolism, NIMH, Bethesda, MD 20205.

In primates, the cortical pathway for processing visual stimuli proceeds from striate cortex (area OC) through surrounding prestriate cortex (areas OB and OA) to the inferior convexity of the temporal lobe (areas TEO and TE). Portions of each of these visual areas are known to be reciprocally connected through the forebrain commissures. In particular, the representation of the vertical meridian at the OC-OB border as well as selected parts of area OA receive commissural inputs via the splenium of the corpus callosum, while extensive portions of areas TEO and TE, important for higher-order visual function, receive their contralateral input via the splenium and anterior commissure. To quantify the contribution to vision made by these commissural systems, we measured rates of local cerebral glucose utilization (LCGU) throughout the cortical visual system in two different surgical preparations: unilateral optic tract section combined with forebrain commissurotomy (n=5) and unilateral optic tract section alone (n=4). The 2-deoxyglucose method was applied one month postoperatively in awake rhesus monkeys actively viewing visual patterns. The commissural contributions to vision were inferred from differences in LCGU between the deprived hemispheres of the two groups.

From the autoradiographs of each brain, representative sections were chosen at 1 mm intervals throughout the extent of the cortical visual pathway. Weighted averages of LCGU for the entire extent of each visual area were then calculated from these sections by means of a computerized image-processing system. In the intact hemisphere there was a progressive decline in LCGU along the cortical visual pathway from a high of 66 in area OC to a low of 47 μmoles/100g/min in anterior TE. This sequential decline in the intact hemisphere was the same for both the animals with tract section plus commissurotomy and those with tract section only. There were also no differences between operated groups in the visually deprived hemisphere for areas OC through TEO, where LCGU averaged 50% of that in the intact hemisphere (ranging from 40% in OC to 60% in TEO). A difference attributable to visual input via the intact commissures was found in TE, however, where LCGU in animals with combined tract section and commissurotomy remained at 60% of that in the intact hemisphere, whereas in animals with tract section only LCGU reached 80% and 90% of the values in the intact hemisphere for posterior and anterior TE, respectively. These results indicate that commissural inputs contribute more to the visual functions of area TE than to those of any other visual area and that commissural imputs alone may be insufficient to support visual function in areas TEO, OA, and the OC-OB border.

230.8 THE LOCATION OF CALLOSAL PROJECTION NEURONS IN RELATIONSHIP TO MAPS OF THE VISUAL FIELD IN CAT CORTEX. Mark A. Segraves and Alan C. Rosenquist. Dept. Anat., Univ. of Penna., Phila., PA 19104.

A previous report (Segraves, Soc. Neurosci. Abstr., 5:807, 1979) indicated that callosal cells in cat lateral suprasylvian visual areas were distributed throughout a more extensive portion of the map of the visual hemifield than was the case for callosal cells in area 17. The experiment described below was intended to delineate directly the distribution of callosal neurons within each visual field representation. In each cat, we used electrophysiological recording methods to map several visual cortical areas and placed small electrolytic lesions to mark receptive field positions. Following this procedure, the posterior 2/3 of the callosum was cut, and a cotton pleget saturated with 50% HRP placed between the cut ends of the callosum. A 48 hr. survival was used, and the tissue reacted with o-dianisidine.

Callosal cells in area 17 were restricted to a portion of the visual field map extending from the vertical meridian (VM) to not more than 5-10° azimuth. Moving from upper or lower field representations towards the horizontal meridian, callosal cells tended to be located nearer and nearer to the VM. In PMLS, labelled cells were located in all portions of the visual field map. This included callosal cells adjacent to lesions marking receptive fields of 60° azimuth. Despite the wide distribution of callosal cells in PMLS, the highest density of labelled cells was in portions of PMLS representing from the VM out to 20° azimuth.

It is likely that the callosal system may be segregated into multiple components. We would like to suggest two components based upon the distribution of neurons within a visual field map. One population of callosal cells located exclusively within a few degrees of the VM may be important for functions like coarse stereopsis and vergence eye movements. A second population of callosal cells that exists in representations of both central and peripheral vision may be important for functions such as the interhemispheric transfer of visual learning. Our evidence indicates that only the first of these two components exists in area 17, whereas both may be present in area PMLS. (Supported by EY02654 & GM00281)

230.9 LOCATION OF THE TERMINALS OF THE INTERHEMISPHERIC CONNECTIONS OF THE CAT'S VISUAL CORTEX. E. H. Murphy, B. R. Payne and N.Berman. Depts. Anat. and Physiol. Med. Coll. PA, Phila., PA 19129.

The location of axon terminals of cells which form the interhemispheric connections of the cat's visual cortex was determined either by degeneration techniques after transection of the corpus callosum (CCX) or by autoradiography after anterograde transport of tritiated amino acids following multiple injections into one or more visual areas. In the autoradiographic material there is a high density of accumulated label over layers I, III and VI. Discrete injections restricted to the 17/18 border, 18/19 border or lateral suprasylvian (LS) areas show that projections to the contralateral homotopic visual areas terminate mainly in layers III and VI and heterotopic projections terminate mainly in layers I and VI. Interhemispheric projections from more laterally placed areas (LS) to contralateral medial areas (17, 18 and 19) are greater than from 17, 18 and 19 to LS. In area 17 debris after CCX is maximal at the border with area 18 and decreases with distance from that border. A comparison with the visuotopic maps of Tusa et al. (1978, 1979) and Albus and Beckman (1980) and our own physiological recordings from some of these animals indicate that the labeled terminals are located in a region of cortex representing between 2^{o} and 4^{o} of visual space lateral to the 0^{o} vertical meridian (VM). For areas 18 and 19, the representation occupied by labeled terminals estends up to 20^{o} lateral to VM and up to 40^{o} or more for LS areas. The extent of the region receiving information from the contralateral hemisphere varies according to elevation in the visual field such that it is minimal in the region of the 0^{o} horizontal meridian and greater above and below. In addition, some animals show regions of dense labeling at the lateral edge of area 18 which may extend into area 19. These results indicate that visual field representations located away from VM receive substantial inputs from the contralateral hemisphere. Stone et al. (1980) have suggested that areas 17, 18 and 19 receive information primarily from the three subIcasses of ganglion cells X, Y and W respectively. Ganglion cells in the temporal retina project mainly to the ipsilateral visual centers although some project contralaterally. The extent of temporal retina which contains contralaterally projecting ganglion cells is least for the X-cell group, intermediate for the Y-cell group and greatest for the W-cell group. Therefore, the representation of the ipsilateral half field is greatest for the W-group and least for the X-group. This correlates with our finding that the region of visual field connected by interhemispheric pathways is largest for area 19 (W-recipient), somewhat smaller for area 18 (Y-recipient) and least for area 17 (X-recipient). Supported by EY02088 & EY02488.

230.10 LOSS OF BINOCULARITY IN AREA 17 AFTER SECTION OF THE CORPUS CALLOSUM: TIME COURSE AND EXTENT OF THE VISUAL FIELD AFFECTED. B. R. Payne, N. Berman and E. H. Murphy. Depts. Anatomy and Physiology, The Medical College of Pennsylvania, Philadelphia, PA 19129.

We have reported previously that transection of the corpus callosum leads to a significant decrease in the number of neurons in area 17 which may be activated binocularly (Payne et al. Science 207, 1980). We now present data concerning the extent of the visual field affected and post-operative time course of this effect. We recorded from 794 units in area 17 in cats after corpus callosum transection (CCX cats) and 558 units from area 17 in normal cats.

In normal cats the region of maximal binocularity in one hemifield is a vertical strip 12^{o} wide adjacent to the 0^{o} vertical meridian (VM) but excluding units with receptive fields within 4^{o} of the center of area centralis. In this strip 78% of units are binocular. The value decreases to 64% between 12^{o} and 20^{o} from VM and 49% at more lateral locations. Thus, the decline in binocularity begins before the monocular segment is reached. In CCX cats, the major loss of binocularity occurs within the 12^{o} vertical strip which corresponds to the area of maximal binocularity in normal cats. This decline is significant (X^2, 1 df $p < .001$). However, at locations more lateral than 12^{o} the proportion of binocular units increases to reach that found in normal animals (61%). Compared to normal cats, CCX cats also show a significant decrease in the number of binocular units in area centralis (X^2, 1 df $p < 0.02$).

Studies of the time course of these changes show that the decrease in the number of binocular units is progressive in the first two weeks after surgery and the maximal loss occurs between 2 and 4 weeks after transection when only 30% of the units can be activated binocularly. At 8 weeks 48% of the units are binocular and at 8 to 10 months 58% of the units are binocular. These values are significantly different from normal (X^2, 1 df, $p < 0.001$ both cases). With longer survival times normal levels of binocularity are observed between 6^{o} and 12^{o} from VM. Thus, the region of maximal binocularity becomes narrower with longer survival times and is localized to within 6^{o} of VM.

These results show that transection of the corpus callosum reduces the proportion of binocular cells and that this effect is most marked in the area of maximal binocularity observed in area 17 or normal cats. The decrease in binocularity is progressive over the first few postoperative weeks. After several months some recovery does occur.

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230.11 BINOCULAR INTERACTIONS IN CAT AREA 18 AND THE 17/18 BORDER. Jill C. Gardner and Max C. Cynader. Dept. of Psychol., Dalhousie Univ., Halifax, N. S., Canada B3H 4J1.

Visual neurons in cat area 18 and near the 17/18 border were tested for their sensitivity to stimuli presented at 7 different binocular disparities. Responses to stimuli moving in-phase (I-P: sideways) and anti-phase (A-P: toward or away from the animal) were compared across the two cortical regions.

The responses of both populations of cortical cells were similar in the following respects: highly sensitive cells were usually found in the supragranular layers and were strongly direction selective. They were driven well through only one eye (ocular dominance (OD) groups 1, 2, 6 and 7) or responded exclusively to binocular and not to monocular stimulation. Non-specific cells were distributed throughout the cortical layers, could be driven well through either eye (OD groups 3, 4 and 5) and were relatively less sensitive to the direction of stimulus motion. Units strongly dominated by one eye showed direction selective inhibition from the non-dominant eye which produced large inhibitory interactions to I-P movement. Sensitivity to binocular disparity was thus greater for stimuli moving I-P than A-P. The degree of binocular facilitation, however, was similar across both conditions.

Though both regions contained neurons with marked disparity sensitivity, units near the 17/18 border showed larger disparity-specific interactions. This difference was attributed to stronger inhibitory interactions at the 17/18 border. To determine whether the greater sensitivity of cells in this region was due to the influence of the corpus callosum, binocular interactions were examined near the intact 17/18 border of cats with large unilateral visual cortex lesions. In these cats, many of the units displayed strong binocular interactions similar to those found in normal cats, but overall, responses in lesioned animals showed reduced sensitivity to binocular disparity. There was a decrease in strenth of the inhibitory component of the response to both I-P and A-P movement and an increase in the number of cells which showed little or no disparity tuning.

These data suggest that the projection of the corpus callosum to the cat 17/18 border has a significant effect on binocular visual responses. Interruption of the callosal pathway with unilateral visual cortex lesions had no effect on facilitory responses but produced a clear reduction in the strength of inhibitory interactions. The callosal pathway thus appears to have an important influence on the inhibitory networks underlying the disparity-sensitive response of visual neurons. (This research supported by USPHS Grant EY02248, and grants from the MRC (MT5201) and NSERC (A9939) of Canada.

230.12 NEURONS SENSITIVE TO DYNAMIC RANDOM-DOT STEREOGRAMS IN AREAS 17 AND 18 OF RHESUS MONKEY CORTEX. G.F. Poggio, Dept. of Physiology, Johns Hopkins Medical School, Baltimore, MD 21205.

The results of recent experiments in alert, visually active macaques using contrast bar stimuli have confirmed the existance in visual cortex of neurons sensitive to standing binocular disparity, and have indicated that mechanisms processing stereoscopic information are present at early stages of binocular interaction in the visual pathways. Although these findings show that positional disparity influences the neuron's response, they do not provide conclusive evidence that disparity alone is sufficient to evoke it and thus leave undetermined the role of these neurons in mechanisms of stereoscopic depth perception. In order to obtain a better understanding of the functional significance of disparity sensitive neurons in A17 & A18, experiments were performed in alert rhesus monkeys in which the disparity sensitivity of cortical neurons was tested with two stimulus configurations. Visual patterns were generated on paired CRT screens using a dot matrix display and presented dichoptically moving or stationary over the neuron's receptive field while the animal steadily fixated a small target. Firstly, binocular disparity sensitivity was assessed with a luminous solid bar figure of selected size and orientation appearing over a background of dynamic noise of lower dot density, at a series of crossed, zero and uncrossed disparities. Secondly, the neuron's sensitivity was tested with pure disparity stereograms: the density difference between figure and background was eliminated; the figure, now embedded in the ground, was no longer present monocularly and the only remaining basis for the neuron's response to the figure, was binocular positional disparity (Julesz, 1971). Preliminary results show that many cortical neurons that respond to disparate contrast stimuli also respond quite selectively, and usually in the same qualitative fashion, to dynamic random-dot stereograms over the same disparity range. All types of stereoscopic neurons we had previously observed - Tuned excitatory, Tuned inhibitory, Near and Far (Poggio and Fischer, 1977) - were found to be sensitive to pure disparity stimuli. For any one neuron, the binocular response to random-dot patterns is usually weaker than the response to contrast figures, but several neurons were encountered that gave equally strong responses to both. Also, the latency of the response to noise patterns is commonly two to three times longer than the latency of the response to solid figures. These observations provide evidence that there are neurons in A17 & A18 of the macaque cortex that are sensitive purely to binocular positional disparity, and suggest that these neurons are part of basic cortical mechanisms of binocular depth perception. (Supported by NIH Grant EY02966).

230.13 CHOLINESTERASE POSITIVE ACTIVITY FOUND IN NEURONS OF THE BASAL FOREBRAIN THAT PROJECT TO VISUAL CORTEX IN THE GREY SQUIRREL (Sciurus carolinensis). Richard Rieck* and Harry J. Gould, III. Dept. Anat., Univ. Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Direct connections between the basal forebrain and the visual cortex were studied in the grey squirrel using horseradish peroxidase (HRP) and acetylcholinesterase (AChE) histochemistry. Discrete pressure injections of HRP were made in the visual cortices of the grey squirrel. Following a three day survival each animal was perfused and the brain was cut perpendicular to the long axis of the cerebral hemispheres.

Neurons of the basal forebrain that were labelled with HRP were observed in both the vertical and horizontal limbs of the diagonal band of Broca (DBB), the lateral preoptic area (LPOA) and the caudal sublenticular substantia innominata (SI). An analysis of cell sizes in the DBB, LPOA, and SI revealed a population of large labelled neurons that project to cortex in addition to the smaller non-labelled neurons in each region. Groups of large neurons in the basal forebrain also demonstrated cholinesterase activity following processing for AChE histochemistry. Double labelling with HRP and AChE histochemical techniques confirmed that the neurons projecting to neocortex from the DBB, LPOA and SI form a component of the cholinesterase positive population of neurons.

Previous double labelling studies have demonstrated that AChE positive neurons of the basal forebrain project to premotor cortex (Hardy, et al., Neurosci. Let., 3:1, 1976; Mesulam and Van Hoesen, Br. Res., 109:152, 1976). From the results of the present study, we conclude that AChE positive neurons of the basal forebrain also give rise to a projection to primary visual cortex.

230.14 ACTIVE DIRECTED GAZE CONTROLS THE EXCITABILITY OF THE LIGHT SENSITIVE NEURONS OF THE INFERIOR PARIETAL LOBULE IN THE WAKING MONKEY. B. C. Motter* and V. B. Mountcastle, The Johns Hopkins University School of Medicine, Baltimore, Md., 21205.

The response fields of light sensitive neurons of the inferior parietal lobule (area 7) may cover large parts of both halves of the visual field, but commonly exempt a central zone of 2-4° radius. This "foveal sparing" and the remarkable sensitivity to extrafoveal stimuli during target fixation may both be due to a precise connectivity linking retina and parietal lobe of the cerebral cortex; alternatively, they may be controlled dynamicly by the act of fixation with or without attention. We tested these alternatives by examining the sensitivity of parietal cells to light stimuli in three behavioral states: (1) during attentive fixation of a luminous target in the detection-reaction time task; (2) during eye pauses in the intertrial interval, as the animal awaited the onset of the target light initiating a new trial; and, (3) during eye pauses in behaviorally quiescent periods when the animal was not engaged in any task.

The receptive fields and response patterns were determined for conditions (1) and (2) simultaneously. For condition (2) the stimulus was the appearance of the fixation target initiating a new trial. This new target, physically identical to the test stimuli of (1), usually evoked an on-to-target saccade, which we take to indicate an attentive state, but one that differs from that of condition (1), in which the animal attentively awaited the dimming of the target light.

Visually evoked responses for the majority of neurons were reduced or absent during the intertrial interval of (2), as compared with those evoked from the same retinal areas by identi cal stimuli during the intratrial interval of (1). When the animal sat quietly, performing no task and attending to no target (3), the stimuli appearing within previously defined response fields were ineffective for many cells, and evoked diminished responses in others. Test lights appearing coincident with the instantaneous line of gaze were ineffective stimuli for the majority of cells, under all three behavioral conditions.

The results indicate that the act of fixation accompanied by directed attention to a particular target facilitates the response of parietal light sensitive neurons to stimuli in their non-foveal receptive fields. Whether foveal sparing is due to a dynamic suppression of responses to foveal lights, or to a precise connectivity alone, is unknown. (Supported by USPHS Grant 5-RO-1-EY03167-02).

230.15 THE DIRECTION OF GAZE INFLUENCES THE RESPONSE OF MANY LIGHT SENSITIVE NEURONS OF THE INFERIOR PARIETAL LOBULE (AREA 7) IN WAKING MONKEYS. R. A. Andersen and V. B. Mountcastle, The Johns Hopkins Un. School of Medicine, Baltimore, Md., 21205.

The properties of many light sensitive cells of the inferior parietal lobule are influenced by the direction of gaze. Thus light stimuli delivered to retinotopically similar points in their response areas produce different responses for different angles of gaze, with the head fixed, even though the response areas are oriented in retinal coordinates.

This effect was studied in two behavioral states. In state 1, light sensitivity was tested with a projected image as the animal attentively fixated a small lighted target whose dimming he had to detect for liquid reward. A difference in the response as a function of line of gaze was found for both stationary and moving stimuli, and was observed unchanged in darkness. In state 2, the animal sat awaiting the appearance of a target light indicating the onset of a behavioral trial. When the target light appeared within the previously established response area of the neuron, the magnitude of the response evoked by it varied with the instan taneous line of gaze at the moment of light onset; the response could vary from maximum to none with a gaze change of 20°. The results indicate that the influence of the line of gaze upon the excitability of the system linking retina and parietal cortex exists both for attentive fixation of a target, and for the eye pauses that occur during the intertrial intervals.

A smaller group of light sensitive cells of the parietal cortex is also active during fixation into a restricted region of space. The response areas of these cells are also oriented in retinotopic coordinates; their gaze fields generally lie in the area in space occupied by the response area when the eyes are in neutral position. Many are vigorously active during slow pursuit movements, when those movements are made within the gaze field of the cell; the excitatory directions are usually identical for tracking and light stimuli. Many of these more complex cells respond differentially with different angles of gaze, as described above.

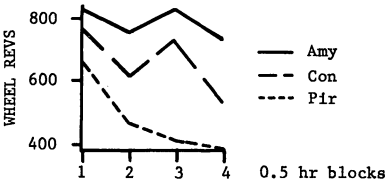
The spatial attribute of the influence of the angle of gaze upon the responses of light sensitive cells, as well as the properties of the smaller group of cells with complex properties, suggest that area 7 contributes to neural mechanisms important for spatial orientation and perception. This idea is consistent with the defects in spatial perception that follow parietal lobe lesions in monkeys and in humans. (Supported by USPHS Grant 5-RO-1-EY03167-02).

231.1 RECIPROCITY OF AMYGDALOID AND PIRIFORM MODULATION OF ACTIVITY IN HYPERACTIVE RATS. Irwin N. Lourie* and Michael M. Krieger* (SPON: Nagendran S. Thampi). Res. Dept., Norristown State Hospital, Norristown, PA 19401.

Sprague-Dawley derived rats (n=18) inbred for hyperactivity using running wheel measures for 26 generations were run for 16 consecutive days in an ad lib access, 12 hr light/dark paradigm. The subjects were then divided into 3 equal groups based on total activity and acquisition rate criteria. Group one received a radiofrequency generated lesion directed at the center of the Amygdala (Amy). Group two received a similar lesion directed at the lateral adjacent piriform cortical area (Pir) and the third group served as a sham operated control (Con). After 16 days of recovery the following experimental program was followed: 1 - Reacquisition to ad lib wheel running (RAW) for 16 days in which daily total activity as well as consecutive 15 min samples during the dark cycle were collected; 2 - A 2 hr forced running paradigm (FR2) run for 2 days in which animals were placed in closed off wheels during the light cycle with 30 min and total time data collected.

There are three constellations of RAW effects: initial rates, maximum activity and dark cycle patterns. Amy and Pir show a tripling and doubling respectively of initial rates up to day 5 after which their rates rapidly dissipate and maximal activity tends to be depressed in the lesioned groups. Each group can be distinguished by its dark cycle patterns with controls peaking at 3, 6 and 9 hrs. Amy shows activation at end of 12 hr cycle and Pir at the beginning. FR2 data shows a distinct and opposite lesion effect. This is shown in the accompanying graph.

There are two known influences on the expression of RAW rates, circadian and estral. These apparently have little influence on FR2 behavior. A possible explanation for these effects lies in the dual olfactory system which has been demonstrated by Raisman (Exp. Brain Res. 14:395, 1972) in which the main and accessory olfactory traits can be distinguished by projections to Pir and Amy respectively. These experiments demonstrate that these systems may also be characterized by differences in their excitatory or inhibitory influences on short term hyperactive behavior.



231.2 UNIT ACTIVITY OF TRIGEMINAL GANGLION NEURONS RECORDED DURING JAW OPENING AND CLOSING. N. F. Capra and G. B. Gatipon. Departments of Anatomy and Pharmacology, University of Mississippi Medical Center, Jackson, Ms 39216.

The identification of temporomandibular joint (TMJ) afferents, in cats with perikarya located in caudal and lateral regions of the trigeminal ganglion, (Romfh et al. 1979), prompted an electrophysiological investigation of the functional properties of neurons in this area. Cats were prepared for surgery under Halothane anesthesia. After placement in a stereotaxic apparatus, each cat was immobilized with Pavulon and was artifically respirated. Prerecording surgery consisted of precollicular decerebration and removal of restricted portions of the left cerebral hemisphere to expose the left trigeminal ganglion. A small portion of the bony tentorium was then removed and the dura investing the ganglion was incised.

A stepping motor was attached to the right mandible via a short metal rod, so that movements of the motor produced small angular deviations of the jaw. The motor was activated by a stimulator which also triggered an oscilloscope. In this manner, unit activity could be directly correlated with jaw movements which occured during a single sweep of the oscilloscope. An effort was made to identify somesthetic receptive fields for those neurons encountered in a single electrode penetration that did not respond to jaw movements. Action potentials recorded from glass or metal microelectrodes were amplified, displayed on an oscilloscope, and channeled to a window discriminator. The discriminator produced a square wave pulse for each action potential and the pulses were recorded on magnetic tape for subsequent analysis.

Three classes of neurons responsive to jaw movements can be described on the basis of data obtained from twenty-five units. Included are: (1) cells that responded to jaw closing movements (9 units); (2) cells responsive to jaw opening movements (5 units); and cells that were sensitive to both opening and closing of the jaw (5 units). The remaining cells were activated by tooth contact, probing of the oral mucosa, cutaneous stimulation near the mandible and TMJ region, or manually induced medio-lateral movements of the jaw. Several of the units that responded to movements were also activated by tactile stimuli.

The results of the present report suggest that a population of neuronal perikarya located in the caudal and lateral region of the trigeminal ganglion are capable of coding neural information related to changes in jaw position and that some of these neurons can also be activated by low threshold mechanoreceptors located outside the TMJ.

231.3 MOTONEURON EXCITABILITY CHANGES BY DESENSITIZATION OF CUTANEOUS RECEPTORS. M.A. Sabbahi, C.J. De Luca and W.R. Powers*. Dept. of Health Sciences, Boston Univ., Boston, and Liberty Mutual Research Center, Hopkinton, MA.

H-reflex recovery curves have been used clinically as a measure of the excitability of the motoneuron pool. However, interpretation of these curves is difficult because of the unknown factors involved in the primary inhibition period and recovery period. In this study desensitization of different skin areas and dermatomes was investigated to determine its effect on the recovery of the motoneuron pool of the soleus muscle.

Experiments were performed on 53 adult normal subjects. The H-reflex was elicited by stimulating the posterior tibial nerve unifocally using one millisecond pulses every five seconds, while recording the soleus evoked response with surface electrodes. Recovery of the motoneuron pool was tested using identical pairs of stimuli with variable interstimulus intervals. Topical anesthesia (20% Benzocaine) was sprayed separately to the skin areas overlying the calf, tibialis anterior, quadriceps, or hamstring muscles. In three subjects the total skin area of the lower limb was sprayed. In another group, topical anesthesia was applied to either L2, L3, L4, L5, S1 or S2 dermatomes and the H-reflex recovery curve was studied at 15 and 30 min. intervals.

The amplitude of the H-reflex was significantly ($p<0.001$) facilitated after anesthesia was applied to different skin areas and dermatomes, except those of the anterior tibial skin area and S1 dermatome. In those cases where the amplitude of the H-reflex was significantly increased after the application of anesthesia, the initial recovery occurred 10-80 msec. earlier than when no anesthesia was applied. This had no correlation with the skin area or dermatome desensitized by the anesthesia. The primary inhibition period continuously decreased after the application of anesthesia. After the initial recovery was obtained the amplitude of the test H-reflex recovered faster post-anesthesia. When the total skin areas of the lower limb were desensitized no summation effect on the recovery cycle was noticed.

From these results, it is possible to speculate that cutaneous afferents input may be an important inhibitory factor influencing the excitability of the motoneuron pool, as measured by the H-reflex recovery curve.

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231.4 FICTIVE SCRATCH REFLEX IN TURTLE: POSTSYNAPTIC POTENTIALS IN MOTOR NEURONS. Paul S.G. Stein, Margaret L. Grossman*, Joel A. Berenbeim*, and Paul R. Lennard. Department of Biology, Washington University, St. Louis, MO 63130.

A low-spinal turtle will produce a scratch reflex in response to gentle mechanical stimulation of the shell (Valk-Fai and Crowe, J. Comp. Physiol. 125:351,1978). The motor neuron activity patterns characteristic of this reflex can also be produced after the preparation is immobilized with the neuromuscular blocking agent, Flaxedil (Stein and Grossman, Soc. Neurosci. Abstr. 5:497,1979). Since these motor patterns are produced in the absence of a "real" movement, they are termed a "fictive" scratch reflex (Berkinblit et al., J. Neurophysiol. 41:1040,1978).

The fictive scratch reflex in turtle is composed of an A phase in which knee extensor (KE) motor neurons (MNs) are active and a B phase in which hip retractor-knee flexor (HR-KF) MNs are active. The KE MNs active near the onset of the A phase are termed A1 MNs and those KE MNs which begin activity late in the A phase are termed A2 MNs. Microelectrodes filled with 4M KAc were utilized to obtain intracellular recordings from A1, A2, and B MNs. The A MNs were identified by their antidromic action potential (AP) in response to stimulation of the nerve to the KE musculature; the B MNs were identified by their antidromic APs in response to stimulation of the nerve to the HR-KF musculature.

During the early portion of the A phase A1 MNs depolarized and discharged APs, and B MNs hyperpolarized. During the late portion of the A phase A1 MNs shifted from depolarization to hyperpolarization, A2 MNs discharged APs, and B MNs shifted from hyperpolarization to depolarization. During the B phase the A1 MNs were hyperpolarized, and the B MNs were depolarized and discharged APs. The trajectory of membrane voltage in the A1 MNs was opposite that of the B MNs, i.e., depolarization in one MN type was co-active with hyperpolarization of the other MN type.

These data suggest that the following synergies exist within the scratch generator: interneurons which depolarize A1 MNs are co-active with interneurons which hyperpolarize B MNs, and interneurons which depolarize B MNs are co-active with interneurons which hyperpolarize A1 MNs.

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231.5 NEUROLOGICAL DEFICITS FOLLOWING POSTARCUATE LESIONS IN MONKEYS. G. Rizzolatti*, M. Matelli* and G. Pavesi* (SPON: J. M. Sprague). Istituto di Fisiologia Umana, Universita di Parma, 43100 Parma (Italy).

We reported previously (Exp.Brain Res., 36:R19, 1979) that neurons in the posterior bank of the arcuate sulcus (inferior limb) can be driven by somatic, or by somatic and visual stimuli (bimodal neurons). Bimodal neurons respond to visual stimuli presented in the peripersonal space of the animal and their visual receptive fields are spatially related to the tactile fields.

In the present experiment we examined the deficits following lesions of the postarcuate cortex in three monkeys (Macaca irus). In two monkeys (S1 and S2) small unilateral lesions were made in the postarcuate cortex where mouth and peribuccal space are represented; in the third (L1) the whole posterior bank of the arcuate sulcus was ablated unilaterally; the same lesion was made in S1 contralateral to the first ablation, one month later. After the operation, S1 and S2 did not show any obvious sensorial or motor deficits except for a tendency of S2 to use the hand ipsilateral to the lesion. When examined in the primate chair it appeared that stimuli touching the face or presented near it on the contralateral side were responded to slowly and did not elicit the normal mouth and face movements. This defect, although reduced (especially in S1), was still present 4 weeks after the operation. Neurological deficits were more severe in L1 and S1 (second ablation). These animals did not use the contralateral limbs in "voluntary" movements, and, in "automatic" movements, they moved without normal dexterity. Head and eyes were deviated ipsilaterally (very slightly in S1). The animals were poorly responsive to tactile and nociceptive stimuli applied to contralateral parts of the body and did not show any clear sign of emotion during the stimulation. L1 neglected visual stimuli in the contralateral hemifield and particularly those presented in the peripersonal space. S1 had only a neglect for these last stimuli. Ipsilateral deviation of the head and the eyes disappeared after a few days; the other deficits, although less severe, were present one month after surgery.

We have previously proposed that neurons in postarcuate cortex elaborate somatic and visual information from the peripersonal space in order to organize patterns of movements. The results of the experiments with small lesions are consistent with this idea. However, the deficits following large lesions suggest that the postarcuate cortex is not only involved in organization of movement but also in perception of stimuli from contralateral side of the body and contralateral peripersonal space. Aided by NIH Grant 2 RO1 EY00577.

231.6 THE EFFECTS OF CORTICAL LESIONS ON VISUAL GUIDANCE OF THE HAND. S. Buchbinder*, B. Dixon*, Y. W. Hwang*, J. G. May* and M. Glickstein. Walter S. Hunter Lab. of Psychology, Brown University, Providence, RI 02912.

We have been studying performance of monkeys with cortical lesions on a task which requires precise orientation of the wrist and fingers, under visual guidance (Haaxma and Kuypers, Brain 98, 1975) in order to analyze which neural pathways may be involved.

In two monkeys, two-stage lesions were made in extrastriate visual areas that project to the pontine nuclei (Glickstein, et al., J. Comp. Neurol., in press). The lesions included both banks of the superior temporal sulcus medial to its confluence with the lateral fissure, the gyrus between these two fissures, and the anterior bank of the parieto-occipital fissure. In three out of four cases, this lesion produced irreversible loss of the animals' ability to guide visually wrist and finger movements of the contralateral hand. In one case, the first operation failed to produce a permanent impairment in the contralateral hand which we believe to be due to an incomplete lesion. Both animals were within normal limits on visual discrimination learning, and both could still use their fingers in a precision grip under tactile control. In one case, the second lesion not only caused impairment in the guidance of the contralateral hand, but further impaired performance with the hand ipsilateral to the second lesion.

In two cases, control lesions were made in the frontal lobe which included both banks of the arcuate fissure and the gyrus enclosed by this fissure. These frontal lesions produced only mild and transient impairment in hand use. In another control case, large bilateral lesions were made of the inferotemporal cortex which caused no deficit in visual guidance of the hand, although visual discrimination performance was impaired.

Thus, lesions which destroyed the cells which relay visual information from the cortex to cerebellum by way of the pons cause lasting impairment of visual guidance of the hand. Control lesions in inferotemporal and frontal cortex do not. The results suggest that the corticopontocerebellar pathway may be involved in visual guidance of the wrist and fingers.

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231.7 DIFFERENCES BETWEEN MOTOR CORTEX PYRAMIDAL TRACT NEURONS (PTNs) AND CORTICORUBRAL NEURONS (CRNs) DURING VOLUNTARY MOVEMENT IN THE MONKEY. C. Fromm*, E.V. Evarts, and J. Kröller*. Lab. of Neurophysiology, NIMH, Bethesda, MD 20205.

Following microelectrode recordings from red nucleus (RN) (Shinoda et al., this vol.), stimulating electrodes were permanently implanted in RN (2 pairs of electrodes) and in PT (1 pair) to allow antidromic identification of 3 classes of motor cortex neurons: PTNs, CRNs and PT-CRNs (i.e., neurons with branches to both PT and RN). CRNs commonly had conduction velocities ranging from 8-20 m/s and were usually antidromically driven by the RN electrode pair which was situated at the dorsolateral boundary of the posterior parvocellular RN. The PT-CRNs belonged mostly to the fast subset of the PTN population (50-60 m/s) and were more often driven by stimulation of the RN electrode pair situated at the caudal pole of the magnocellular RN. 22 CRNs, 27 PT-CRNs and 196 PTNs were extensively studied using the same paradigm as in the prior RN recordings. In general, the low frequency irregular discharge and the rather slight changes of discharge with active movement shown by the CRNs were markedly different from the regular tonic discharge and intense movement-related modulation of the PTNs. In order to compare CRNs and PT-CRNs with PTNs in exactly the same motor cortex loci, analyses were restricted to a subgroup of 98 PTNs which had been recorded in the same 38 penetrations with the CRNs and PT-CRNs. A major difference between the 3 subsets was in their time of discharge with respect to movement onset: 90% of the PTNs changed frequency before start of the movement and 10% at or after movement, whereas only 26% of CRNs changed before movement, the remaining 74% showing changes only at or after movement onset; the PT-CRN population was intermediate. This same conclusion was reached by comparing timing of neurons within each of the individual penetrations (Ps) taken separately. For 10 Ps, PTNs clearly preceded CRNs, with only 3 Ps showing overlap and no Ps showing CRNs prior to PTNs. For 9 Ps, PTNs preceded PT-CRNs and in 8 Ps, PTNs and PT-CRNs overlapped. The 3 subgroups also differed with respect to effects of different steady state loads: whereas different loads were associated with marked changes of discharge frequency in a majority (67%) of PTNs, the effects of different loads were slight and found in only 30% of PT-CRNs and virtually absent in CRNs. Another difference was that the incidence of short (<50 ms) latency responses to passive ramp displacements was lower in the CRNs (23%) as compared to the PTNs (62%).

These findings point to clearly different functional roles for CRN and PTN outputs from motor cortex, and are consistent with the characteristics of RN discharge observed during prior RN recordings in the same monkey.

231.8 UNIT ACTIVITY IN MONKEY RED NUCLEUS DURING SKILLED MOVEMENTS. N.J. Kohlerman, A.R. Gibson and J.C. Houk. Dept. of Physiology, Northwestern Medical School, Chicago, Illinois 60611.

The rubrospinal tract is the primary component of the lateral descending motor pathway (Lawrence and Kuypers, Brain 91:15-36). Magnocellular RN neurons receive their major input from deep cerebellar nuclei as well as a projection from motor cortex. To clarify mechanisms of descending motor control, we studied response patterns of rubral units during controlled movements.

A rhesus monkey has been trained to maintain an oscilloscope trace within target boundaries. The target either steps or ramps to various positions, and the monkey must reacquire the target. Each device requires movements primarily about one joint; movements about finger, wrist, elbow or shoulder joints can be studied separately. We have collected data on 85 well isolated units in arm related areas of the red nucleus during the use of one or several of the devices. These units show increases from low resting rates (~25 spikes/sec) to rates of over 200 spikes/sec during movements. Optimal relations between movements and discharge rate are generally seen on only one device; i.e., a cell responding well to wrist rotations shows variable and weaker relations to elbow movements. Most neurons fire optimally to finger or wrist movements; only one cell was optimally related to elbow movements. On the optimal device, unit discharge is tightly locked to movement, and the units fire preferentially to either flexion or extension. Increases in neural discharge lead the movement by a consistent latency. Typical lead times are 120 ms, while some units have lead times over 200 ms, and others show leads as short as 66 ms. Latency of increase in discharge rate is well correlated (r as high as .98) with latency of movement over a reaction time range of 300 ms to several seconds. Duration of discharge increase also correlates well (r as high as .98) with duration of movement. Discharge rate correlates well (r≈.8-.9) with the velocity of movement, and the increment in the number of spikes correlates well (r≈.8-.9) with the amplitude of movement. The relation between discharge rate and velocity plots as a straight line over an approximately 10 fold range in velocity and 7 fold range in rate. No tonic discharges relating to holding position have been observed, nor are there any changes in tonic discharge when the devices are force loaded.

Our data suggest that, in arm related areas of red nucleus, unit discharges are command signals for movement about finger, wrist or elbow joints. Work by other investigators suggest that these signals are generated in the cerebellum, although the relative contributions of cerebellar and cortical inputs to the red nucleus remains to be clarified.

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231.9 ACTIVITY OF ZONA INCERTA NEURONS IN THE BEHAVING PRIMATE.
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Dept. of Physiology, Johns Hopkins School of Medicine, Baltimore, Md. 21205

The functional properties of neurons in the Zona Incerta (ZI) were examined during the course of studies in the subthalamic region of the primate. 75 ZI neurons were isolated in 35 histologically verified penetrations in 3 rhesus monkeys. Their responses to active movements and "passive manipulations" of the limbs were examined. 29 neurons (39%) were related to arm, 8 (11%) to leg, and 13 (17%) to orofacial movements, and 6 (8%) to visual stimuli or eye movements. The remaining 19 (25%) neurons were not clearly related to movement or to visual or somatic stimulation. Of the 37 neurons related to limb movements 3 (8%) were clearly modulated by passive manipulations; only three of the neurons related to arm movements were related to finger, wrist or elbow movements. A striking finding of this study was the large proportion of neurons (18/75) which were activated when the animal reached for objects of interest. Cells within single or adjacent penetrations often showed similar functional properties. This clustering of neurons with similar properties suggests the possibility of a somatotopic organization, but the limited number of neurons studied precludes an immediate resolution of this issue.

The activity of ZI cells related to arm movements was also studied during the performance of a visuomotor tracking task in which the monkey was required to follow a visual target with side-to-side movements of the arm. The manipulandum was a lightweight handle which the animal could grasp and move along a horizontal path. The display consisted of 2 rows of light emitting diodes. The upper row indicated the position of the target and the lower the position of the handle. The animals first held in a starting position for at least 1 sec., and then made step movements in response to a sudden jump of the target lamp to a new position. Animals performed this task using mainly forearm musculature. Of 23 neurons studied 11 (48%) were clearly related to task performance; 5 (22%) showed directional effects. Nine neurons were clearly related to arm movements outside the context of the task, but were not active within the the task. The preferential activation of a subset of ZI neurons during reaching suggests an involvement of this structure in projection movements of the limbs into extrapersonal space. The results of this study together with the fact that ZI is a target of cerebellar projections indicate that ZI participates in motor function. Its exact role in motor control remains to be elucidated.

231.10 WHAT IS THE ROLE OF VESTIBULAR INPUTS IN POSTURE CONTROL?
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The role of the vestibular system in the control of postural equilibrium is difficult to assess because it is probably less sensitive to body motions than are the other two sensory modalities which also provide redundant orientational information; namely, vision and somatosensation. Because vestibular inputs are not subject to the unexpected, external perturbations common within the other two modalities (e.g., motion of objects in the visual surrounds and/or motion or compliance of the supporting floor), vestibular inputs might be critically important at a higher level of control. One example of this higher control would be the adaptive reorganizing of visual and somatosensory control strategies to suit particular task conditions. This hypothesis of adaptive control has been tested by evaluating the equilibrium controls in a group of seven patients with clinically well documented vestibular deficits (but otherwise neurologically normal) during two different tasks requiring: (1) direct vestibular control of equilibrium and (2) reorganization of the somatosensory and visual controls.

Direct vestibular control was assessed while subjects stood upon a movable platform and within a movable visual surround using a technique which stabilized the rotational position of the ankle joints and of the visual surrounds.

The capability of patients to adapt to appropriately unsuspected conflicts between visual and somatosensory inputs was assessed by transiently moving the platform and/or visual surrounds.

Patients with complete loss of vestibular inputs were unstable during the task requiring direct vestibular control and were also unable to adapt appropriately during visual-somatosensory conflicts (although adaptive changes were clearly evident). Patients with partial vestibular lesions, while still able to stabilize when direct vestibular controls were required, demonstrated clear deficits in their ability to adapt appropriately during sensory conflicts. Some of these patients chose a highly adaptive (but often inappropriate) strategy of control, while others maintained a fixed strategy.

([1]Supported by NIH Grant #NS-13286-04)
([2]Supported by NIH Grants #NS-12261 and #NS-00148)

231.11 ANALYSIS OF DISCHARGE PATTERNS OF SINGLE HYPOGLOSSAL NERVE FIBER DURING HYPERCAPNIA AND HYPOXIA. J. Mitra.
Case Western Reserve University, Cleveland, Ohio.

Recent studies have indicated that the integrated electrical activity of the hypoglossal nerve has both phasic and tonic activity. The phasic activity is curvilinearly related to phrenic nerve activity during hypercapnia and hypoxia (Weiner et al. FASEB Abst. 1980; ATS Abst. 1980). The purpose of the present study was to investigate the effects of CO_2 and low O_2 on the behaviour of single hypoglossal motoneuron. The study was done on chloralose anesthetized cats. We found three types of discharge patterns during eupnea. Type I - low CO_2 threshold respiratory modulated phasic units. Type II - non phasic with higher threshold for CO_2 and hypoxia. Fibers of this group showed respiratory modulation at higher CO_2 level and hypoxia. Type III - non phasic and did not show respiratory modulation at any of the levels of CO_2 (6 to 10%) and hypoxia (12% O_2) we studied and remained so throughout the experiment. These fibers increased discharge rate progressively with increase in CO_2 level or hypoxia.

It seems to us that the curvilinear relationship of hypoglossal activity is due to involvement of Type I and Type II fibers. The tonic activity may be due to Type III fibers.

232.1 DEVELOPMENT OF SYNAPTIC EFFICACY AT AN IDENTIFIED MOLLUSCAN SYNAPSE. Peter A. Pawson and Ronald Chase. Dept. Biology, McGill Univ., Montréal, Que., H3A 1B1.

The terrestrial snail Achatina fulica produces large clutches of eggs (>100) which hatch synchronously. The large size of the embryos permits the early identification of individual neurones by unique electrophysiological and morphological criteria. We have taken advantage of these conditions to study the development of a monosynaptic chemical synapse between two giant neurones located in the sub-oesophageal ganglia. The transmission was studied by intracellular stimulation of the pre-synaptic neurone (V2) while simultaneously recording the evoked EPSPs in the post-synaptic cell (RPr1). The period of analysis extended from late embryonic stages (pre-hatching) to sexual maturity (7 months).

We examined the passive membrane properties of the post-synaptic cell to determine whether developmental changes in these properties might contribute to a perceived change in synaptic efficacy. The I-V plot exhibits the same degree of linearity through out the period of study. A semi-log plot of the cell's input resistance vs. age shows a linear decline over the same period. Spontaneous EPSPs in young RPr1 neurones are faster rising and have larger amplitudes, indicating less electrotonic attenuation of synaptic potentials in younger animals. This property is shared by the V2-RPr1 EPSP, i.e. it has a faster rise-time and shorter half-width in young snails.

During the initial weeks post-hatching, transmission at the V2-RPr1 synapse is characterized by the frequent absence of evokeable EPSPs, even in high Ca^{++} and low Mg^{++} Ringers, or when using a stimulus frequency (0.2Hz) which produces marked facilitation of the EPSP in older animals. The absence of the evoked EPSP cannot be accounted for by RPr1's passive properties, as the synapse is electrically closer at this stage. At later times, when the EPSP is readily elicited at a low frequency (0.02Hz) of stimulation, the most prominent change is a progressive increase in the degree of frequency dependent facilitation.

We performed a quantal analysis on the developing synapse to determine pre- and post-synaptic contributions to the changes in synaptic efficacy. Values for quantal content (M) and quantal size (Q) were determined from amplitude histograms of the evoked EPSPs, from an analysis of failures and from an analysis of variance of the EPSPs. Q shows a 4-fold decrease from the time of hatching to 6 months of age, paralleling in order of magnitude and time course the decline in RPr1's input resistance. M shows a progressive, several-fold increase with age; especially in terms of the facilitated quantal content. The results demonstrate a developmental increase in efficacy at a specific chemical synapse, due mainly to changes in pre-synaptic release.

232.2 DEVELOPMENT OF SYNAPTIC PLASTICITY: THE EMERGENCE OF POST-TETANIC POTENTIATION AT IDENTIFIED SYNAPSES IN APLYSIA. H. Ohmori* and S.G. Rayport. Division of Neurobiology & Behavior, Depts. Physiology & Psychiatry, Columbia University, P & S, New York, N.Y. 10032.

Despite the extensive information now available on synapse formation, little is known about the development of synaptic plasticity. As part of a larger study designed to investigate the ontogeny of various forms of plasticity in Aplysia, we have begun to analyze the emergence of post-tetanic potentiation (PTP) in the inhibitory synapses made by cholinergic cell L10 onto its follower cells and of the excitatory postsynaptic potential onto R15 (RC1-R15) resulting from threshold stimulation of the right connective (Frazier et al., 67; Barondes et al., 77).

We first examined PTP by looking at the inhibitory synapses L10 makes onto left-upper-quadrant cells in animals ranging from 2 milligram juveniles (stage 11) to 300 gram adults (stage 13). An L10 spike was evoked every 5 sec until a stable control PSP was achieved, then L10 was stimulated at 5 hz for 30 sec. The amount of PTP was measured as per cent increase of the post-tetanus PSP over control. In early stage 11 there was no PTP (98 $\pm$ 11%) even though the synaptic connection was present and produced a significant PSP of 11.3 $\pm$ 7.3 mV with the membrane hyperpolarized 50 mV below the PSP reversal potential. PTP first appeared in late stage 11 and gradually increased until it reached a maximum of 230 $\pm$ 60% in stage 12 animals.

As PTP developed, the synapse itself became functionally less effective although it increased in size. In early stage 11, the input impedance of the postsynaptic cells was 270 $\pm$ 60 MΩ ; it decreased by a factor of 40, to 7.2 $\pm$ 3.3 MΩ in stage 13 adults. Over the same developmental range, the amplitude of the control PSP decreased about 3-fold (from 11.3 $\pm$ 7.3 to 3.6 $\pm$ 2.7 mV), suggesting that the synaptic current (the ratio of the control PSP to the input impedance of the follower cell) increased about 12 times during this phase of development.

We next investigated the development of PTP in the RC1-R15 connection and found that PTP emerges here synchronously with that in the L10 synapses and has an essentially similar developmental program.

Although we do not know when in development these synapses first form, synapse formation long precedes the emergence of plasticity in both synapses. Indeed, the complete expression of the phenotype of these synapses emerges in several developmental steps that extend over a number of weeks and encompass a significant portion of the total time of the animal's development, about 50 of 120 days. Thus there is a discrete interval when synaptic transmission is well developed but not capable of modulation by activity and this implies that the mechanisms underlying PTP are distinct from those involved in synapse formation.

232.3 INNERVATION OF ANTENNAE AND LEGS IN DROSOPHILA: WILD-TYPE AND HOMEOTIC MUTANTS. S. Green* (SPON: R. Greenspan) Div. of Biol., Calif. Inst. Technol., Pasadena, CA 91125

Homeotic mutations transform one appendage into another. The innervation of such appendages is compared with the wild-type in order to study: (1) How does the afferent projection select its targets in the CNS? (2) How does CNS development depend on an appropriate afferent projection? (3) Is position in the CNS determined by the same sets of genes that determine position in other tissues?

The mutation spineless-aristapedia (ss^a) transforms distal antenna to tarsus. Filling neurons with cobalt from wild-type and transformed antennae, I find that the projections are identical in spite of the fact that the tarsus contains different types of sensillae than antennae. This is all the more striking considering the extreme dissimilarity between the simple tarsal sensory projection to the thoracic ganglion and the complex antennal projection to the brain. The mutation antennaless removes one or both antennae. The morphology of brains from ss^a and antennaless flies appears wild-type in silver stained preparations.

HRP fills from each of the three different legs gives a characteristic pattern of motorneurons and sensory axons in the thoracic ganglion. The abdominal legs caused by the mutation bithoraxoid send their afferents into the CNS along an abdominal nerve or, occasionally, along the metathoracic (third) leg nerve. In either case, sensory neuropil of the metathoracic neuromere is the target but the precise pattern depends on the route taken. Occasionally metathoracic leg motorneurons innervate these abdominal legs, initially taking a 'normal' route through the metathoracic leg nerve. Results from bithoraxoid and ss^a suggest that the sensory neurons are largely guided to the vicinity of their targets by their path and will make functionally appropriate connections only if brought to close proximity with the target.

Mutations in the bithorax genes (BX) affect position determination in the thorax and abdomen. The combination of BX genes we use transforms metathorax to mesothorax. The motorneurons of the metathoracic neuromere of these animals resemble those of the wild-type mesothorax in morphology and relative position. Therefore, position in the nervous system, the cuticle, and possibly the mesoderm as well is determined in parallel by this set of genes.

232.4 REORGANIZATION OF THE MOTH NERVOUS SYSTEM DURING METAMORPHOSIS. R.B. Levine and J.W. Truman Dept. of Zoology, Univ. of Washington, Seattle, WA. 98195

During metamorphosis the holometabolous insects undergo significant changes in morphology and behavior. The nervous systems of these insects must adapt accordingly. In an investigation of this process we have focused on the motor neurons innervating the abdominal musculature of Manduca sexta. The intersegmental muscles of the abdomen are of embryonic origin and persist throughout larval, pupal, and early adult stages. They are innervated by the same excitatory motor neurons at all stages. The basic dendritic morphology of these motor neurons changes very little during metamorphosis, although there is a slight increase in the extent of dendritic branching. Other larval muscles degenerate at the onset of pupation. Of the motor neurons which innervate them, some degenerate during the pupal stage, while others are maintained and later innervate newly-generated adult muscles. The dendrites of motor neurons in the latter group undergo significant structural reorganization during pupation.

The motor neurons, including those undergoing dendritic reorganization, continue to be electrically excitable and to receive synaptic inputs throughout metamorphosis. Some inputs are maintained throughout post-embryonic life. One is from abdominal stretch receptors. These sensory neurons excite ipsilateral motor neurons monosynaptically, and inhibit contralateral motor neurons through a polysynaptic pathway. Other sources of innervation are present for only part of the insect's life in accordance with the different behaviors in which the motor neurons participate at different stages. An example is the excitation derived by the intersegmental motor neurons from sensory hairs embedded in the cuticular "gin traps". This polysynaptic pathway mediates a pupal-specific defensive behavior(Bate, 1973). Thus while much of the abdominal nervous system is conserved during metamorphosis, new elements are added to the basic framework. All of the motor neurons present in the adult were also present and functional in the larva, although some have been respecified. Some aspects of neuronal circuitry remain constant, while other pathways are unique to a particular stage in the animal's life.

232.5 EXPERIMENTAL ANALYSIS OF A SOMATOTOPIC MAP IN THE CRICKET NERVOUS SYSTEM. R.K. Murphey and S. Johnson*. Dept. of Biol. Sci. SUNY Albany, Albany, N.Y. 12222.

Last year in this space I demonstrated the existence of a somatotopic map between the peripheral and central nervous system (CNS) of crickets. We have now stained most of the sensory neurons in this equilibrium detecting system with cobalt. These studies have demonstrated that the position of the terminal arborization in the CNS is a joint function of the position of the sensory cell body on the circumference of the cercus and its proximo-distal position on the cercus. The receptors are arranged in columns running proximo-distally on the cone-shaped cercal appendage. All of the sensory neurons within a column have similar axon trajectories and similar target regions in the CNS. For example, a neuron (#60) in one column enters the ganglion medially in the cercal nerve and terminates in ventro-medial neuropile. All the neurons in this column terminate in this same ventro-medial area of neuropile.

We have begun our analysis of the rules by which sensory neurons find their target areas by surgically manipulating the cercus (see also Walthall and Murphey this volume). We removed the right cercus from a specimen and implanted it in the left socket of another specimen. This asks the question: How does a regenerating neuron interact with a target region which is the mirror image of its normal target? Typically the surgery altered the axon trajectory. For example a neuron which normally entered the ganglion medially entered laterally after surgery. However, this same neuron arborized in the target area appropriate for its position on the cercus. These results demonstrate first that the axon trajectory can be altered by surgery; second, that transplanted right neurons do not cross the midline to innervate their normal target area; third, in spite of the surgery a right sensory cell terminates in the area of left neuropile homologous to its normal target area. Since these sensory neurons were present before the surgical exchange, it appears that we cannot reprogram an identified neuron by merely altering the medio-lateral coordinates of its target area. Errors in the regeneration process were observed but they occurred with equal frequency in the left-to-right transplant and in the right-to-right transplant used as a control. The physiology of the postsynaptic neurons is consistent with these morphological results (Sakaguchi and Murphey, this volume). The results suggest that the developmental instructions, obtained by sensory neurons when they differentiate in the periphery, are very stable and that these insect sensory neurons do not alter their connectivity when faced with an altered set of body axes. Supported by NSF and NIH research grants to R.K.M.

232.6 BIRTH ORDER VS. POSITIONAL CUES IN THE REGENERATION OF A TOPOGRAPHIC PROJECTION IN THE CRICKET CNS. W.W. Walthall and R.K. Murphey. Dept. of Biol. Sci. SUNY Albany, Albany, N.Y. 12222.

An orderly array of clavate hairs is located at the base of the cricket cercus. These uniquely identifiable receptors are associated with single sensory neurons whose axons form a topographic projection in the terminal abdominal ganglion (Murphey, Neuroscience Abstract #841, 1979). The receptor array is organized into columns which run approximately parallel to the longitudinal axis of the cercus. All of the sensilla within a column have the same circumferential position, and their associated axons project to and terminate within approximately the same region. Yet within one of these regions the terminal projections are not evenly distributed.

The sensory neurons of the older, more distal clavate hairs project farther into the area designated by the cell's circumferential position and the younger more proximal hairs are restricted to more peripheral regions of the same area. Two possible mechanisms for this aspect of the projection pattern are time of arrival (birth order) and the sensory cell body's proximo-distal position. To distinguish between these possibilities the axons of the cercal nerve were severed, either by removing and then reimplanting the cercus, or by cutting or crushing the cercal nerve. This was done to remove any competitive advantage that older hairs might possess as a result of their early arrival in the CNS.

The morphology of single sensory axons that have regenerated their terminal arbors revealed the reconstruction of a normal projection pattern. In other words, the terminal arbor was still correlated with the cell body's position in the proximo-distal axis of the cercus. The regenerating axons are not following their old paths since they sometimes take radically different paths into the target region. Furthermore, extracellular recordings from the second order interneurons of treated animals revealed the restoration of correct physiologic connections by the regeneration process. Thus, it appears that a regenerating axon extracts important guidance cues from its cell body's proximo-distal position, and birth order or competitive interactions do not play a role in the reconstruction of this map. However, this result could be explained by differential rates of axonal regeneration, and experiments are underway in which the neurons of selected hairs will be killed and the axons of the remaining receptors subsequently forced to regenerate in the absence of neighboring hairs. Supported by NIH grant R01NS1557101 to R.K.M.

232.7 RE-ESTABLISHMENT OF FUNCTIONAL NEURONAL CONNECTIONS FOLLOWING TRANSPLANTATION OF A SENSORY APPENDAGE IN THE EQUILIBRIUM DETECTING SYSTEM OF THE CRICKET. Donald S. Sakaguchi* and R.K. Murphey (SPON: Charles Edwards). Dept. of Biol. Sci., SUNY Albany, Albany, N.Y. 12222.

The cricket (Acheta domesticus) has a pair of large bilaterally symmetrical sensory appendages at the tip of its abdomen called cerci. Peripheral sensory neurons associated with club-shaped sensilla called clavate hairs are located at the base of each cercus. The clavate sensory receptors are equilibrium detectors which signal information regarding the animal's position in the gravity field (Bischof, 1975). We have recently demonstrated that these receptors excite at least two pairs of large bilaterally symmetric interneurons (IN) whose axons can readily be recorded in the paired connectives just anterior to the genital ganglion. One of these neurons has been injected with the dye Lucifer Yellow and its cell body shown to be located at the anterior-lateral edge of the ganglion. When the specimen's position is altered from its normal up-right position, the IN are activated. Rolling the animal to its right excites the right equilibrium interneurons and simultaneously silences the left equilibrium interneurons. The complementary response occurs when the animal is rolled to its left. If the clavate hairs are immobilized with vaseline, the IN response to positional changes is blocked.

The cerci can be transplanted in various positions or orientations. If a cercus is amputated and then reimplanted in its original socket, functional and selective restoration of the original connectivity is demonstrated. When a right cercus from a donor animal is grafted onto the left socket of a host, the medio-lateral axes are reversed with respect to the body of the host. Recordings from the host IN revealed that connections were re-established with the appropriate type of IN ipsilateral to the graft. (These results corroborate the findings of Palka and Schubiger, 1975, in which they examined the cricket filiform hair system.) From the reimplantation results we conclude that regeneration in this system will re-establish normal connectivity. The right-left exchange experiments show that left equilibrium interneurons will be reinnervated by the clavate afferents from a transplanted right cercus. Physiologically the animal behaves as though it has two right cerci. This implies that mismatching axes of a sensory appendage in relation to the normal body position does not alter the resulting connectivity diagram. The sensory neuron is thus developmentally specified in such a manner that it restores functional connections with the appropriate type of interneuron despite a drastic alteration from its normal innervation pathway. Supported by NIH Grant R01NS1557101 to R.K.M.

232.8 COMPENSATORY GROWTH AND REINNERVATION OF AN AUDITORY INTERNEURON AS A CONSEQUENCE OF PARTIAL DEAFFERENTATION. Ronald R. Hoy and Sharon R. Rollins*. Section of Neurobiology & Behavior, Cornell University, Ithaca, N.Y. 14853.

In earlier studies we observed the effect of witholding auditory afferent innervation from an unique identified auditory interneuron in the prothoracic ganglion of a cricket Teleogryllus oceanicus during postembryonic development (Hoy, Casaday, &Rollins Soc. Neurosci. Abst. 4). Unilateral deprivation of innervation from auditory afferents during postembryonic development has bizzare consequences for the growth of the interneuron's dendritic field; specifically, the normally ipsilateral dendritic projection of the neuron becomes a contralateral projection, due to the aberrant growth of its medial dendrites into the contralateral half of the auditory ganglion.

The neuron, interneuron-1 (int-1),was described in Teleogryllus by Casaday & Hoy (J.Comp.Physiol. 121: 1-13, 1977) as having a unique morphology and the unusual physiological characteristic of being inhibited by 5 kHz tones; later, Moiseff & Hoy (Soc. Neurosci. Abst. 5, 1979) reported that int-1 is excited by ultrasound. These unique properties serve as a physiological "fingerprint" for its identification; thus far, int-1 is the only acoustic interneuron that we have found with these properties.

We now report that the aberrant growth of dendrites in response to unilateral deafferentation occurs even in adult animals. There is no response to acoustic stimulation from an unilaterally deafferented int-1 for about one week following amputation of the ear. However, during the second postoperative week the deafferented int-1 begins to respond to sound, with the vigor of its responses increasing over the next several weeks. Recovery of response properties occurs in the sense that the cell is inhibited by 5 kHz tones and excited by ultrasound. While the quality of the response in the reafferented neuron is normal, it does not appear to achieve the same level of excitability to ultrasound as does its contralateral, nondeprived, homologue. The restoration of the qualities of the auditory response implies that the crossover dendrites achieve a normal quality of synaptic input from the afferents of the contralateral ear. They apparently successfully compete with its nondeprived contralateral homologue for afferent innervation.

232.9 LONG TERM SURVIVAL OF ANUCLEATE GLIAL SEGMENTS DURING NERVE REGENERATION. E.J. Elliott* and K.J. Muller. Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210.

As a first step in studying the role of glia in axon regeneration in the medicinal leech, we have studied the behavior of the glial cell itself after nerve injury. Identified neurons in segmental ganglia of the leech c.n.s. will regenerate axons severed by a crush of the connectives that link adjacent ganglia, and within one or two months will reconnect specifically with their normal targets. Crushing a connective also severs processes of the single large glial cell that ensheathes the axons of each connective, and thus divides the cell into a nucleated segment and an anucleate glial segment or stump.

We have found that the nucleated segment of glial cell remains intact, with no sign of degeneration or of cell division. Surprisingly, the anucleate stump also survives for up to a year, maintaining both its resting potential and a normal morphology as determined by e.m. examination. During this time, the glial stump remains isolated from the nucleated portion. Horseradish peroxidase injected into the stump diffuses readily within it but does not travel into the nucleated segment, indicating that the divided portions do not fuse. Nor do the stump and nucleated regions become electrically coupled, as has been shown during regeneration of some neurons in the leech. When intracellularly injected, Lucifer Yellow dye crosses between most electrically coupled neurons or glia in the leech, but does not pass between the glial stump and nucleated segment. Moreover, current injected into glial cytoplasm on one side of a crush is not detected with a nearby second intracellular electrode in glial cytoplasm on the other side of the crush. Thus, the nucleated glial segment and the anucleate glial stump remain separate but morphologically intact and physiologically normal over the time period (3-8 weeks) required for functional axonal regeneration.

After some time, which varies among animals from 2 months to over a year, the anucleate glial stump atrophies, the numerous glial filaments diminish, and electrode penetration becomes difficult. Within about 3 months the nucleated portion of the injured glial cell sends processes across the crush which ensheathe some axons. Axons are also ensheathed by processes of microglia that have invaded or proliferated within the connective and seem to be phagocytic. In an attempt to determine whether the persistence of the glial stump retards growth of the nucleated glial cell, we have begun selectively to destroy glial stumps by intracellular injection of protease. These experiments, and others in which the entire glial cell is destroyed, should also provide direct information on the role of glia in axon regeneration. (Supported in part by a National Multiple Sclerosis Society Fellowship to E.J.E. and NIH grant NS 15014.)

232.10 SELECTIVE NEURITE ATROPHY DURING DEVELOPMENT OF CELLS IN THE LEECH CNS. Bruce G. Wallace. Dept. Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305

The central nervous system of the medicinal leech consists of a chain of 21 free segmental ganglia linked together and to fused head and tail ganglia by connectives. In the adult, the morphology of several identified neurons varies from ganglion to ganglion in a characteristic fashion: the arborization of cells in ganglia near the anterior or posterior ends of the chain is more extensive than that of their homologues in centrally located ganglia. For example, an annulus erector (AE) motor neuron located in any one of the 2nd through the 17th free segmental ganglia arborizes in the neuropile of that ganglion and sends branches out each of the contralateral roots to innervate muscles in the body wall. The branching pattern is similar for the AE cell in the first free segmental ganglion, except that in addition it sends a branch rostrally through the contralateral connective into the head ganglion and out through several roots from the head ganglion to the periphery. Likewise, the AE cell in ganglia 18 sends branches caudally through the contralateral connective and innervates the body wall through roots from several more posterior ganglia.

The morphology of mechanosensory cells responding to light touch (T cells) varies in a similar way. Each T cell in ganglia from the middle of the chain regularly sends a process out one or both ipsilateral roots as well as down the anterior and posterior ipsilateral connectives and out through roots from adjacent ganglia. The arborization of cells in ganglia near the head and tail spans more than three ganglia. Thus, in addition to the normal projections, a T cell near the head innervates the skin through roots from a second more rostral ganglion, a T cell near the tail through roots from several more caudal ganglia.

The creation of this branching pattern has been investigated by injecting cells in ganglia from embryonic leeches with the fluorescent dye Lucifer Yellow. Early in development homologous cells all along the ganglionic chain have a similar morphology, more extensive than that found in the adult. For example, in every segmental ganglion, AE motor neurons send processes down both the anterior and posterior contralateral connectives. T cells all send processes into the connectives past the adjacent ganglia. During development these "extra" processes fail to increase significantly in caliber and near the end of embryogenesis they disappear. Cells in ganglia in the middle of the chain lose both the anterior and posterior branches, cells near the head lose only the posterior branches, cells near the tail only the anterior branches. In this way, a segmental gradient in the morphology of homologous cells is established during development by the selective atrophy of neurites.

232.11 ROLE OF SENSORY ACTIVITY IN BEHAVIORAL RECOVERY OF THE DIRECTIONAL ESCAPE RESPONSE IN THE COCKROACH. S.F. Volman, J.M. Camhi, and N. Vardi. Dept. of Neurobiology and Behavior, Cornell University, Ithaca N.Y. 14850

Behavioral plasticity has been demonstrated in the escape behavior of the cockroach Periplaneta americana (Vardi and Camhi, Neurosci. Abst. 1979). These insects respond to small, abrupt wind puffs by turning away from the wind source. For instance, wind from the front left normally elicits a large turn to the right. The wind is detected by a bilateral pair of abdominal appendages called cerci, and the escape response is mediated partly by a group of individually identified giant interneurons. If one cercus is ablated, or if its fine filiform hairs are chronically immobilized with Eastman 910 adhesive, the animal initially makes inappropriate turns. For example, if the left cercus is removed, the animal now responds to wind from the front left by turning to the left, toward the wind source. By about 30 days after cercal ablation or covering, the animals' turns are significantly corrected; that is, they more often turn appropriately away from a wind source on the deafferented side. This recovery is correlated with changes in the response properties of the giant interneurons.

Animals in previous experiments showed similar recovery regardless of whether one cercus had been silenced by ablation or by immobilization of the hairs. Thus we hypothesized that a long term imbalance of activity in the two cercal sensory nerves might be adequate to initiate the recovery process. To test this idea, we prepared late instar nymphs with the left cercus ablated and the right cercus covered with adhesive. Of these animals, we tested the seven individuals that molted to adulthood between 28 and 36 days after cercal treatment. The small bud that appeared at molting on the ablated side was removed immediately, as in previous experiments. The glued side now had a new, free set of filiform hairs. In the behavioral test, these animals showed no recovery of turning; that is, they performed as if they had been deafferented on the left side for one day rather than one month.

In another experiment, animals were confined individually in small plastic cups after just ablating one cercus. These cups afforded minimal ambient wind and, since there was practically no walking space, minimal relative wind. (In all other experiments, animals had been kept in large, communal cages with screened sides.) After one month, these animals had recovered significantly less than the ones kept in large cages. Another group was ablated on one side and kept in the same cups but given wind therapy (extra ambient wind) for 8-10 hr/day. After 30 days, their behavioral recovery was significantly greater than that of cockroaches in cups but without wind.

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232.12 NEURAL BASIS OF BEHAVIORAL CORRECTION FOLLOWING UNILATERAL CERCAL ABLATION IN THE COCKROACH. Noga Vardi and Jeffrey M. Camhi. Sect. Neurobiology and Behavior, Cornell Univ., Ithaca, N.Y. 14850

Cockroaches (Periplaneta americana) respond to minute wind puffs by turning away from the wind source and running. They use this wind-mediated response to escape from the strikes of natural predators. The wind receptors are filiform hairs located on the cerci — two abdominal appendages. These receptors excite 7 bilateral pairs of giant interneurons (GI's) which appear to be involved in mediating the directional motor output of the legs. If one ablates the left cercus and tests the turning behavior the next day, most of the turns are to the left, regardless of wind direction. After one month, however, the animals correct, making more right turns in response to wind from the left.

Correlated with this behavioral change we find a pronounced enhancement in the responses of 5 of the 7 left GI's one month after ablation.* The enhanced response of the left GI's is driven by receptors of the right cercus since: a) the response is eliminated after immobilizing sensory hairs on the right cercus, and b) in one group of animals we ablated the left cercus and after a month ablated the right cercus, then tested the behavior the next day. In a second group we ablated both cerci and tested the behavior on the next day. The percent of responses was low in both groups and not significantly different. This suggests that enhancement of input from non-cercal receptors had not occurred during the one month of correction.

We wished to determine whether the observed enhancement in the number of spikes in the deprived (left) GI's by itself could explain the observed behavioral correction. We recorded from the right GI's of behaviorally corrected animals. In spite of the enhancement of the left GI's, on average they gave fewer action potentials than did the right GI's in response to wind from the left. Thus the number of spikes could not by itself explain the corrected behavior. Therefore, we also investigated whether the left GI's showed recovery in terms of latency. We recorded the total spike activity of the left connective and the right connective in response to wind puffs from the left front, using two pairs of hook electrodes situated at the same relative position on the nerve cord. One day after left cercal ablation, in only 12% of the trials did the first spike occur in the left connective. At one month the first spike occurred in the left connective on 54% of the trials. This difference is statistically significant (χ^2 test, $p < 0.005$). The percent recovery based on these latency measurements is close to the percent behavioral recovery and therefore may account for our behavioral results.

This work was supported by NSF grant BNS 79-09663.

*A preliminary account based on two GI's has already been presented (Vardi and Camhi, Neurosci. Abst., 1979).

233.1 IDENTIFICATION OF SYNAPTIC BOUTONS FOR PEPTIDERGIC TRANSMISSION IN SYMPATHETIC GANGLIA. L. Y. Jan*, Y. N. Jan* and M. S. Brownfield* (SPON: B. Libet). Dept. of Physiology, Univ. of California, School of Medicine, San Francisco, CA 94143

In sympathetic ganglia of the bullfrog a peptide which resembles luteinizing hormone-releasing hormone (LHRH) probably functions as the transmitter for the late slow excitatory postsynaptic potential (epsp), a signal that lasts several minutes, because (i) Radioimmunoassays indicated that a LHRH-like peptide was contained in sympathetic ganglia. Five days after preganglionic axons were cut, this substance disappeared from ganglia but tripled in spinal nerves proximal to the cut region; (ii) The LHRH-like substance can be released from ganglia with isotonic KCl or with nerve stimulation, a release that requires Ca^{2+}; (iii) LHRH acts directly on sympathetic neurons and produces a depolarizing response lasting for minutes; (iv) The LHRH response and the late slow epsp are associated with similar changes in membrane conductance and excitability of the postsynaptic cell, and they interact with the cholinergic epsp's in a parallel manner; (v) Both responses are blocked by certain analogs of LHRH, which antagonize the effects of LHRH in the rat (see Jan, Jan & Kuffler, Proc. Natl. Acad. Sci. USA, 76, 1501-1505, 1979; ibid, in press, 1980).

To directly examine the possibility that the LHRH-like substance is contained in presynaptic elements, we stained sympathetic ganglia immunohistochemically by using various antisera specific for LHRH and the peroxidase-antiperoxidase technique of Sternberger. The LHRH-like immunoreactivity was found in synaptic boutons around sympathetic neurons. No staining was obtained if the primary LHRH antisera were pre-adsorbed with LHRH. Cutting preganglionic fibers for the late slow epsp also abolished staining for the LHRH-like immunoreactivity. Therefore, the LHRH-like substance is probably contained in presynaptic nerve terminals for the late slow epsp.

An interesting feature of the late slow epsp is its delayed onset and slow time course. Typically, a late slow epsp lasts for several minutes, which is about 10000 times slower than the conventional fast synaptic potentials. Several factors may attribute to the slowness of this peptidergic synaptic potential. For instance, the distance between the peptidergic terminal and the postsynaptic cell body could be an important factor. Physiological and anatomical experiments have been done to address this question. Results from these experiments will be discussed.

233.2 RIA AND HPLC EVIDENCE FOR THE PRESENCE OF METHIONINE ENKEPHALIN AND CHOLECYSTOKININ IN THE NEURAL RETINA OF SEVERAL VERTEBRATE SPECIES. L. E. Eiden, M. C. Beinfeld* and R. L. Eskay*. Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

The amount (ng/retina) of immunoreactive (IR) cholecystokinin (CCK) in the retina of several vertebrate species, as determined by radioimmunoassay (RIA) of methanolic (90%) extracts of tissue, is as follows: 15-day-old chick, 0.04; monkey, 0.6; rat, 0.2; frog, 1.7; cow, 3.4. The quantity (ng/retina) of IR-methionine enkephalin (Met Enk), as determined by RIA of 2 N acetic acid, boiled extracts of tissue, is as follows: rat, less than 0.005; monkey, less than 0.01; cow, less than 0.1; frog, 4.6; 5-day-old chick, 7.8. The addition of increasing volumes of retinal samples containing IR-CCK or IR-Met Enk to each of the standard assay systems, resulted in an inhibition of binding of labelled peptide that paralleled the standard curve preparation. In order to characterize further the IR-retinal peptides, pools of retinal tissue were extracted and dried down. The residue was resuspended in distilled water and passed through a Waters C_{18} Sep Pak. Each Sep Pak was eluted with either a solution of acid-ethanol (Met Enk) or a solution of 90% methanol (CCK). The eluate was evaporated to dryness and the residue was resuspended in distilled water. An aliquot of this solution was injected on either a Supelco reverse phase C_{18} column or a molecular sieve column (Varian 2000 SW micropack). The C_{18} column was eluted with acetonitrile-phosphoric acid-triethylamine buffer; whereas, the molecular sieve column was eluted with 0.1 M sodium phosphate buffer, pH 7.0. One ml fractions per min were collected, lyophilized, and reconstituted in assay buffer for RIA. On the basis of reverse phase HPLC, the Met Enk-like IR in retinal tissue was found to have the same retention time as synthetic Met Enk. Furthermore, on the basis of reverse phase HPLC and a molecular sieve column, the CCK-like IR in the bovine retina was found to be the sulfated octapeptide (CCK8).

233.3 THE IDENTITY AND HYPOTHALAMIC ORIGIN OF CHOLECYSTOKININ IN RAT BRAIN AND POSTERIOR PITUITARY AND ITS POSSIBLE ROLE IN REGULATION OF NEUROSECRETION. M.C. Beinfeld*, D.K. Meyer*, and Michael J. Brownstein* (SPON: Y.P. Loh). Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

The distribution of cholecystokinin (CCK) was measured in rat brain utilizing a RIA and was found to be in agreement with previous studies of CCK distribution by the technique of immunohistochemistry. CCK was present in high concentrations in cerebral cortex and striatum and widely distributed in the central nervous system. The CCK concentration in posterior pituitary was similar to cerebral cortex. No CCK was detected in intermediate and anterior lobes of the pituitary.

The CCK-like immunoreactivity in rat brain was found to be sulfated octapeptide (CCK8) on the basis of Sephadex G-50 and HPLC chromatography. The predominant CCK-like material in posterior pituitary was also CCK8. In contrast to previous reports on porcine pituitary (Rehfeld, J., Nature 271:771, 1978), no gastrin was found in rat pituitary.

In order to determine the origin of CCK fibers in the posterior pituitary the CCK content of posterior pituitary was measured after pituitary stalk sectioning or electrolytic lesioning of the paraventricular (PVN) nucleus. Pituitary stalk sectioning resulted in complete loss of CCK. PVN lesioning resulted in about 60% loss of CCK. These results indicate that all of the pituitary CCK has an extrapituitary source and that about 60% of it is made in PVN.

To determine whether CCK8 might be secreted in parallel with vasopressin and/or oxytocin, the posterior pituitary CCK content was determined in lactating rats and rats given 2% saline to drink. In lactating rats pituitary CCK was reduced to 17% of control and in salt treated animals CCK was reduced to 20% of controls. Since CCK was reduced under conditions where release of vasopressin and oxytocin are altered, it is possible that CCK may be involved in the regulation of vasopressin or oxytocin neurosecretion.

233.4 DISTRIBUTION OF SOMATOSTATIN, SUBSTANCE P, AND NEUROTENSIN IN THE BASAL GANGLIA OF THE HUMAN. P.E. Cooper, M.H. Fernstrom, S.E. Leeman and J.B. Martin. Depts. of Neurol. and Physiol., Harvard Med. Sch. and Mass. Gen. Hosp., Boston, MA 02115.

Abnormalities of the CNS neurotransmitters dopamine, acetylcholine and GABA have been implicated in the pathophysiology of Parkinsonism, Alzheimer's disease, and Huntington's chorea respectively. Experimental evidence indicates that small molecular weight peptides are widely distributed in selective neuronal pathways in the CNS where they are postulated to exert both neuroendocrine and neurotransmitter/neuromodulator functions. It is possible that abnormalities in peptide distribution and/or concentration, analogous to those already documented for other neurotransmitters, may contribute to the clinical-pathological features of a variety of neurological disorders. Before assessment of peptide levels in pathological specimens, it is important to determine the distribution and concentration in normals and to assess stability. In addition, if appropriate animal models of disease are to be developed, peptide mapping carried out in the human should be compared to that in disorders induced in animals.

Ten brains were obtained post-mortem from patients (6 males, 4 females) with negative neurological histories, dying of non-neurological disorders. The mean age was 56 yr (range: 39-82) and the interval between death and autopsy was 13h (range: 4.25-20). Samples were homogenized in acid solution and somatostatin, substance P and neurotensin were measured by specific radioimmunoassay (RIA). The following results were obtained:

AREA SAMPLED	SOMATOSTATIN	SUBSTANCE P	NEUROTENSIN
	(pmol/g tissue-wet ± SEM)		
Caudate - head	107.5 ± 15.9	112.6 ± 12.1	2.9 ± 0.4
Putamen	86.1 ± 13.4	81.3 ± 11.3	2.5 ± 0.3
Globus pallidus			
- internal	15.9 ± 4.9	518.0 ± 151.4	9.8 ± 1.1
- external	18.3 ± 3.1	123.8 ± 23.8	9.7 ± 1.3
Subthalamic nuc.	99.5 ± 26.3	20.7 ± 3.3	9.7 ± 3.7
Amygdala	339.4 ± 47.0	25.9 ± 3.0	5.4 ± 1.1
Substantia nigra	23.8 ± 2.9	921.6 ± 155.9	23.4 ± 2.0

For each peptide there was a unique pattern of distribution in the basal ganglia. For a single peptide in a single region, variations from brain to brain were small. There was no correlation between peptide levels and age, interval between death and autopsy, or storage time prior to assay. Recoveries of peptide added to brain before RIA were >90%. Human post-mortem brain is suitable for RIA measurement of peptides. If valid comparisons are to be made between individual brains and groups of brains, the quantitative technique of RIA is preferrable to immunohistochemistry. The pattern of peptide distribution in the human is similar to that seen in a variety of animal species.

233.5 THE PRESENCE OF SUBSTANCE P-LIKE IMMUNOREACTIVITY IN THE ANTERIOR PITUITARY GLAND OF THE GUINEA PIG. Louis DePalatis, Raymond H. Ho and Robert P. Fiorindo (SPON: R. Rosenberg). Departments of Anatomy and Physiology, The Ohio State University, Columbus, Ohio 43210.

We have used the indirect peroxidase anti-peroxidase method of Sternberger to localize Substance P-like immunoreactivity (SPLI) in the anterior pituitary gland of the adult guinea pig. Animals were fixed by intracardiac perfusion with Bouin's fluid for 15 min. after which the pituitary glands were removed and immersion-fixed in Bouin's fluid for an additional 24 hrs. Immunohistochemical processing was done on 5 μm tissue sections. SPLI was localized within the cytoplasmic compartments of anterior pituitary cells in both the male and female guinea pig. The SPLI-positive cells were visualized predominantely in the medial region of the rostral portion of the anterior pituitary gland with fewer numbers of immunoreactive cells in the lateral and caudal regions. The processing of adjacent tissue sections for the localization of various glycoprotein hormones revealed that the SPLI endocrine cells also contained immunoreactive TSH, but not FSH or LH. SPLI was not visualized in anterior pituitary gland cells of the domestic fowl, frog and turtle, or of other rodent species, i.e., rat and mouse. The specificity of the immunostaining was established in control experiments in which SP- and rat-TSH-antisera pretreated with an excess of the respective antigens did not localize the aforementioned immunoreactivities on adjacent sections. Incubation of tissue sections with SP-antiserum that was pretreated with 10 μg/ml of either luteinizing hormone-releasing hormone, somatostatin, methionine enkephalin, crude bovine neurophysins, r-TSH, r-LH, r-FSH, r-GH, or r-prolactin revealed that the antiserum did not significantly cross-react with any of these antigens. Thus, we conclude that the guinea pig anterior pituitary gland contains a SP-like product that is found within thyrotrophs. This finding in the guinea pig anterior pituitary suggests that the SPLI may be indicative of: 1) the presence of the immunoreactive substance bound to intracellular receptor sites in the thyrotrophs or 2) the endogenous synthesis of a SP-like compound by thyrotroph cells in this species. (Supported by the Snyder Fund and the Graduate School, The Ohio State University).

233.6 γ_3-MELANOCYTE STIMULATING HORMONE (γ_3-MSH): ELECTROGRAPHIC, THERMOREGULATORY, AND BEHAVIORAL EFFECTS. S.J. Henriksen, A.L. Benabid, S. Madamba, F.E. Bloom, and N. Ling*. A.V. Davis Ctr. for Behav. Neurobiol. and *Neuroendocrinology Laboratories, The Salk Inst., La Jolla, CA 92037.

γ_3-MSH is a twenty-seven residue peptide (1) whose sequence, bounded by basic residues, is contained within the cryptic portion of the recently sequenced ACTH/β-LPH precursor pro-hormone (2). Although the sequence of γ_3-MSH resembles that of α-MSH and β-MSH, γ_3-MSH has neither significant melanocyte stimulating activity nor hypophysiotropic activity (1). In order to investigate possible physiological actions of γ_3-MSH we have administered this peptide centrally to rats and subsequently monitored electrographic, thermoregulatory, and behavioral sequelae. Sprague-Dawley rats prepared with cortical and subcortical electrode arrays as well as a cannula aimed at the lateral ventricle, were given intraventricular or intracisternal injections of γ_3-MSH (1-10 nM in 10 μl Ringers) synthesized utilizing solid phase methodology. Ventricular injections of γ_3-MSH (5-10 nM) resulted in the development of a striking behavioral syndrome including: transient behavioral hyperactivity; "barrel-rolling"; cortical desynchronization; and profound hypothermia. Doses of γ_3-MSH over 10nM resulted in apparent anoxia and subsequent death. Lower doses resulted in transient "barrel-rolling" and a longer lasting (up to 2 hrs) hypothermia. Peak temperature decreases following γ_3-MSH occurred 15-30 min. after administration. In rats maintained in ambient temperatures of 24° C, rectally monitored temperature decreases of over 4 ° C were observed following γ_3-MSH. Intracisternally administered γ_3-MSH appeared to produce greater effects on temperature than on behavioral activity. Chloral hydrate and halothane anesthesized rats had an attenuated temperature response to γ_3-MSH. Since immunohistochemical studies in our lab (Bloom et al, in preparation, and McGinty et al. this volume) have localized numerous γ_3-MSH immunoreactive cell bodies in the arcuate zone of the rat hypothalamus, these data suggest a possible role of γ_3-MSH in thermoregulation. 1) N. Ling et al. Life Sciences 25, 1773, 1979; 2) S. Nakanishi et al. Nature 278, 923, 1979 (Supported by DA 01785).

233.7 EVIDENCE THAT DEACETYLATED α-MSH IS A BEHAVIORALLY INACTIVE PRECURSOR OF α-MSH PRESENT IN RAT AND HUMAN BRAIN. Thomas L. O'Donohue*, Gail E. Handelmann*, Ted Chaconas*, David S. Olton, Russell L. Miller* and David M. Jacobowitz. Dept. of Pharmacology, Howard University, Wash., D.C,; Dept. of Psychology, Johns Hopkins Univ., Baltimore, Md.; Lab. of Clin. Sci., NIMH, Bethesda, Md. 20205.

An extensive system of neurons containing α-MSH-like immunoreactivity has been identified in the rat and human brain. Four peaks of α-MSH-like immunoreactivity in the rat and human brain were identified by the use of high pressure liquid chromatography and a radioimmunoassay for α-melanocyte stimulating hormone. The most hydrophobic peptide eluted at the same time as standard α-MSH, an N-acetylated tridecapeptide amide. The second most hydrophobic peptide coeluted with deacetylated α-MSH in the rat and human brain and rat pituitary gland. The other two more hydrophyllic peptides are methionine sulfoxide forms of the acetylated and deacetylated forms of the α-MSH which are produced artifactually during extraction.

In order to determine relative potencies of the α-MSH and deacetylated α-MSH, the effect of these peptides on three behavioral tests sensitive to MSH-like peptides were studied. Intraventricular administration of 2 μg or 6 μg of α-MSH in the rat produces, in a dose-response fashion, vigorous grooming behavior and the stretch-yawn-syndrome which is characterized by an unusual sequence of vigorous bouts of stretching and yawning. In contrast, deacetylated α-MSH failed to elicit either behavior at either dose. The 6 μg dose of the deacetylated α-MSH sulfoxide was active in inducing the grooming response while α-MSH sulfoxide was inactive. α-MSH and α-MSH sulfoxide also markedly increase the rate of acquisition of a Y-maze visual discrimination task and rats administered α-MSH (40 μg/kg ip.) learned the task in less than one-half the number of trials required by control rats. Neither the deacetylated α-MSH nor the deacetylated α-MSH sulfoxide were active in the test.

These data indicate that the deacetylated form of α-MSH has to be acetylated for activation. It is therefore proposed that an intraneuronal acetylating system may determine the activity of released α-MSH.

233.8 COEXISTENCE OF GASTRIN FAMILY PEPTIDE WITH ACTH, α-MSH, OXYTOCIN OR DOPAMINE IN THE SAME CELL. A STUDY IN THE CNS, HYPOPHYSIS AND GUT OF RAT, OX, DOG, MONKEY AND HUMAN. J.J. Vanderhaeghen and F. Lotstra* Department of Pathology (Neuropathology), Free Univ. of Brussels, Brugmann Univ.Hospital, Queen Elisabeth Medical Foundation, 1, av. Jean Crocq, B - 1020 Brussels, Belgium.

Peptidic material related to the gastrin family as been discovered in the brain of several vertebrates (Vanderhaeghen, J.J., Signeau, J.C. & Gepts, W.(1975)Nature, 257, 604-605.) and was later shown to consist mainly in the COOH terminal oct. of CCK (Dockray, G.J. (1976), Nature, 264, 568-570.) in its complete biologically active sulfated form (Robberecht, P., Deschodt-Lanckman, M. & Vanderhaeghen, J.J. (1978), Proc.Natl.Acad.Sci. USA, 75, 524-528). Antral forms of the gastrin family have been reported in the porcine hypophysis. Recently immunohistochemistry has localized gastrin family peptide in rat hypophyseal posterior lobe fibers and in hypothalamic neurosecretory magnocellular cell bodies of supraoptic, circular and paraventricular nuclei (Vanderhaeghen, J.J., De Mey, J., Lotstra, F. & Gilles, C.(1978), Acta Neurol.Belg. 79,62-63), (Loren, I., Alumets, J., Hakanson, R. & Sundler, F. (1979) Histochemistry, 59, 249-257), (Vanderhaeghen, J.J., Lotstra, F., De Mey, J. & Gilles ,C. (1980) Proc. Natl. Acad.Sci. USA, 77, 1190-1194) as well as in cell bodies of A10, A9 and A8 dopamine regions of Dahlstrom and Fuxe.(Vanderhaeghen, J.J., Lotstra, F., De Mey, J. & Gilles, C. (1980) Proc. Natl. Acad.Sci. USA, 77, 1190-1194). Gastrin family peptides have also been reported to coexist in the same cell with other peptides or neurotransmitters in the gut and pancreas. Using PAP technique combine with serial sections and double staining procedures we report the presence of gastrin family peptide in hypophyseal anterior and intermediate lobe cells sometimes together with respectively ACTH or α-MSH in the same cell (ox, dog, monkey and human). Gastrin family peptide in the hypothalamic neurosecretory magnocellular nuclei coexists frequently with oxytocine. After stereotaxic mesencephalic lesion obtained by injection of 6-OH dopamine it will be showned that gastrin family peptide diminishes in dopamine mesencephalic nuclei and their rostral projection (Nucleus Accumbens, Caudate Putamen, Tuberculum Olfactorium). The significance of coexistence of gastrin family peptide with several other peptides or neurotransmitters in one cell will be discussed.

233.9 NEUROPHYSIN FIBERS IN RAT AND MOUSE CINGULATE CORTEX. G.E. Hoffman. Dept. Anatomy, Univ. Rochester, Sch. Med. Dent., Rochester, NY 14642.

Localization of neurophysin has been successfully used to trace the projections of the peptide neurons containing oxytocin and vasopressin. Most of the studies have utilized paraffin-embedded brains. Thus, some of the more diffuse projections of these systems may have been missed due to the difficulties in tracing single axons for long distances in the relatively thin sections. For this reason, neurophysin systems were examined in thick sections of freshly fixed rat and mouse brains. To accomplish this, animals were anesthetized with pentobarbital, heparinized and perfused transaortically with saline followed by 10% neutral formalin or Zamboni's fixative. The brains were removed and sectioned on a vibrating microtome at 50 or 75 um. The sections were rinsed in PBS and incubated with anti-rat neurophysin (provided by Dr. A Robinson) at a dilution of 1:1000 for 24 hrs. at 4°C. The sections were further processed for immunocytochemistry by the procedure of Grzanna et al. The results, in addition to confirming previous projections of the neurophysin systems, revealed a projection to the cingulate cortex. Beaded neurophysin fibers were observed in all layers of the rostral half of the cingulate cortex. At the medial surface of the cingulate cortex scattered neurophysin-containing fibers were found within the induseum griseum. The possible source of these fibers, as determined from Halasz knife cuts, as well as their association with vasopressin or oxytocin will be discussed. The presence of neurophysin fibers in the cingulate cortex may explain the role of these peptides in memory and learning.

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233.10 CHOLECYSTOKININ RECEPTOR BINDING IN MAMMALIAN BRAIN. R.B. Innis and S.H. Snyder. Dept. Pharmacology, Johns Hopkins School of Medicine, Baltimore, Maryland 21205.

Cholecystokinin (CCK), a peptide hormone of 33 amino acids which stimulates pancreatic secretion, is found in high concentration in mammalian brain. CCK has been localized within neurons of the central and peripheral nervous systems and has been shown to have potent excitatory electrophysiological actions on central neurons. To further support CCK's role as a peptide neurotransmitter or neuromodulator, we have investigated high affinity receptor binding of CCK to mammalian brain membranes.

^{125}I-Bolton Hunter labeled CCK (^{125}I-BH-CCK) is the only iodinated form of CCK which demonstrates high affinity binding to membranes prepared from rat pancreas or guinea pig brain. Specific binding is markedly enhanced by incubating tissue with 10^{-4} M bacitracin, a proteolytic inhibitor. Monovalent cations at about 100 mM inhibit 50% of specifically bound ^{125}I-BH-CCK, and 5 mM calcium or magnesium enhances binding 2-3 fold. In both brain and pancreas, ^{125}I-BH-CCK binds saturably with a dissociation constant (K_D) of 5×10^{-10} M. The specificity of binding, demonstrated by displacement studies with numerous peptides and other putative neurotransmitters, supports the role of this binding site as the biological receptor. In close parallel to their potencies in stimulating pancreatic secretion, CCK-8 is 2-4 times more potent than CCK-33 in displacing ^{125}I-BH-CCK from both tissues, and the desulfated form of CCK-8 is virtually inactive. Although human gastrin I and pentagastrin show the expected low affinities for the pancreatic receptor binding, they are almost as potent as CCK-8 at the brain receptor. Because gastrin, pentagastrin and CCK share the same C-terminal pentapeptide sequence and because the tetrapeptide of CCK (CCK-4) is the predominant molecular form in the brain, one may speculate that the binding in brain represents the CCK-4 receptor. However, a Hill coefficient of 0.7 for displacement by CCK-33 and a biphasic dissociation curve in brain and pancreas supports the existence of at least two receptor sites for CCK.

The connection between CCK's actions in the pancreas and adenylate cyclase is unclear. We have shown that guanyl nucleotides modulate receptor binding in pancreas and brain in a manner typical of other peptide hormones whose actions are associated with stimulation of adenylate cyclase. For example, GTP and GppNHp inhibit 50% of specifically bound ^{125}I-BH-CCK at a concentration of about 2 μM, although GMP, ATP and ADP are virtually inactive. We have also shown that analogs of cGMP inhibit binding in both brain and pancreas in close parallel to their potencies in antagonizing CCK stimulated amylase release by the pancreas.

233.11 THE EFFECT OF CYCLO (LEU-GLY) ON DRUG-INDUCED DOPAMINE RECEPTOR HYPERSENSITIVITY. Jack Lee*, Ronald F. Ritzmann and Hemendra N. Bhargava*, Dept. Physiol. Biophys. & Dept. Pharmacog. Pharmacol., Univ. Ill. Med. Ctr., Chicago, IL 60612.

The effect of cyclo (Leu-Gly) (0.18 μmole/mouse) on the development of behavioral supersensitivity of dopamine (DA) receptors induced by (a) chronic morphine treatment (b) chronic haloperidol administration and (c) lesioning of neurons by injections of 6-hydroxydopamine (6-OHDA) was investigated in male Swiss Webster mice. The sensitivity of DA receptors was assessed by determining the responses (locomotor activity and body temperature) to apomorphine (APO). Morphine was administered by s.c. implantation of a morphine pellet (each containing 75 mg of free base) for 3 days. Cyclo (Leu-Gly) was injected s.c. 2 hr prior to pellet implantation. This procedure induces a high degree of tolerance to and physical dependence on morphine. Haloperidol (1 mg/kg/day) was injected daily for 21 days. Cyclo (Leu-Gly) was injected 1 h prior to each haloperidol injection. Lesions of DA neurons were produced by injecting ip pargyline (75 mg/kg) followed 30 min later by injecting desmethylimipramine (40 mg/kg) (ip). After an additional 30 min each mouse received an intracerebral injection of 6-OHDA (75 μg). Seventy two hours later mice were injected with cyclo (Leu-Gly) (0.18 μ mole/mouse). The injections of cyclo (Leu-Gly) were repeated daily for the next 11 days. Appropriate controls (placebo pellet or saline injections) were run at the same time.

The DA receptor sensitivity was measured at (a) 24 h after morphine or placebo pellet removal (b) 48 h after the last haloperidol injection and (c) 14 days after 6-OHDA injection, using an appropriate dose of APO. All the 3 methods used produced supersensitivity of DA receptors as evidenced by enhanced locomotor and hypothermic response to APO compared to their controls. Administration of cyclo (Leu-Gly) prior to morphine pellet implantation, haloperidol injection or after 6-OHDA injection prevented the development of DA receptor supersensitivity. This was evidenced by the blockade of enhanced responses to APO.

Several studies link the development of neuroleptic induced dyskinesias and morphine induced tolerance and dependence with enhanced sensitivity of brain DA receptors. Since DA receptor supersensitivity was blocked by cyclo (Leu-Gly), this agent or related compounds may be useful in treating dyskinesias and opiate tolerance-dependence process. (Supported by NIH grants DA-02542 and DA-02598 and Ill. Dept. Ment. Hlth. & Develop. Disabil. #904-02).

234.1 COMPRESSION OF PREGANGLIONIC AXONS INTO AN EXPERIMENTALLY REDUCED SYMPATHETIC GANGLION. A. J. Smolen and T. Lindley*. Dept. of Anatomy, Med. Coll. of Pa., Philadelphia, PA 19129.

The mechanisms by which various neural centers achieve numerical balance with the target neurons which they innervate has been studied extensively in the developing (or regenerating) avian and amphibian nervous systems. It generally has been concluded that removing the normal target results in an exaggerated loss of its afferent neurons, and conversely, that experimentally increasing the size of the target results in the maintenance of an increased number of afferent neurons.

Similarly, it has previously been shown in the superior cervical sympathetic ganglion (SCG) of the neonatal rat that, after cutting all of the postganglionic axons, there is a nearly complete direct retrograde cell death of the ganglionic neurons. In this case, the preganglionic axons are completely deprived of their target, and so undergo a transneuronal retrograde loss.

The present experiment was undertaken in order to define the effect on the preganglionic input of removing only a portion of their target neurons in the SCG, at a time before most of the ganglionic synapses are formed. We cut the internal carotid postganglionic nerve in rats at 3 days after birth, a time when there is only about 20% of the normal adult number of synapses in the ganglion. We left intact the other major postganglionic nerve, the external carotid nerve. Unoperated littermates served as controls. Two months later we removed the ganglia and their preganglionic chains and, using morphometric methods, obtained estimates of the numbers of ganglionic neurons, synapses, and preganglionic axons.

In the operated animals there was a marked reduction in the number of ganglionic neurons, with a somewhat smaller reduction in the number of synapses. There was no change in the number of preganglionic axons.

	CONTROL	OPERATED	
Ganglionic neurons	13,960±300	6,230±620	P<.01
Ganglionic synapses	4,370,000±100,000	2,840,000±410,000	P<.05
Preganglionic axons	5,900±1,030	5,640±350	N.S.

From these results we conclude that reducing the target at this stage of development does not result in a retrograde transneuronal loss of a proportion of the preganglionic axons. Instead, all of the preganglionic axons survive, and are compressed onto a smaller number of target neurons. There is, however, no marked hyperinnervation of the remaining ganglion cells. Thus, each preganglionic axons makes fewer synapses in the reduced ganglion than it would in the normal ganglion.
(Supported by NIH Grant NS 15952).

234.2 DEVELOPMENTAL PLASTICITY OF THE CORTICOSPINAL TRACT (CST) FOLLOWING MIDTHORACIC SPINAL CORD "OVER-HEMISECTION" IN THE NEONATAL RAT. D. R. Bernstein* and D. J. Stelzner, Department of Anatomy, SUNY Upstate Medical Center, Syracuse, NY 13210.

Animals receiving midthoracic spinal cord (T_8-T_{10}) "over-hemisection" - including right hemicord and left dorsal funiculus (df) - at birth, survived 3-12 mo. prior to stereotaxic unilateral ^{3}H-proline injection of either sensori-motor cortex (SMI). Similarly injected normal adult rats served as controls. After 48 hrs., rats were sacrificed and the brain and spinal cord examined using standard autogradiographic techniques. The injections labeled and the contralateral corticospinal tract (CST) within the df in controls. Labeled axons were also found sparsely in the contralateral lateral funiculus (LCT) and central gray below the df. Thus the "over-hemisection" given to neonates severs the matrix that most developing CST fibers would traverse at a later stage. Following injection of the appropriate SMI cortex, the right or left CST was studied. The left CST appeared normal rostral to the lesion zone and labeled fibers bypassed the lesioned left df by swinging through the left gray matter to form a greatly expanded left LCT in the intact matrix at the lesion zone. Caudal to the lesion, tract fibers: a) remained primarily in the LCT, but also in the left dorsal and central gray; b) were not found in either df; c) terminated correctly within the left gray matter, as well as in the appropriate but "wrong-side" gray where label density is nearly equal bilaterally. In contrast, the right CST was observed to distribute normally to within 1-1.5 segments above the fully transected right hemicord where label distribution spread to fill the entire hemicord creating a neuroma-like formation. None, or only minor, label was observed caudal to the lesion zone.

In a second experiment the right SMI cortex was ablated at the same time that the thoracic lesion was made, either at birth or at 6 days of age. By the later period nearly all CST fibers have grown through the pyramidal decussation and at least some of the axons have grown through thoracic spinal cord. The right CST was found to project around the thoracic lesion site in animals operated at both ages. Thus the left CST axons in some manner exclude the right CST axons from growing around the "over-hemisection". The projection of the right CST axons in this instance was similar to the left CST described above.
(Supported by Grant NS 14096)

234.3 EXPANSION OF THE CROSSED PARABIGEMINO-TECTAL PROJECTION IN ONE-EYED RATS. J. A. Stevenson and R. D. Lund. Dept. Anatomy, Medical Univ. of South Carolina, Charleston, S. C. 29403.

The parabigeminal nucleus (PBG) of the rat projects bilaterally to superficial (retino-recipient) layers of the superior colliculus (SC). In the albino rat the PBG is composed of three subunits. The ipsilateral PBG-SC projection arises from dorsal and ventral subnuclei and terminates across the full extent of SC. The crossed projection originates from cells of the middle subnucleus, and its terminal field is restricted to the anterior half of the SC in normal animals (Watanabe and Kawana, '79).

Degeneration studies after PBG lesions and examination of PBG cell labeling after horseradish peroxidase (HRP) injections into SC of adult pigmented rats demonstrate a PBG-SC projection generally similar to that of albinos. The termination of the crossed projection is restricted primarily to the anterior half of SC. However, light degeneration limited to the medial bank of SC was seen extending toward, but not reaching, the posterior pole of SC. Animals subjected to unilateral eye removal at birth showed an expanded crossed PBG-SC projection to the SC contralateral to the removed eye. While heaviest medially, this projection to superficial layers filled nearly the entire extent of SC. Only a small area at the postero-lateral border was free of degeneration following PBG lesion. PBG cell labeling following HRP injections in the posterior half of such SC confirm the expanded projection and demonstrate its origin in the appropriate PBG subnucleus.

This result demonstrates plasticity in a secondary visual pathway as an indirect effect of an early retinal lesion. It suggests that either interactions between retinal and PBG afferents to SC normally restrict the field of PBG termination or that the abnormal ipsilateral retinotectal projection, caused by early eye removal, induces expansion of the crossed PBG-SC terminal field.
(Supported by USPHS Grants EY 05381 and EY 03414 from the National Institutes of Health.)

234.4 TEMPORAL SENSITIVITY OF THE VISUAL PATHWAYS IN NORMAL AND MONOCULARLY DEPRIVED CATS. Kim R. Jones* and M.A. Berkley. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

Single unit recordings from the LGN of monocularly deprived (MD) cats are remarkable in that few Y-cell responses can be observed in the deprived laminae. However, it has recently been reported that in the optic radiation of these cats, single-unit penetrations display a normal complement of Y-cells driven by the deprived eye (Eysel, et al., Exp. Brain Res., 34: 521 (1979). This finding has prompted the suggestion that Y-cell numbers in the deprived geniculate are in fact normal, and that the loss seen physiologically is the result of a sampling bias reflecting preferential shrinkage of Y-cell cell bodies.

Since knowledge of the physiological state of the geniculate following visual deprivation is of some importance, the present experiment was designed to measure activity in the LGN in such a way so that cell size was not a significant factor. Accordingly, the temporal modulation sensitivity in both normal and MD cats was assessed using an averaged evoked potential technique.

A sinusoidally-modulated luminous stimulus, variable in both frequency and modulation depth, was used to elicit geniculate responses. By presenting a number of modulation depths at each frequency of stimulation, a linear function relating the amplitude of the evoked response to the log of the modulation depth could be obtained for each individual frequency. The zero-response intercept of this function was taken as the modulation threshold. Plotting these thresholds as a function of the stimulating frequency resulted in a temporal modulation sensitivity function, or TMSF.

Comparison of geniculate TMSFs in normal cats revealed no significant differences after stimulation of either eye. In MD cats, however, the TMSF derived via stimulation of the deprived eye was significantly depressed when compared to that derived via stimulation of the normal eye. To localize this temporal deficit, TMSFs were also derived from evoked potentials recorded from optic tracts and visual cortices of MD cats. No differences in sensitivity between the normal and deprived eyes were seen in the optic tract TMSFs, but the TMSFs derived from visual cortex exhibited even greater differences than were observed in the LGN. It was concluded that monocular deprivation causes deficits in the processing of temporal information that are first evidenced in the geniculate and further intensified at the level of visual cortex, a conclusion consistent with a loss of Y-cell function in LGN.

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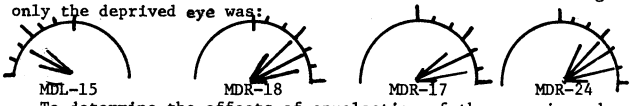
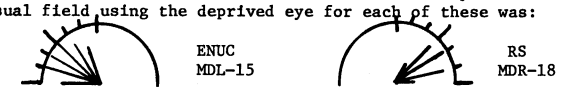
234.5 THE EXTENT OF THE VISUAL FIELD IN MONOCULARLY DEPRIVED CATS AND ITS PERMANENCE. Douglas C. Smith, Robert N. Holdefer*, Tom M. Reeves, and Jeanne A. Dorsett*. Dept. of Psychology, Southern Illinois University-Carbondale, Carbondale, IL. 62901.

We have been measuring visual orienting behavior in monocularly deprived (MD) cats for two reasons. First, recent investigations by von Hof-van Duin (1977) and Heitlander and Hoffmann (1978) have reported visual orienting in MD cats using the deprived eye over a much larger portion of the visual field (i.e, entire ipsilateral hemifield) than that reported by Sherman(1973) (45-90 deg. ipsilateral). Although the methods of testing were different, Heitlander and Hoffmann found visual orienting in a portion of the binocular visual field using Sherman's methods. The second reason was to determine whether the removal of the experienced eye might result in an expansion of the deprived eye's visual field correlated with the increase in the percent of cells responding to the deprived eye known to occur in striate cortex and the superior colliculus following this manipulation (Kratz et. al.,1976; Hoffmann and Cynader, 1977). Visual field perimetry was done using Sherman's (1973) techniques. MD was 7 mo in duration and all cats had perfect closures. The percent correct at each guideline (15 deg. apart) was determined using a minimum of 60 trials. Each cat was tested through the experienced eye, binocularly and through the deprived eye in this order. The monocular visual field for each of the 4 MD cats tested using only the deprived eye was:



To determine the effects of enucleation of the experienced eye a reverse-suture (RS) control was used for comparison. The visual field using the deprived eye for each of these was:



Comparison of the postenucleation orienting behavior of the enucleated cat (MDL-15) with its baseline performance (above) shows an increase in the visual field occurred following enucleation of the experienced eye which does not occur following RS. This increase was apparent in the first session postenucleation. An additional cat in each condition is being tested and the results will be presented. We conclude that the extent of the visual field using the deprived eye is limited to the ipsilateral monocular visual field and that enucleation apparently leads to an increase in the visual field which does not occur following RS.

234.6 THE EFFECT OF EARLY UNILATERAL EYE ENUCLEATION ON BILATERALLY PROJECTING RETINAL GANGLION CELLS IN HAMSTERS. Karen Hsiao* and Gerald E. Schneider (SPON: W. Rosenblith). Department of Psychology, M.I.T., Cambridge, MA 02139.

Neonatal monocular enucleation in hamsters results in an enhanced ipsilateral projection from the remaining eye (Finlay et al, JCN, 1979). In order to determine what portion of the enhanced ipsilateral projection was due to collateral sprouting of contralaterally projecting optic axons, a double labelling technique using horseradish peroxidase (HRP) and nuclear yellow (NY) was employed.

Hamsters at ages between 0 and 3 days underwent removal of one eye. As adults at least 8 weeks old, their optic tracts were sectioned below the lateral geniculate nucleus. A NY pellet was placed in one optic tract cut, and an HRP pellet was placed in the other. The optimum transport times to the retina, 12-14 mm away, for NY and HRP were 48 hr and 24 hr, respectively. The hamsters were perfused, and their retinas flat-mounted and processed using the TMB method (Mesulam, 1978). The retinas were scanned under UV epi-illumination. The positions and numbers of the ipsilaterally projecting retinal ganglion cells (RGCs), and of the doubly labelled cells, which represented bilaterally projecting RGCs, were noted.

The normal hamster retina contains 770(SD=150) ipsilaterally projecting RGCs, only 4(SD=2) of which project bilaterally. Ipsilaterally projecting RGCs are concentrated in a crescent-shaped area in the temporal retina, and are dispersed among contralaterally projecting RGCs. Hamsters with early unilateral eye enucleations experienced nearly a two-fold increase in the number of ipsilaterally projecting RGCs, bringing the total number to 1370(SD=230), and at least a four-fold increase in the number of bilaterally projecting RGCs, bringing the total number to 18(SD=6). The bilaterally projecting RGCs tended to be situated in the region outside the temporal crescent in the outer third of the retina, and particularly in the upper retina.

Although the number of ipsilaterally projecting RGCs increased by about 600 in hamsters with neonatal eye enucleations, only about 14 of these projected bilaterally in the adult. Thus, approximately 2% of the enhanced ipsilateral projection appears to be due to collateralization of the contralateral projection at the chiasm. It seems possible that the enhanced ipsilateral projection results mainly from a diminished incidence of cell death in the developing population of ipsilaterally projecting RGCs.

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234.7 A CROSSED CORTICOTECTAL PROJECTION AFTER EARLY TECTAL LESION IN THE HAMSTER. Steven L. Wertheim* and Gerald E. Schneider (SPON: N. Hebben). Dept. of Psych., M.I.T., Cambridge,MA 02139.

In the normal adult hamster the projection from the visual cortex to the superior colliculus (SC) is strictly ipsilateral. In order to investigate possible plasticity in the corticotectal projection, we have performed unilateral heat lesions of the superficial layers of the SC on the day of birth in ten Syrian hamsters. In three of these animals, the eye ipsilateral to the tectal lesion was removed simultaneously. At maturity the projection from the visual cortex ipsilateral to the lesion was traced with the Fink-Heimer technique. The projection from the contralateral eye was traced in the same animal with the autoradiographic method.

We have found a crossed projection to the undamaged SC from the visual cortex in these animals, in addition to the previously described anomalous recrossing retinotectal projection. The crossed corticotectal projection shares several distinctive properties with the recrossed retinotectal projection in the same animal. Most of the aberrant corticotectal fibers cross the midline within the bundle of recrossing retinofugal fibers. In the animals with tectal lesion alone, the crossed corticotectal projection is restricted to a medial zone of the contralateral SC, as is the anomalous retinal projection to that SC. In two animals with relatively strong crossed corticotectal projections, the lateral borders of the two aberrant projections appear to coincide. This correspondence is less precise in cases with a weaker crossed cortical projection. The rostro-caudal extent of crossed corticotectal termination is more limited than that of the aberrant retinal projections. There is no clear tendency for these aberrant cortical and retinal terminals to avoid overlap. However, definite segregation is seen in the tissue remaining on the side of the lesion where there are dense anomalous projections from both sources.

Ipsilateral eye removal in combination with tectal lesion results in an increased lateral distribution of crossed corticotectal, as well as retinotectal, fibers. The crossed cortical projection does not reach the lateral border of the SC as does the aberrant retinal projection. It appears restricted to the dorsal part of the superficial gray layer, thus avoiding the main region of termination of the other visual cortex.

These results indicate that normal and anomalous development of corticotectal and retinotectal fibers may be influenced by similar factors, and that the distribution of corticotectal axons may be strongly influenced by retinofugal axons.

(Supported by M.I.T. Undergraduate Research Opportunities Program, NSF Graduate Fellowship and NIH grant EY00126.)

234.8 EFFECTS OF NEONATAL LESIONS OF SOMATOSENSORY CORTICES IN THE POSTNATAL DEVELOPMENT OF CALLOSAL CONNECTIONS. R. Caminiti and G. M. Innocenti. Inst. of Physiol., University of Ancona, Italy and Inst. of Anat., University of Lausanne, Switzerland.

In the neonate cat, neurons projecting through the corpus callosum occupy the entire tangential extent of the sensory areas and subsequently become confined to restricted regions (callosal efferent zones).

The distribution of S1 (first somatosensory area) and S2 (second somatosensory area) neurons projecting to the contralateral S2 was studied with horseradish peroxidase (visualized with tetramethylbenzidine) in normal adult cats and in cats aged between 129 and 248 days in which the injected S2 area had been deprived of some of its input by an earlier lesion (on postnatal days 3 to 30; day of birth = day 0) of ipsilateral S1, alone (8 cats) or combined with a lesion of contralateral S2 (4 cats). In animals with S1 lesions, as in the normal controls, labeled neurons were selectively distributed to the regions of the trunk representation and to parts of the forelimb and hindlimb representations; however, the wide acallosal region in the forepaw representation was smaller than normal and it contained scattered labeled neurons in three of the four animals whose S1 had been lesioned during the first postnatal week. In these animals, the distribution of labeled neurons in the contralateral S2 was apparently normal. Furthermore, the additional lesion of this area during the first postnatal week (one animal) did not increase the degree of filling in of the normally acallosal parts of S1.

The filling in observed in these experiments is very modest considering the number of afferent axons to S2 eliminated by the lesion and considering the widespread distribution of neurons projecting through the corpus callosum in neonatal S1. It suggests however, that competition between callosal and other axons may be to some extent responsible for the disappearence of juvenile neurons which project through the corpus callosum.

234.9 ULTRASTRUCTURAL STUDY OF DEVELOPMENTAL CHANGES IN SYNAPTIC ORGANIZATION OF THE CHICK VESTIBULAR SYSTEM: TANGENTIAL NUCLEUS. K. D. Peusner. Department of Anatomy, Jefferson Medical College, Philadelphia, PA. 19107.

The tangential nucleus is a component of the avian lateral vestibular complex (Cajal). The largest primary vestibular afferents, the colossal vestibular fibers, form very large axosomatic endings, the spoon endings, on the principal cells of the chick tangential nucleus in late embryos (Peusner and Morest). In 15 and 16 day embryos, the lengths of spoons often exceed the length of the principal cell soma, measured at 32 um, and spoons form immature and mature vesiculated synapses and gap junctions at the spoon-principal cell interface. In addition, the principal cell somata are contacted by a small number of small-sized terminals of unknown fiber origins as early as 8-9 days. But even by 16 days in the embryo, these small-sized terminals, which contain both round and elongated synaptic vesicles, form only morphologically immature synaptic vesicle complexes with the principal cell. A few small-sized terminals may insert themselves into rare enlargements of the extracellular space at the spoon-principal cell interface. Undoubtedly, the spoon is the major afferent input to the principal cell soma in late embryos. However, from hatching through adult ages, spoons undergo a progressive transformation in ultrastructural constituents and atrophy such that in young adults, spoon remnants measure less than 5 um in length and contain extremely electron-dense axoplasm. Atrophied and degenerating spoon processes join the principal cell by numerous and lengthy gap junctions. In hatchlings, small-sized terminals are a regular feature, inserted into enlargements of the extracellular space at the spoon-principal cell interface. Although spoons have atrophied considerably in young adults, the principal cell exhibits somatic surfaces encapsulated by small-sized terminals. These small-sized terminals interlock with neighboring terminals. All small-sized terminals contain both round and elongated synaptic vesicles and form nearly symmetrical synaptic membrane complexes with the principal cell. In conclusion, this study documents a major developmental change in synaptic organization of the adult vestibular system. The evidence supports developmental remodeling of synaptic endings in the adult central nervous system. Supported by USPHS grant #1 RO1 NS15633.

235.1 LONG-TERM BEHAVIORAL AND NEURONAL CHANGES INDUCED BY SECTION OF INTERGANGLIONIC CONNECTIVES IN LEECHES. B. Granzow, E. Freed* and B. Kristan. Department of Biology, University of California, San Diego, La Jolla, CA 92093

Although swimming motor output can be generated in a completely isolated nerve cord of the leech, a single segmental ganglion cannot generate swimming motor output when its connections with the rest of the nerve cord are severed. However, a ganglion which is isolated by cutting both its anterior and posterior connectives, if left in situ with its lateral nerve roots intact, will regain its ability to generate swimming motor output within ten days of isolation (Kristan and Guthrie, Brain Res., 131:191-195, 1977). Behavioral observations of the operated leeches revealed that over a period of several days the body wall segment which is innervated by the isolated ganglion gradually develops definite alternating dorsal and ventral longitudinal muscle contractions which are accompanied by body flattening. This swimming motor pattern can occur 'spontaneously' or be evoked by tactile and electrical stimuli applied to the skin of that segment. The swimming motor output (consisting of alternating bursts in dorsal and ventral motoneurons) has also been recorded from chronically isolated ganglia, both when the ganglion remained connected to its body wall and when the ganglion was completely isolated. In the latter case the motor output was evoked by intracellular stimulation of the swim-initiating interneuron, cell 204, and was the same as that generated by the whole isolated nerve cord both qualitatively and quantitatively. We are now analyzing the cellular bases of the changes which occur, to account for the ability of the chronically isolated ganglion to generate the swimming motor output. We are using intracellular recordings to test for changes in efficacy of connections between swim related neurons and HRP injections to look for anatomical changes of these cells. We have evidence for strengthening of excitation both in the pathway from mechanoreceptors to cell 204 and from cell 204 onto the central pattern generators.

235.2 VISUALIZATION OF LIVING, INTRACELLULARLY STAINED NEURONS USING A SILICON INTENSIFIED TARGET CAMERA. S. B. Kater and R. D. Hadley. Dept. of Zoology, Univ. of Iowa, Iowa City, Iowa 52242.

Visualization of neurons stained intracellularly with fluorescent markers relies upon exciting the fluorophore with intense ultraviolet or blue light. The intensity of the exciting source determines the intensity of the emitted fluorescence. There are two problems commonly associated with this technology, especially when employing it with living neurons: 1) intense illumination can be fatal to living cells, and 2) intense illumination can produce rapid fading of the fluorophore, rendering it an inadequate marker in both living and fixed materials. Such problems are especially limiting under circumstances where one observes changes in neuronal morphology over time as for growing neurons.

We employ the fluorophores Lucifer Yellow and 6-carboxy-fluorescein to examine the growing processes of living, regenerating neurons of the snail Helisoma. Buccal ganglia are maintained in organ culture and regenerating identified neurons are filled with dye by intracellular iontophoresis. The growing processes can then be observed in situ both within the neuropile and specified nerve trunks. Extremely low levels of exciting light are employed (≠1/1000 the amount used for normal fluorescence photomicroscopy) and essentially no image is seen by eye directly through the microscope oculars. An attached Silicon Intensified Target camera produces a vivid image of sufficient quality to study the dynamics of neuronal path-finding involved in specific synaptic formation in this system (fine processes, etc.). Appropriate controls indicate that with 6-carboxy-fluorescein we can safely monitor the behavior of growing neurons in the environment of the neuronal and glial elements with which associations normally are made. Thus, studies on the dynamics of neuronal growth on a normal biological substrate can be performed using a nondamaging optical technique.

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235.3 DEGENERATION AND REGENERATION OF A CENTRAL PATHWAY IN THE GOLDFISH AN EM STUDY. R.L. Levine and G.M. Bray. Dept. Biol., McGill Univ., Montréal, P.Q., Canada H3A 1B1 and Neurol. and Neurosurg., McGill Univ., Montréal Gen. Hosp.

After unilateral tectal lobe removal in the goldfish, the efferent pathways from that lobe undergo Wallerian degeneration (Levine, R.L., ARVO '80). In addition, the optic fibers which were severed by the lesion regenerate into most of the degenerating tectal efferent tracts (Lo, R.Y.S. and Levine, R.L., J. Comp. Neurol., 1980). We have studied these two processes, i.e., degeneration and regeneration, in a particular pathway, i.e., the tractus rotundus.

In the normal animal the tractus rotundus is a bundle of fine (<1.5μ diameter) myelinated fibers which runs between the nucleus rotundus and the vertical limb of the horizontal commissure. In the animals we examined (7.5-8.0 cm length) the bundle was 100μ in diameter and contained approximately 20,000 axons. The thalamomammillary bundle lies immediately adjacent to the tractus on its medial surface and consists of several hundred small (1.5-3μ) myelinated axons. Finally, between 100 and 200 larger myelinated axons (3-5μ) form a shell around the dorsal and lateral aspects of the tractus rotundus.

After tectal lobe removal there is a rapid degeneration of nearly all of the fibers in the tractus rotundus on the operated side. Myelin degeneration is seen throughout the bundle by three days postoperative. Phagocytosis of the degenerating myelin also occurs rapidly and the majority of the debris has been removed by 14 days postoperative. Neither the thalamomammillary tract nor the large lateral fibers associated with the tractus show a major degenerative response to tectal removal. Concurrent with the degeneration in the tractus rotundus, optic fibers begin to regenerate into it. The first optic axons are seen as small fascicles among the glia and myelin debris at 7 days postoperative, and by 32 days postoperative they occupy most of the tractus.

Even at this early stage of investigation, some important generalizations may be made. First, the regenerating optic fibers appear to be confined to the boundaries of the degenerating tractus rotundus. They do not invade the immediately adjacent, parallel bundles which are not degenerating. Secondly, at this time, we have seen no cells in the tractus which appear similar to astrocytes (as they have been described in other lower vertebrates (Stensaas, L.J., J. Neurocytol., 6: 269, 1977). These observations accord with those of Achúcarro (1915) who found that astrocytes in the teleost brain tend to remain near the ependymal lining of the ventricular system. A corollary of this is that we have not seen radially arranged glial 'channels' comparable to those described by other workers in regenerating central paths in the lower vertebrates.

235.4 GROWTH RESPONSE MECHANISMS IN REACTIVE SYNAPTOGENESIS Stephen D. Collins and Robert L. Gulley Dept. Anat. Sch. Med. Case Western Reserve Univ. Cleveland OH 44106 and N.I.H., Bethesda MD. 20014

Many investigators have reported reorganization of synaptic connections in the adult CNS following partial deafferentation. Reorganization may involve the growth of new connections or the rearrangement of existing synapses. A quantitative morphological study of a well defined primary sensory nucleus which undergoes synaptic reorganization was performed to determine the mechanisms of response of intact afferents following partial deafferentation. In the rostral pole of the anteroventral cochlear nucleus of the guinea pig, four morphological types of boutons synapse on the somas of a homogeneous population large spherical cells. The four classes of boutons (primary auditory or E-bouton, O-bouton, F-bouton and S.R.-bouton) appose 46, 13, 5 and 4% of the spherical cell perimeter, respectively(Schwartz&Gulley,'78). Ablation of the cochlea causes rapid and complete degeneration of the large calyceal E-bouton which is followed by an increase in the total apposition of O- and F-bouton classes. The O-boutons double and the F-boutons triple their total apposition three weeks after cochlear ablation. No change in average spherical cell diameter occurs prior to 30 days post-cochlear ablation(Collins&Gulley,'79)

The cochlear nuclei from 20 guinea pigs at survival times of 7,14,21,30 and 180 days were studied in the electron microscope. The perimeters of spherical cell somas and the appositional length of each bouton apposing the somas were measured. The average number and the average length of O-boutons and F-boutons apposing a standard length of spherical cell membrane were determined. Two distinct growth mechanisms were observed for the two bouton classes. The number of O-boutons remained approximately constant through the 180 day study period while the median length of O-boutons increased 80%, from 0.9 microns to 1.6 microns. In contrast, the median length of F-boutons increased only slightly, while the number of F-boutons increased 142%.

These results demonstrate a specific mechanism of response by individual classes of neurons in a primary sensory nucleus to partial deafferentation of a common postsynaptic neuron. The F-bouton class adds, either by collateral sprouting or paraterminal sprouting, new boutons following calyceal terminal degeneration whereas the O-bouton class undergoes bouton enlargement, a form of contact synaptogenesis. The growth mechanism specificity of each neuronal class may determine innervation characteristics in injured and developing nervous systems.

235.5 TOPOGRAPHIC ORGANIZATION OF NORMAL AND REGROWING PYRAMIDAL TRACT AXONS. K. Kalil, M. Volz,* and T. Reh. Dept. of Anatomy and Neurosciences Training Program, Univ. of Wis., Madison, WI 53706.

In the infant hamster, severed pyramidal tract axons regrow along an anomalous route to innervate their normal terminal areas in the dorsal column nuclei and the cervical spinal cord (Kalil and Reh, 1979, Science 205). However, it is not known whether the cortical cells giving rise to the new pathway occupy the same cortical region as the normal pyramidal tract neurons and whether the topography of the normal corticospinal projection is maintained by the regrowing pathway.

In normal adult hamsters the topographic organization of the pyramidal tract neurons was plotted by injecting horseradish peroxidase (HRP) into localized regions of the dorsal column and trigeminal nuclei, and the cervical and lumbar enlargements of the spinal cord. In addition, injections of HRP directly into the medullary pyramid revealed the entire extent of cortical neurons giving rise to the pyramidal tract. This region extends rostro-caudally over a surprisingly large region of the cerebral cortex from the very frontal pole of the hemisphere to a level approximating the parietal cortex and mediolaterally from the midline to the rhinal sulcus. This cortical area encompasses MI, SI and SII but also extends rostrally into a region which would include the premotor and frontal cortices.

Within this region the topography of the sensorimotor cortex is similar to that described in the rat (Wise et al., 1979, Neurosci). The lumbar region is confined to a narrow posteromedial strip of cortex extending no more than 2mm. from the midline. The representation of the cervical cord lies rostral and lateral to the lumbar area and does not overlap it. The trigeminal and DCN projections arise from a large zone of the sensorimotor cortex lateral to the cervical projection zone. In all cases the cells are heavily labeled and occupy a continuous band in the deep part of layer 5 only.

Parallel HRP experiments are in progress on adult animals with infant lesions of the pyramidal tract to determine whether regrowing axons maintain this topography.

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235.6 FUNCTIONAL ROLE OF REGROWING PYRAMIDAL TRACT FIBERS IN THE NEONATAL HAMSTER. T. Reh and K. Kalil. Neuroscience Training Program and Dept. of Anatomy, Univ. of Wis., Madison, WI 53706.

We have shown in previous reports that axons of the pyramidal tract regrow when severed in the infant hamster. The regrowing fibers follow an abnormal route, but nevertheless reach and form synapses in their normal terminal areas, the dorsal column nuclei and the dorsal horn of the spinal cord (Kalil and Reh, 1979, Science 205:1158-1161; Reh and Kalil, 1979, Soc. Neurosci. Abstr. 2157). We now present evidence that this new pathway may be functionally useful in the animal's behavior.

Several qualitative and quantitative tests have been employed to determine whether the infant lesioned animals recover or retain any of those abilities mediated by the pyramidal tract. Previous studies of motor behavior in rodents with adult pyramidal tract lesions have shown deficits in the fine manipulatory movements of the forepaws. Hamsters with adult lesions can no longer hold and rotate sunflower seeds while eating, but instead use the affected forelimb as a passive support while the normal paw attempts to grasp and rotate the seeds. These animals also have difficulty in accurate limb placement when crossing difficult terrain, and they no longer display the tactile placing response.

We examined neonatally lesioned animals for deficits in these behaviors: seed handling, accurate limb placement, and tactile placing. The animals were filmed with a 16mm camera and the film was analyzed for both qualitative and quantitative behavioral deficits. Animals receiving pyramidal tract lesions in the first two weeks of life, in contrast to those lesioned as adults, do not show qualitative behavioral deficits in the tests, since they manipulate seeds in a normal manner and show normal tactile placing responses and accurate limb placement of both fore- and hindlimbs. However, preliminary evidence suggests some quantitative differences between neonatally lesioned and normal hamsters. The present results show that new axonal growth following neonatal pyramidotomy may be important in the recovery or sparing of motor function. Experiments are in progress to determine whether the recovered behaviors are lost when the new pathway is destroyed in the adult animal.

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235.7 TWO AXONALLY TRANSPORTED PROTEINS ASSOCIATED WITH GROWING AXONS IN RABBITS. J. H. P. Skene* and M. Willard* (SPON: C. J. Cornbrooks). Depts. of Anatomy-Neurobiology and Biochemistry, Washington Univ. Sch. of Med., St. Louis, MO 63110.

To explore the molecular events which control axon growth, we have compared the proteins transported rapidly along growing and non-growing axons in neonatal and adult rabbits. We labeled retinal ganglion cell proteins by intraocular injection of ^{35}S-methionine in rabbits of various ages and recovered labeled rapidly transported proteins in the optic nerves 3 hours after isotope injection; the labeled proteins were analyzed by one- and two-dimensional electrophoresis and fluorography. We found two polypeptides (GAP-23, mol. wt. 23K; GAP-43, mol. wt. 43K) which were labeled in neonatal optic nerves, but whose labeling relative to total rapidly transported radioactivity declined precipitously 7-21 days after birth. When these polypeptides could be detected in adult rabbit optic nerves, their relative labeling was less than 1/9 the level found in neonates. GAP-23 and GAP-43 resemble two rapidly transported polypeptides (GAP-24; GAP-43) induced during optic nerve regeneration in toads. GAP-23 and GAP-43 are also transported in regenerating hypoglossal nerves of adult rabbits; GAP-43 is induced by axotomy, while GAP-23 is transported in undamaged motor axons and not further induced by injury. CNS (optic nerve) axotomy in adult rabbits does not re-induce transport of the GAPs, and the injured axons do not regenerate. We interpret these observations as follows (the "GAP hypothesis"): 1. a neuronal "growth state" can be defined as a discrete program of gene expression, which includes expression of genes for the GAPs; 2. the growth state can be subdivided into a "synaptogenic state" characterized by transport of GAP-23 but not GAP-43, and an "axon elongation state" requiring both GAPs; 3. the failure of mammalian CNS neurons to express GAP genes after axotomy underlies their failure to regenerate damaged axons.

235.8 NERVE GROWTH FACTOR INCREASES ORNITHINE DECARBOXYLASE ACTIVITY IN GOLDFISH RETINAL EXPLANTS. Michal Schwartz, Shinichi Kohsaka* and Bernard W. Agranoff. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

Prior crush of the goldfish optic nerve greatly enhances neuritic outgrowth upon subsequent explantation of the retina. In the present study, we demonstrate that the addition of β-NGF from mouse salivary gland to the explants increases the activity of ornithine decarboxylase (ODC), a rate-limiting enzyme of polyamine synthesis. ODC activity in post-crush retinal explants is maximal 6 h after explantation in the presence of either NGF (1 μg/ml) or of fetal calf serum (10%). The observed increase (5 to 6-fold) following addition of NGF is inhibited by the presence of antisera to NGF or the ODC inhibitor, diaminopropane (1.0 mM). Post-crush retinal explants have higher initial ODC levels (Kohsaka and Agranoff, abstracts, this meeting) and demonstrate a larger response to the NGF addition than do control retinas. The results suggest a difference in affinity and/or the number of receptors for NGF or NGF-like molecules in the two retinal preparations. The results are of interest in view of the reported enhancement of goldfish ganglion cell morphological responses to axotomy following intraocular injection of NGF (Turner et al., Society for Neuroscience Abstracts 5, 684, 1979). A possible role for ODC in nerve growth is discussed in view of an apparent dissociation of effects of NGF on ODC activity and neurite outgrowth in cultured PC-12 cells.

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235.9 GLIAL SCAR FORMATION IN THE BRAIN OF ADULT RATS IS INHIBITED BY IMPLANTS OF EMBRYONIC CNS TISSUE. Lawrence F. Kromer. Dept. of Neurosciences, UCSD, La Jolla, CA 92093.

Axonal connections are reported to develop between intracephalic implants of embryonic CNS tissue and the CNS of the adult rat host (Björklund & Stenevi,'76,'77; Kromer, et al.,'80a, 80b). Since axonal growth occurs between the two tissues, the question arises whether an intervening zone of astroglial processes is present or whether the embryonic CNS implant prevents the formation of a glial scar in this transition area.

Embryonic hippocampal (HPC) tissue (taken from 7-25mm fetuses, approximately embryonic days 10-18) was transplanted into a cavity produced by transecting the fornix-fimbria at the rostral end of the HPC in adult female albino rats. The implants were allowed to survive in situ for 2-6 months. Several specimens were reacted with antiserum against glial fibrillary acidic protein (GFA) and prepared according to the peroxidase-anti-peroxidase method, others were prepared for electron microscopy(EM), and a third group of implants was injected with horseradish peroxidase (HRP) in order to anterogradely label axons.

The HRP experiments indicated that axons originating in the implant can enter the adult host CNS at regions where the implant had fused with areas of the host brain that contained neuropil consisting of few myelinated axonal bundles, e.g. the molecular layers of the dentate gyrus, subiculum, and cingulate cortex. However, HRP labeled axons appeared to be deflected from the densely myelinated fiber tracts of the corpus callosum and fimbria. Specimens which were incubated with GFA antiserum indicated that there was no astrocytic reaction in the host HPC or cingulate cortex and no glial scar formation was present along the border between the neuropils of the implant and the host. In the EM material there was no astroglial or microglial barrier between the host neuropil and the HPC implant. Moreover, in many specimens it was impossible to identify the precise border between the two tissues since there was a continuous neuropil of axons and dendrites. Conversely, at regions where the implant had fused with the myelinated fiber tracts of the host, there were layers of astrocytic processes separating the implant from the host CNS.

The results suggest that implants of embryonic telencephalic tissue placed in the telencephalon of an adult rat (homotypic tissues) can: 1) prevent an astrocytic reaction in the host telencephalon and 2) inhibit the formation of a glial barrier along the transition zone between the implant and host neuropils but not between the implant and densely myelinated areas of the adult host. Moreover, axons from the implant can grow across the border of the implant and host neuropils but not into myelinated fiber tracts of the adult host. (Supported by NIH grant NS-15270.)

235.10 BRAIN TRANSPLANTS IN AN ANIMAL "MODEL" OF HUNTINGTON'S DISEASE. H. Kimura*, P.L. McGeer, Y. Noda* and E.G. McGeer. Kinsmen Laboratory of Neurological Research, University of B.C., Vancouver, B.C., Canada. V6T 1W5.

Recent reports (1) indicate that embryonic brain tissue transplanted to CNS sites in the adult can not only survive but can also develop functional connections. We now report that neostriatal cells from neonatal rats form viable transplants in the striata of adult host animals in which striatal neurons have undergone degneration following injection of kainic acid (KA). Four to six weeks after the unilateral injection of 5 nmoles KA into the striatum of 300 g male Wistar rats, neonatal (1 day) striatal cells were injected into the lesioned area. Four weeks later, animals were examined for surviving transplants by three different methods: histological examination, metabolism following parenteral ^{14}C-deoxyglucose (DG) administration, and histofluorescent techniques. Histological examination revealed a small core of neurons in various stages of development in the center of the gliotic and otherwise neuron-free striatal tissue. The transplanted neurons were more darkly stained than surrounding endogenous counterparts and showed new vascularization. ^{14}C-DG autoradiograms showed a greater accumulation of label in the transplanted tissue than in the surrounding lesioned striatum. The autoradiographic density of the transplanted tissue was similar to that in the contralateral (unlesioned) striatum. These experiments indicated that transplanted tissue survived and was metabolically active in the host striatum.

Furthermore, fluorescence histochemistry for catecholaminergic neurons showed particularly bright fluorescence in varicosities situated in and around the transplanted neuronal cell mass. Such fluorescence is seen in neonatal animals and indicates formation of new neuronal connections. Thus, transplanted neurons may not only survive but may become innervated by the dopaminergic terminals spared by the KA lesion.

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(1). Perlow, M.J. et al. Soc. Neurosci. 9, 2318 (1979). Bjorklund, A. & Stenevi, U. Brain Res. 138, 259-70 (1977). Stenevi, U. et al. Acta Physiologica Scand. suppl. 452, p. 39 (1977).

236.1 VASCULAR AFFERENT CONNECTIONS IN THE FELINE CENTRAL NERVOUS SYSTEM. F. J. Thompson, J. R. Wald*, D. A. Lerner*, A. Blackwelder* and M. DiGaetano*. Dept. of Neuroscience, Univ. of Florida Coll. of Med., Gainesville, FL. 32610.

Recently, details of the projection of low threshold venous afferent fibers to the spinal cord (Thompson and Barnes, Brain Res. 177:561, 1979) and to the motor cortex (Thompson et. al., J. Autonom. Ner. Syst., in press) were reported. The present studies have specifically examined connections of peripheral vascular afferents to lumbar motoneurons and to motor cortex neurons.

The experiments were carried out on α-chloralose anesthetized cats and also decerebrate-spinal cats. The vascular afferents studied were electrically activated in the wall of a segment of femoral vein (hindlimb studies), and in the wall of the bracheal-cephalic vein (forelimb studies).

Facilitatory connections to lumbar motoneurons were revealed by recording electrical activity in the ipsilateral hindlimb muscles. EMGs elicited by femoral venous afferent stimulation were recorded in both flexor and extensor muscles acting on the thigh, shank, and foot: gracilis m., biceps femoris m., sartorius m., tensor fascia latae m., vastus lateralis m., anterior tibial m., medial gastrocnemius m., and lateral gastrocnemius m. The facilitation has also been examined in single motoneurons identified by antidromit stimulation of the muscle nerve.

Cortical mapping experiments have shown that (forelimb) bracheal-cephalic venous afferents project to the cortex of the lateral sigmoid gyrus; the (hindlimb) femoral venous afferents project to the cortex of the medial postcruciate gyrus. These two regions represented the primary projection foci, and overlap the cortical regions known to be motor cortex for the fore- and hindlimb musculature respectively. Microstimulation in the depth of the lateral sigmoid cortex in the region of cortical neurons activated by stimulation of (forelimb) bracheal-cephalic venous afferents, produced activation of musculature in same forelimb. Cortical neurons activated by bracheal-cephalic venous afferent stimulation were identified to project into the pyramidal tract by antidromic stimulation of the medullary pyramid.

These studies indicate that limb venous afferents activate effector neurons of the somatomotor system. The involvement of these connections in the reflex control of skeletal muscle venous counterpressure will be discussed. (Supported by NIH, NHLBI, 1 RO1 HL/HD 25614-01).

236.2 SYMPATHOINHIBITORY EFFECTS ELICITED BY STIMULATION OF BARORECEPTOR A- AND C- FIBERS. A.T. Zielinski* and G.L. Gebber. Dept. of Pharmacol./Toxicol., Michigan State Univ., E.Lansing, MI 48824.

A comparison was made of inhibition of renal sympathetic nerve discharge (SND) produced by selective activation of baroreceptor A- and C-fibers of the rabbit aortic depressor nerve. Inhibition of SND locked to single shocks applied to the aortic nerve was viewed as a computer-averaged positive potential. Aortic nerve activity was routinely monitored. A-fibers were selectively activated with low intensity (5-10 V) square wave pulses of 0.1 ms duration. Higher intensity triangular pulses of longer duration were used to block conduction in A-fibers during concomitant activation of C-fibers. Outward current flow at the cathode activated both A- and C-fibers; however, conduction in A-fibers was selectively blocked at the more centrally located anode due to the arrival of action potentials near the peak of inward current flow. The onset latency of inhibition of SND produced by A-fiber stimulation was 139±7 ms while the duration of inhibition was 190±7 ms (9 experiments). Corresponding values for the onset latency and duration of inhibition produced by C-fiber stimulation were 262±12 ms and 212±16 ms, respectively. Differences in axonal conduction velocity could not account for that difference (123 ms) between the onsets of inhibition of SND produced by A- and C-fiber stimulation. In this regard, antidromic conduction times from the medulla to the electrode on the aortic nerve for A- and C-fibers were separated by only 78±4 ms. This observation suggests that baroreceptor A- and C-fibers engage separate central circuits. This contention is supported by the results of experiments in which the medullary depressor region was stimulated. It was found that onsets of inhibition of SND evoked from different medullary sites in the same rabbit could vary by as much as 60 ms. Inhibition of SND elicited by A- or C-fiber stimulation was unaffected by midcollicular decerebration. Thus, the difference (≈45 ms) in the central delays of inhibition evoked by A- and C-fibers cannot be explained by a forebrain loop in the C-fiber pathway. Renal nerve responses elicited by stimulation of descending spinal excitatory tracts were depressed during inhibition of spontaneous SND produced by A- or C-fiber activation. The degree of depression of the spinal → renal nerve response accompanying aortic nerve stimulation was greater than that observed following C_1 spinal transection. These results suggest that activation of either fiber type leads to spinal inhibition. (Supported by PHS Grant HL-13187.)

236.3 BRAIN STEM NEURONAL TYPES WITH ACTIVITY PATTERNS RELATED TO SYMPATHETIC NERVE DISCHARGE (SND) IN THE BARORECEPTOR DENERVATED CAT. Susan M. Barman and Gerard L. Gebber. Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

Using the method of spike-triggered averaging, two categories of brain stem units whose spontaneous discharges were related to inferior cardiac SND were identified in cats with sectioned carotid sinus, aortic depressor and vagus nerves. The discharges of S units (N=47) were related only to SND, while those of SE units (N=18) were related both to SND and EEG activity. Each of the 2 categories of neurons could be subdivided into 2 groups depending upon whether their discharges occurred during the rising or falling phase of 2-6/s slow waves of SND. S and SE units were intermingled primarily in nucleus reticularis ventralis at caudal medullary levels and in nucleus reticularis parvocellularis at mid- and rostral medullary levels. Autocorrelograms of S unit discharges and inferior cardiac SND contained the same rhythm (2-6 c/s). This rhythm was different from that contained in both SE unit discharges and EEG activity in experiments in which crosscorrelation analysis failed to reveal a relationship between cortical activity and SND. In other experiments, sympathetic nerve and cortical rhythms were locked to each other. Only SE units were located in these cats, and the rhythm contained within the autocorrelograms of their discharges was the same as that observed in SND and EEG activity (delta-theta range). These data suggest that 1) S units comprise or receive inputs from the brain stem oscillator responsible for the 2-6 c/s rhythm in SND; 2) SE units are contained in the brain stem "sympathetic" network since SND could be entrained to a cortical rhythm in some baroreceptor denervated cats; and 3) SE units are connected in series with S units since only SE units were found in experiments in which SND was entrained to a cortical rhythm. (Supported by PHS Grant HL-13187 and a Michigan Heart Association Grant-in-Aid.)

236.4 BIOCHEMICAL EVIDENCE FOR THE ROLE OF GLUTAMATE AS A NEUROTRANSMITTER OF BARORECEPTOR AFFERENTS. M.H. Perrone* W.T. Talman, and D.J. Reis. (SPON: M.D. Coughlin) Laboratory of Neurobiology, Department of Neurology, Cornell University Medical College, New York, NY 10021.

The middle third of the nucleus tractus solitarius (NTS) is the principal site of termination of primary baroreceptor afferents. We sought to determine if this region is innervated by fibers containing glutamate and if so if those fibers arose from vagal afferents, some of which are from baroreceptors. Male Sprague-Dawley rats were decapitated and a 1 mm saggital section of the brainstem was removed at the level of the obex. Samples of the NTS were removed with microbore punches (500-1000 μ i.d.) and homogenized in 0.32 M sucrose. The uptake of [^{3}H]-l-glu measured in the low speed supernatant from the NTS was characterized by high affinity (Km = 2.6 μM), Na^+-dependence, and sensitivity to osmotic shock. The uptake (pMoles/mg protein/min) of 1μM [^{3}H]-l-glu in the NTS (202±12; n=4; mean±SEM) is greater ($p<.05$) than that in homogenates of adjacent lateral NTS or hypoglossal nucleus or the spinal trigeminal nucleus (51±10; n=5). It is almost two-fold greater than that in the septum (77±10; n=4) or nucleus (98±12; n=4) accumbens and is of the same magnitude as that of the hippocampus (219±17; n=4), regions presumed to be heavily innervated by glutamatergic fibers. Unilateral ablation of the nodose ganglion, the site of cell bodies of vagal afferents, leads within 24 h to a 40% reduction ($p<.01$) in the V_{max} (but not the K_m) of high affinity uptake of l-glu restricted to the middle third of the NTS bilaterally. Sucrose density centrifugation demonstrated that the decreased uptake in NTS is confined to the synaptosomal fraction. There was no change in the Na^+-dependent uptake of 1 μM ^{3}H-GABA. Endogenous glutamate levels were decreased ($p<.001$) 35% in the NTS 7 days after unilateral extirpation of the nodose ganglion without change in the content of GABA, glycine, aspartate, or glutamine. We conclude that some vagal afferents terminating in NTS are glutamatergic. These findings are consistent with the view (Talman et al, Neurosci. Abst., 1980) that the neurotransmitter of baroreceptor afferent fibers is glutamate.

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236.5 PHARMACOLOGICAL EVIDENCE FOR L-GLUTAMATE AS THE NEUROTRANSMITTER OF PRIMARY BAROREFLEX AFFERENTS. W. T. Talman, M. H. Perrone*, and D.J. Reis. Laboratory of Neurobiology, Dept. of Neurology, Cornell University Medical College, New York, NY, 10021.

To examine the possibility that L-Glutamate (L-Glu) is a neurotransmitter of baroreceptor afferent nerve fibers, we determined in rat the effects on mean arterial pressure (MAP) and heart rate (HR) of microinjections into the nucleus tractus solitarii (NTS) of: (a) L-Glu; (b) its agonists, D-Glu, kainic acid (KA), and L-Aspartate (L-Asp); and (c) an antagonist, glutamate diethylester (GDE). Unilateral microinjection of 0.1 µl of L-Glu, D-Glu, L-Asp, KA, GDE, or saline were made into NTS of 60 adult rats anesthetized with ∝-chloralose. L-Glu elicited a dose dependent fall of MAP and HR. The threshold dose was 5 ng (3×10^{-11} moles). The maximal response (at 1000 ng) was a fall of MAP of 37±3.5 mm Hg (mean ± SEM) and slowing of the HR by 53±10.9 beats/ min. The relative potencies were KA > L-Glu ≥ L-Asp > D-Glu. The effective sites for L-Glu were restricted to the intermediate third of the NTS. Unilateral microinjection of GDE (15 µg; $6X10^{-8}$ moles) into the NTS did not change MAP or HR. However, GDE totally blocked the response to L-Glu. Before GDE, L-Glu (50 ng; $3X10^{-10}$ moles) significantly ($p<0.001$) lowered MAP (mmHg) by 23±4.1 from 83±3.7 to 59±7.4 and HR (beats/min) by 25±7.2 from 320±8.2 to 295±1.0, but after GDE the microinjection of L-Glu caused no significant change in MAP (0±0.8) or HR (-1±1.2). The simultaneous microinjection of 15 µg GDE into the NTS bilaterally totally abolished ($p<0.01$) the reflex fall in HR induced by raising MAP 40-50 mmHg by phenylephrine i.v. Within the 2 minutes after bilateral microinjections of GDE, MAP rose from 99±7.2 to 153±8.4 mmHg. The time courses of the antagonism of the L-Glu response, the inhibition of the baroreflex, and the hypertensive response were the same. We conclude that the microinjection of L-Glu or its agonists into NTS simulates baroreflexes, that the effect is blocked by a specific L-Glu antagonist GDE, and that GDE produces signs of arterial baroreceptor blockade. The study suggests (a) that there are L-Glu receptors on neurons in NTS mediating baroreflexes and (b) that L-Glu is naturally released in NTS to exert transient inhibition of sympathetic neurons and excitation of cardiovagal neurons. Taken with the biochemical data (Perrone, et al., Neurosci. Abst., 1980) it offers further support that L-Glu is the neurotransmitter of baroreceptor afferent nerves.

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236.6 SPINAL CORD REGULATION OF SYMPATHETIC OUTFLOW IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS. Frank W. Marcoux and David Whitehorn. Dept. of Physiol. & Biophys., College of Med., Univ. of Vermont, Burlington, VT 05405.

The organization of sympatho-excitatory and inhibitory pathways playing down upon the spinal preganglionic neurons and their local circuitry has, to date, been studied most in the cat. Central hyper-responsiveness of sympathetic outflow components has been associated with hypertension as expressed in the spontaneously hypertensive rat (SHR). Measurements of the reactivity of spinal sympathetic circuits to direct stimulation were made to elucidate the role which spinal neurons play in the overall sympathetic hyper-responsiveness seen in the SHR.

Age-matched male SHR and normotensive controls (WKY), weighing 250-350g, were anesthetized (α-chloralose, 100 mg/kg) and artificially ventilated. Monopolar stimulation (500µA, 100µs pulses delivered at 80Hz for 0.5s) was applied to the cervical (C2-C3) dorsolateral funiculus (DLF) and sympathetic responses were recorded from a branch of the pre-celiac splanchnic nerve (activity was filtered, 300-3000Hz, and digitally integrated). The portion of the response due to activation of descending spinal elements alone was defined as that part which was unchanged in magnitude following ipsilateral dorsolateral quadrant section central to the stimulating electrode (the first 40-60ms of the response with 30-50ms onset latency). The remainder of the response, which was diminished in magnitude following dorsolateral quadrant section, was considered to be mediated largely by supra-spinal elements.

Comparison of the magnitude of the spinal sympathetic response as a percent of spontaneous activity between 8 SHR and 8 WKY showed a tendency for hyper-responsiveness in the SHR (310±35%) over WKY (268±35%). Values are given as the mean ± S.E. Similar results were observed when anesthesia blood pressure levels were adjusted by phenylephrine infusion (1-2 µg/min) to levels more representative of awake blood pressure for each strain. When the ganglionic blocker, hexamethonium, was given (25mg/kg) and only pure preganglionic nerve recordings were compared, the tendency for SHR spinal hyper-responsiveness was reduced.

Complete ipsilateral hemisection central to the stimulating electrode often revealed an enhanced spinally mediated sympathetic response compared to that observed in the dorsolateral quadrant sectioned cord. This suggests that preganglionic neurons are normally under a tonic inhibitory influence driven supra-spinally and descending in the ventral cord. Supported by NIH HL 24110.

236.7 THE A5 CATECHOLAMINE GROUP AS A MEDIATOR OF PRESSOR RESPONSES IN RAT. J.J. Neil* and A.D. Loewy (SPON: R.M. Burde), Dept. of Anatomy & Neurobiology, Washington Univ. School of Med., St. Louis, MO 63110.

Electrical stimulation of the A5 catecholamine cell group in the rat leads to a rapid blood pressure increase with no change in heart rate. Anatomically, this cell group has been shown to project to the intermediolateral cell column (IML) of the spinal cord using anterograde labeled amino acid transport. The object of this study is to determine if stimulation of the A5 cell group activates the sympathetic nervous system via the direct spinal cord pathway.

First, midbrain hemisections were performed in adult rats just rostral to the A5 cell group (to eliminate axons of passage). Two weeks later, these animals were stimulated at A5 with a monopolar electrode both before and after acute spinal cord transection. Pressor responses were elicited in all animals stimulated (5-25µA, 100Hz, 1 msec pulses, 5 sec trains), and spinal cord transection at the C8 level abolished the pressor responses at stimulus intensities up to 50-100µA. Both electrode position and the extent of hemisection were verified histologically in these and all following animals.

Secondly, A5 stimulation was carried out in midbrain-hemisected animals which had been guanethidine sympathectomized. Four of seven animals were adrenalectomized acutely before A5 stimulation. In all animals, no pressor response or heart rate change could be elicited with A5 stimulation. The extent of sympathectomy was evaluated in each animal via electrical stimulation of a pithing rod inserted into the spinal canal at the end of each experiment.

Finally, in order to determine if the pressor response is mediated via the A5 projection to the IML, midbrain-hemisected rats were injected intraspinally with 2µl of 12mg/ml 6-hydroxy-dopamine bilaterally at C8. After four weeks these rats were stimulated at A5. Pressor responses could be elicited from all animals stimulated, even though spinal norepinephrine in the same animals was less than 3% of controls using the trihydroxyindole fluorescent catecholamine assay.

These results suggest that the A5 pressor response is mediated via the sympathetic nervous system, but that the direct A5-IML projection is not necessary for the response. Hence, it seems likely that one of the other A5 projections activates a non-noradrenergic pathway leading to the IML. (Supported by USPHS-grants NS12751 and GM07200, and a grant-in-aid (80 723) from the American Heart Association)

236.8 PATHWAYS MEDIATING SYMPATHO-SYMPATHETIC REFLEXES OF CARDIAC ORIGIN L.C. Weaver, S. Donoghue*and R. Oehl* Depts. of Physiol., Mich. State Univ., E. Lansing, MI, and Univ. of Birmingham, B'Ham, U.K.

Afferent neurons contained within cardiac sympathetic nerves can initiate excitation of central sympathetic outflow. While it is known that cardio-cardiac sympathetic reflexes can be mediated solely within the spinal cord, the central pathways important for excitation of other sympathetic neurons have not been elucidated. Therefore, in this investigation, the contributions of spinal and supraspinal pathways to cardiac sympathetic afferent (CSA) excitation of renal nerve activity were ascertained. CSA neurons were chemically excited by epicardial application of bradykinin or potassium chloride in chloralose anesthetized, vagotomized, sino-aortic denervated cats. Multifiber renal nerve responses were compared prior to and following midcollicular decerebration or high cervical spinal cord transection. Activation of CSA nerves caused excitation of renal nerve activity and pressor responses in intact cats. Responses were unchanged or increased following decerebration. After cord transection, reflex excitation of renal nerve activity was diminished; however, since basal activity also was decreased, the reflexes constituted the same percent change as had been observed in the intact cat. Concentrations of chemicals required to elicit reflexes were increased 2-10 fold after cord section. Pressor responses to CSA stimulation were insignificant in spinalized cats. When excitability of preganglionic neurons was increased by electrically stimulating excitatory tracts in the cervical cord, spinally mediated reflex changes in renal nerve activity were more apparent. These results illustrate that supracollicular neurons are not essential to these excitatory sympathetic reflexes and that at least a portion of these reflexes can be mediated solely within the spinal cord. It was considered possible that (1) medullary neurons simply provide tonic facilitation of reflexes localized within the spinal cord, or (2) the CSA's influence medullary neurons to initiate facilitation of spinal reflexes, or (3) the CSA's excite medullary neurons to initiate supraspinal reflexes which are dependent of spinal reflexes. The last two possibilities require that CSA neurons have synaptic influences on medullary neurons involved in control of sympathetic preganglionic activity. Such influences were investigated in cats in which inferior cardiac nerves were stimulated electrically and the medulla 2 mm rostral and caudal to the obex searched with microelectrodes for responsive neurons. Neurons excited with latencies from 10-300 ms were distributed widely in areas such as the Nucleus Tractus Solitarius, Spinal Trigeminal Nucleus, Inferior Olivary Nucleus and throughout the reticular formation. These results are consistent with the contention that medullary neurons actively contribute to the excitatory reflexes initiated by cardiac sympathetic afferent neurons. (Supported by NIH grant HL21436).

236.9 CARDIAC VAGAL AND SYMPATHETIC DISCHARGES DURING STIMULATION OF HYPOTHALAMIC "DEFENSE AREA". Kiyomi Koizumi and Mark Kollai. Depts. of Physiol., Semmelweis Med. Univ., Budapest and State Univ. of New York, Downstate Med. Ctr., Brooklyn, NY 11203.

We have previously shown that the activity of vagal and sympathetic fibers innervating the heart changes reciprocally in one situation, but in other circumstances co-activation of the two systems occurs (J. aut. nerv. Syst., 1:33,'80). We wish to investigate this relationship during stimulation of the hypothalamic "defense area". In chloralose anesthetized, artificially ventilated dogs, recordings were made from both cardiac vagal and sympathetic nerves simultaneously. The phrenic nerve activity, blood pressure, heart rate and blood flow to the hind limb muscles were also monitored. Stimulation of the hypothalamic "defense area" produced a strong pressor response, tachycardia and 5-8 fold increase in muscle blood flow which was blocked by atropine. There was an immediate cessation of vagal discharges and enormous increase in sympathetic activity which continued throughout the stimulation. At the cessation of the stimulus, vagal discharges suddenly increased greatly, while sympathetic discharges almost ceased. This reversal in response was partly due to baroreceptor excitation and partly due to the direct "after-effect" of the stimulus, since such response, though smaller, was still observed after complete baroreceptor denervation. The inhibition of vagal activity produced by the hypothalamic stimulation was strong enough to prevent appearance of a baroreceptor reflex during stimulation even when the blood pressure reached over 200 mmHg, and strong vagal excitation caused by electrical stimulation of a carotid sinus nerve was also inhibited during such stimulus. Stimulation of the same "defense area" with single or a short train of pulses evoked inhibition of vagal discharges followed by mild excitation of the activity, while in response of sympathetic nerve reverse sequence occurred. The results show that a clear-cut reciprocal control between vagal and aympathetic nerves occurs in "defense reactions", though the pattern can be modified by the extent of the activity in baroreceptors involved. (Supported by grants from Hung. Min. Health 1-07-0301-06-1/K and USPHS NS-847; Fogarty Int'l. Ctr. FO6 TW 374).

236.10 AREA SPECIFIC CONTROL OF PRESSOR-CARDIOACCELERATORY ESB DURING INSTRUMENTAL CARDIOVASCULAR CONDITIONING IN THE MONKEY (Macaca mulatta) J. A. Joseph and B. T. Engel. Gerontology Res. Ctr., NIA, Baltimore, MD 21224.

Each of four monkeys was operantly conditioned to slow (S) or speed (F) heart rate (HR), or had its HR monitored (NF). Each experimental session was divided into a baseline (256 sec) and a conditioning or monitoring session (1024 sec) which was further subdivided into 16, 64 sec segments. HR, systolic (SBP) and diastolic (DBP) blood pressures were recorded throughout each experiment on a beat-by-beat basis, and averages for baseline and each 64 sec segment were automatically computed. Animals were signalled to slow HR by a red cue light or to speed HR by a green cue light. Correct performance was signalled by a yellow light and incorrect performance was punished by a 10 ma, .45 sec shock to the tail delivered on an 8 sec, fixed interval schedule. NF was unsignalled and unreinforced. During each even numbered segment of each session an animal received electrical stimulation to the brain (ESB) in one of four regions: (I) lenticular nucleus, globus pallidus, ventromedial tegmentum; (II) anterior or dorsomedial hypothalamus; (III) posterior or lateral hypothalamus; (IV) subthalamic nucleus. Stimulation was chosen on the basis of pilot studies with each animal such that stimulation resulted in an increase (Δ) of HR of 10-20 beats/min and an increase in BP of 10-20 mm Hg. There were significant interactions between experimental conditions and ESB when changes in HR, SBP or DBP from baseline to each stimulation (ST) or non-stimulation ($\overline{ST}$) segment was analyzed using a 2 (ST; $\overline{ST}$) by a 3 (S, F, NF) mixed model, analysis of variance: (A) For each animal during slow sessions Δ HR was significantly attenuated when ESB was given in regions I or III but not when regions II or IV were stimulated; (B) During speeding Δ HR either was enhanced or was similar to Δ HR during NF but this varied among animals; (C) BP was not reliably altered during ST--i.e., there were no consistent effects across animals. Thus, these experiments show that animals can alter the effect of ESB through behavioral conditioning, and that the degree of alteration can be very great. Results for a typical animal are shown below for Δ HR (in BPM). Note that the scores in this table are changes from baseline. Thus, ST effects are superimposed on pre-existing performance levels. Compare ST with $\overline{ST}$ across conditions.

	S		NF		F	
REGION	ST	$\overline{ST}$	ST	$\overline{ST}$	ST	$\overline{ST}$
I	-32.3	-33.8	1.1	-7.3	20.5	13.5
II	- 3.9	-21.2	51.1	29.8	42.0	26.4
III	-14.1	-14.1	-1.0	-10.8	22.5	8.6
IV	11.0	-10.3	23.0	9.6	29.8	14.0

236.11 GLOBAL INCREASE IN CEREBRAL BLOOD FLOW INDEPENDENT OF LOCAL GLUCOSE METABOLISM ELICITED BY ELECTRICAL STIMULATION OF FASTIGIAL NUCLEUS IN RAT. M. Nakai, C. Iadecola, L.W. Tucker, and D.J. Reis. Laboratory of Neurobiology, Dept. of Neurology, Cornell Univ. Med. College, New York, NY.

Electrical stimulation of the rostral fastigial nucleus (FN) of rabbit will increase cerebral blood flow (CBF) up to two-fold in the parietal cortex, measured by H_2-clearance (MacKenzie et al., Neurosci. Abs., 1977). In the present study we sought to determine whether FN stimulation in rat also increases CBF and, if so, whether the increase in CBF is: (a) restricted to cerebral cortex or is more widespread; and (b) associated with a corresponding increase in glucose metabolic rate (GMR). Rats were anesthetized (chloralose), paralyzed and ventilated. The FN was stimulated electrically. Blood pressure was maintained constantly in the autoregulated range (130 mm Hg) in stimulated and controlled animals. Local CBF and GMR were determined autoradiographically by the ^{14}C-iodoantipyrine (IAP) technique or ^{14}C-2-deoxyglucose (2-DG) methods, respectively. In controls, CBF closely corresponded to GMR in all 24 brain regions sampled (R = 0.951). With FN stimulation, CBF increased in all 24 areas averaging 183 $\pm$ 4.5% (n=7)($p < .025 - .005$). Increases in CBF ranged from 157% (cerebellar grey) to 226% (sensory-motor cortex) of control. Most marked changes were throughout the cerebral cortex but also substantially in white matter, basal ganglia, and midline thalamic and brainstem nuclei. The GMR increased in only 12 of 24 areas averaging 130.7 $\pm$ 3.9% ($p < .05$, n = 8) and ranging from anterior thalamus (116%) to central thalamic nuclei (166%). Most of the cerebral cortex was not changed. Most increases were seen along the major projection pathway of FN. FN stimulation also increased CBF when measured by the IAP technique using regional dissection. The increases averaged 209 $\pm$ 17% ($p < .005$, n = 7) and ranged from 117% (hippocampus) to 302% (parietal cortex) of control. The two- to three-fold increases of CBF seen in all areas of the brain were not affected by acute sympathectomy and were only slightly attenuated by spinal cord transection. We conclude that stimulation of projections originating in or connecting with FN will evoke a powerful and global vasodilatation of cerebral vessels not necessarily associated with altered cerebral metabolism. The vasodilation is probably mediated by intrinsic pathways in brain.

(Supported by NIH grant HL18974)

236.12 THE EFFECTS OF CENTRAL CATECHOLAMINERGIC LESIONS ON DEVELOPMENT OF THE CARDIAC SYMPATHETIC AXIS. R. Deskin*, E. Mills*, W.L. Whitmore*, F.J. Seidler* and T.A. Slotkin. (SPON: A. Tadepalli). Dept. Pharmacology, Duke U. Med. Ctr., Durham, N.C. 27710.

In order to assess what role the central nervous system (CNS) plays in cardiac growth and development, neonatal rats were treated intracisternally with 6-hydroxydopamine (6-OHDA) to destroy central catecholaminergic nerve terminals. The extent and duration of the lesion was estimated by measurement of whole brain tyrosine hydroxylase activity, which was markedly reduced throughout the entire course of development. Specificity for the CNS was confirmed by the marked reduction of accumulation of radiolabelled monoamines into brain synaptosomal fractions, compared to no effect on uptake into preparations from peripheral neurons (heart synaptic vesicles). Cardiac development was impaired in the 6-OHDA-treated animals, as assessed by the ratio of heart/body weight (an index of organ growth) and by ornithine decarboxylase activity (an index of cellular maturation). The defects did not appear until the second to third week of postnatal age; because this corresponds to the age at which tonic sympathetic control of the heart first appears, baroreceptor-mediated cardiac sympathetic reflexes and basal autonomic input to the heart were evaluated. Although the reflexes developed normally as assessed both physiologically (carotid occlusion test) and biochemically (stimulation of cardiac ornithine decarboxylase by hydralazine-induced hypotension), there was evidence for abnormalities in both parasympathetic and sympathetic tone. Vagal input was elevated in the treated rats, as shown by a subnormal heart rate which became normal after vagotomy. Salt load, which normally causes a centrally-mediated increase in sympathetic outflow, had a severely attenuated effect in the lesioned animals. Thus, destruction of central catecholaminergic nerve terminals in the neonate does not alter maturation of the sympathetic neurons per se, but results in an abnormality in the balance of parasympathetic and sympathetic tone, which may be responsible for subsequent specific defects in cardiac development and in cardiovascular function. Pretreatment of the animals with desmethylimipramine prior to 6-OHDA produced a relatively specific lesion of dopaminergic nerve terminals, and results obtained for cardiac growth and ornithine decarboxylase were virtually identical to those obtained with 6-OHDA alone, suggesting that the effects on central dopamine are more important than on norepinephrine in producing defective cardiac development. (Supported by USPHS HL-24115, HL-NS-22861 and DA-00006).

236.13 CARDIAC GNAGLION CELLS LABELED BY QUINUCLIDINYL BENZYLATE.
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An attempt was made to study the localization of muscarinic receptors in the necturus cardiac ganglion cells. For this purpose, radioactive quinuclidinyl benzylate (H^3-QNB-16.4 Ci/mmol) was employed. H^3-QNB at a concentration of 10 nM blocked the hyper-polarizing response of the gangion cells to Betanachol. The blocking effect of QNB was partially reversible after prolonged wash-out. For the morphological studies, cells were exposed to 20 nM of H^3-QNB for one hour. The cells were then frozen, lyophylized, embedded in epon and sectioned for light microscopy. The sections were coated with Kodak fine grain emulsion and allowed to develop for six months. Care was taken to avoid exposure of the sections to water or water vapor. The autoradiograms showed a clear labeling of the cytoplasm of the ganglion cells. Similar labeling was also found in experiments where 25 μM of atropine were added in addition to 20 nM H^3-QNB. In view of the higher oil-water partition of QNB, it is suggested that in our experiments an intracellular precursor of the muscarinic receptor was labeled by H^3-QNB.

237.1 NYSTAGMUS GENERATION IN THE PRESENCE OF STATIC AND ROTATING GRAVITY VECTORS. T. Raphan, B. Cohen, D. Dennett*, V. Henn*. Depts. of Neurology, Mt. Sinai School of Medicine, New York, N.Y. 10029, and University of Zurich, Switzerland.

Rotation in darkness about an axis coincident with the gravity vector (vertical axis rotation,VAR) evokes per rotatory nystagmus whose slow phase velocity decays to zero with a time constant of 12-30 sec. If head velocity is set to zero after per rotatory nystagmus has disappeared, the characteristics of the per and post rotatory nystagmus are approximately equal. These characteristics are maintained when monkeys are tilted as long as the axis of rotation is coincident with the gravity vector. Optokinetic nystagmus (OKN) and optokinetic after nystagmus (OKAN) induced when monkeys are tilted from the vertical have approximately the same characteristics as the OKN and OKAN induced when the animals are upright. OKAN is somewhat modified for angles of tilt close to 90°. This indicates that a static gravity vector i.e. one that does not rotate relative to the head has little or no effect on the horizontal per and post rotatory nystagmus. OKN and OKAN are similarly unaffected by a static gravity vector. In contrast, rotation in darkness about an axis tilted with regard to gravity (off vertical axis rotation, OVAR) causes indefinite prolongation of nystagmus. This effect occurs at angles of tilt from 5-90°. Steady state velocities increase approximately linearly with rotational velocity and are maintained up to about 50-60°/sec. There is oscillation in slow phase velocity associated with head position and dependent on the speed of rotation. If the monkey is rotated about a vertical axis and then the axis is tilted after the velocity of the per rotatory nystagmus has decayed to zero, slow phase velocity rises to a steady state value with a time constant of 10-15 sec. When the animal is returned to the VAR position the nystagmus decays with about the same time constant as it rose. The OVAR post rotatory response has a peak velocity that is the difference between the VAR post rotatory velocity and the slow phase velocity when the head was stopped at the end of OVAR stimulation. The time constant of the post rotatory response is about 5 seconds and is similar to the characteristics of post rotatory nystagmus during visual suppression. Tilts during post rotatory nystagmus and OKAN also cause suppression of slow phase velocity similar to that of visual suppression. The data indicate that continuous changes in head position with regard to gravity activates the velocity storage integrator to sustain the nystagmus. Stopping after OVAR and tilts during post rotatory nystagmus or OKAN suppress the integrator. These characteristics contribute to better ocular compensation for head movement in space.

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237.2 CONSEQUENCES OF MONOCULAR DEPRIVATION OR CORTICAL LESIONS UPON MONOCULAR HORIZONTAL OPTOKINETIC NYSTAGMUS IN THE CAT. N.P. Strong*, R. Malach* and R.C. Van Sluyters. School of Optometry, University of California, Berkeley, CA 94720.

It has been proposed that there are different central pathways mediating monocular optokinetic nystagmus (OKN) in the two horizontal directions. One pathway transmits information concerning stimuli moving from temporal-to-nasal (N) and the other information about stimuli moving in the opposite, nasal-to-temporal (T) direction (Hoffmann, K-P., in Developmental Neurobiology of Vision, R. Freeman (ed), 63-72, 1979). Previous work has indicated that the quality of monocular OKN in these two directions may be different in both time course of appearance and susceptibility to deprivation; and it has been suggested that both bilateral cortical lesions (Wood, C.C., Spear, P.D. and Braun, J.J., Brain Res., 60:231-237, 1973) and monocular deprivation (MD) (van Hof-van Duin, J., Arch. Ital. Biol., 116:471-477, 1978) disrupt the pathway for T-OKN from both eyes at the cortical level, whereas the generation of N-OKN is independent of cortex.

In a series of control experiments, we have studied the ontogenesis of monocular OKN in kittens using DC electrooculographic recording techniques that permit quantitative analysis of OKN performance. The amount of asymmetry in N- versus T-OKN was determined in normal kittens of various ages using measurements of both slow-phase gain and beat frequency. In comparing the consequences of MD or cortical lesions upon monocular OKN, we have found that the effects of MD are confined solely to the deprived eye and that T-OKN is disrupted much more than N-OKN. Unilateral cortical lesions involving removal of the entire posterior neocortex produce an equivalent breakdown of T-OKN through the eye ipsilateral to the lesion; however N-OKN through this eye is not severely disturbed.

237.3 NEURONAL ACTIVITY IN THE FLOCCULUS DURING OPTOKINETIC NYSTAGMUS AND AFTER-NYSTAGMUS IN THE ALERT MONKEY. W. Waespe*and V. Henn* (SPON: M.B.Bender). Dept. of Neurol., Zuerich Univ., USZ, Zuerich 8091-CH.

Single cell activity in the flocculus was recorded extracellularly during vestibular and optokinetic stimulation. Animals were rotated about a vertical axis in light or darkness, or a fullfield optokinetic drum was rotated around the stationary monkey at constant velocities between 15 and 160 deg/sec. Neurons were classified as Purkinje-cells (P-cells) and Non-Purkinje-cells (Non-P-cells). Non-P-cells, reflecting the input activity to the flocculus, showed a behavior similar to neurons in the vestibular nuclei (Waespe & Henn, Exp. Brain Res., 27, 523-538, 1977). They had a long decay time constant (10-50 sec). There was an equal number of type I and type II neurons. All Non-P-cells were also modulated during optokinetic stimulation. Changes in activity were similar when vestibular nystagmus (VN) and optokinetic nystagmus (OKN) were in the same direction except that during optokinetic stimulation neuronal activity increased monotonically only up to constant velocities of 60 deg/sec whereas OKN slow phase velocity reached higher values. During optokinetic after-nystagmus (OKAN) neuronal activity reflected slow-phase velocity. Only about 10-15% of P-cells were modulated by these stimulus paradigms. Simple spike activity of P-cells was only weakly modulated during vestibular stimulation. About half of these P-cells were also influenced by optokinetic stimulation. However, in contrast to the Non-P-cells, P-cells were modulated only at velocities of 60 deg/sec or more. Furthermore they were activated by vestibular and optokinetic stimuli in the same direction, which elicited VN and OKN into opposite directions. During OKAN P-cell activity showed no relation to the slow-phase velocity of nystagmus.

The results suggest a complementary mode of information processing in the flocculus and vestibular nuclei during optokinetic stimulation. P-cells respond to stimulus values beyond the working range of the vestibular nuclei neurons. The data support findings of lesion studies which show that after flocculectomy monkeys were unable to produce OKN with slow-phase velocities of more than 40-60 deg/sec, the saturation velocity of the vestibular nuclei neurons.

237.4 CHANGES IN CEREBELLAR ACTIVITY DURING ADAPTIVE GAIN CONTROL OF THE VESTIBULO-OCULAR REFLEX (VOR) OF GOLDFISH, J.J. Michnovicz*, J.O. Schairer*and M. V. L. Bennett, Div. Cellular Neurobiology, Dept. Neuroscience. A. Einstein Col. Med., Bronx, New York 10461.

Goldfish have been shown to undergo a relatively rapid adaptive gain control of the VOR when presented with different combinations of vestibular and optokinetic stimuli (Schairer and Bennett, Soc. Neur. Abs. 3: 485, 1977). Upon ablation of the cerebellum, the fish loses this adaptive capacity, while the VOR functions essentially as before. In order to elucidate the role of the cerebellum in this behavior, extracellular recordings were made in the vestibulo-cerebellum during adaptive modification of the VOR.

Restrained, unanesthetized goldfish were placed in a clear tank which was secured to a movable platform and surrounded by a vertically striped optokinetic drum. Rotation was in the horizontal plane at 0.125 hz. Eye position was monitored with a magnetic search coil. VOR gain is expressed as eye velocity/table velocity. Instantaneous firing rates were calculated using a spike discriminator, and gain of the unit was measured as impulses per second/table velocity. Records were taken under four conditions: 1) dark, in which the platform was rotated in the dark; 2) training toward VOR gain of 1, in which the platform was rotated in the light with the drum stationary; 3) training toward 0, in which the fish and drum were rotated together in the light; 4) training toward 2, in which the platform and drum were rotated in the light equally and oppositely.

The effects of optokinetic stimulation on cerebellar units responding to rotation were of two types, immediate and retained. Six units immediately changed in firing frequency as the light was switched on so that the fish could see the drum moving with it (training toward 0). Four units increased in firing rate an average of about 60% while the VOR gain dropped about 70%. Two units decreased in firing rate about 40% in the light when the VOR gain decreased about 70%. One unit showing an increase was followed for over 3 hours. During this period, training toward 0 was maintained for 90 minutes. At the end, the gain of the VOR in the dark had decreased by 69% from its original value, while the firing rate had risen to a level 67% above its original value in the dark. Subsequent training in the opposite direction (toward 2) showed corresponding reversal of changes in both VOR gain and firing gain. A second unit that showed an immediate gain increase in the light gradually increased further in gain during a 1 hour session of training toward 0, but was not tested in the dark. The relative rapidity of these changes may allow further analysis of the cellular mechanisms of this form of learning.

237.5 PLASTICITY OF EYE MOVEMENT DIRECTION IN THE VESTIBULO-OCULAR REFLEX (VOR). L. W. Schultheis* and D. A. Robinson (SPON: W. M. King). The Wilmer Institute, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

It has been shown that signals from each semicircular canal reach all extraocular muscles. A head rotation vector is encoded in the planes of the canals on primary vestibular afferents and in the planes of the eye muscles on motoneurons. The projections from the vestibular to the oculomotor nuclei constitute a transformation from the canal to the muscle coordinate systems. These projections insure that the eye moves in the right direction as well as speed. Just as the eye speed relative to head speed has been shown to be plastic, we wanted to determine whether eye direction was also plastic.

Subjects nodded their heads vertically and tracked a target which was displaced horizontally in proportion to vertical, angular head displacement. It is known (Science, 160: 429, 1968) that after 10 min exposure a stationary target appears to move horizontally during vertical head rotation. This illusion is consistent with the idea that vertical head movements were creating reflex horizontal eye movements. We recorded eye movements in five subjects in such a situation and confirmed this hypothesis. The cross-axis ratio (horizontal eye velocity/vertical head velocity measured in the dark) could be increased from zero (normal) to 0.2 in about 0.5 hr. Efforts to obtain a similar result in the monkey, however, using Dove prisms at 45° to associate vertical, retinal image slip with horizontal head rotation gave negative results (Neurosci. Abstr., 5: 371, 1979).

Nevertheless, for further neural studies, we tried to demonstate this phenomenon in the cat. The head was immobilized by a skull implant and eye and head movements recorded by the magnetic field-search coil method. The animals were oscillated in pitch inside an optokinetic drum which moved horizontally in proportion to vertical head rotation. After 5 hr the cross-axis ratio in three animals was 0.24 (range 0.18 to 0.28). The change was plastic: the ratio did not change after 1-3 hr in darkness. The ratio returned to zero within 0.5 hr upon rotation with the drum stationary. The cross-axis ratio for a fixed exposure time increased if the drum displacement was increased relative to head displacement. Plastic changes did not occur in stroboscopic illumination (3.7 flashes/sec).

These results indicate that in humans and cats the VOR can be twisted so that vertical canal stimulation produces a horizontal component of eye movements.

237.6 DIFFERENCES IN DYNAMICS OF MOTOR OUTPUT TO EYE AND NECK MUSCLES. J. Goldberg*, G. Bilotto, B. W. Peterson, and V. J. Wilson. The Rockefeller University, New York, N.Y. 10021

The dynamics of the vestibuloocular reflex (VOR), vestibulocollic reflex (VCR), and cervicocollic reflex (CCR) were investigated in precollicular decerebrate cats using horizontal angular rotations in which angular position was modulated by single sinusoids or summed multiple sinusoids in the frequency range 0.05 - 4 Hz. Using rotational amplitudes of 1 - 20°, we obtained linear sinusoidal modulation of eye position and head position which at higher frequencies was in a direction approximately opposite (compensatory) to that of the applied rotation.

Dynamics in the VOR system were investigated by recording single unit activity of antidromically identified abducens motoneurons with tungsten microelectrodes and horizontal eye position with bitemporal Ag-AgCl EOG electrodes. The abducens motor signal displayed the characteristics of a first order lead system when measured against horizontal eye position: phase lead tended toward 90° at higher frequencies and gain increased at 20 db/decade. Estimates of the time constant of the lead term ranged from 0.5 to 1.7 seconds.

Dynamics in the neck motor system were investigated by recording EMG activity of dorsal neck muscles under three conditions: whole body rotation with the head fixed to the turntable, body rotation with the head free to move, and body rotation with the head fixed in space. Neck motor signals measured with respect to platform position (or to compensatory head position when the head was free) displayed the characteristics of a second order lead system under all three conditions: phase lead at the upper end of the frequency range (4 Hz) exceeded 120° and was still increasing, gain rose at 40 db/decade. Estimated time constants of the two lead terms ranged from 0.05 to 0.15 seconds. This dynamic behavior was unaffected by a bilateral section of the medial longitudinal fasciculus at the level of the obex (see also Wilson et al., J. Neurophysiol. 42 (1979) 331-346). Thus the phase lead does not depend on the integrity of the three-neuron VCR arcs, which traditionally have been thought to carry the high frequency phase-advanced signal.

These results indicate that the horizontal gaze control system produces output signals with different dynamics to drive the eyes and head even in response to identical vestibular stimuli. As noted by others, the extraocular motor signals appear to be well adapted to overcome the predominantly viscous load presented by the eye mechanics. The neck motor signal would similarly appear to be well suited to compensate for the predominantly inertial load of the head-neck system. Supported by grants EY 02249, EY 00100, NS 02619.

237.7 THE CERVICOOCULAR REFLEX OF THE RABBIT AND ITS LINEAR SUMMATION WITH THE VESTIBULOOCULAR REFLEX. N. H. Barmack, V. E. Pettorossi* and M. A. Nastos* Neurological Sciences Institute of Good Samaritan Hospital & Medical Center, Portland, Oregon 97210.

The vertical vestibuloocular reflex (VVOR) has a higher gain than does the horizontal vestibuloocular reflex (HVOR) during low frequency, sinusoidal, whole-body rotation. This difference can be ascribed to the contribution of the utricular otoliths to the VVOR. During normal head movements, neck proprioceptors might partly compensate for this reduced low frequency gain of the HVOR. We have measured the horizontal (HCOR) and vertical (VCOR) cervicoocular reflexes of rabbits evoked by sinusoidal oscillation of the body about the fixed head (±10 degs, 0.005-0.800 Hz). When the body of a rabbit was rotated horizontally clockwise (as viewed from above) in total darkness, clockwise conjugate eye movements were evoked. When the body was rotated about the longitudinal axis onto the right side, the right eye rotated down in its orbit and the left eye rotated up. The mean gain of the HCOR (eye velocity/body velocity) rose from 0.21 at 0.005 Hz to 0.27 at 0.020 Hz and then declined to 0.06 at 0.3 Hz. Eye position led body position by 18 degs at 0.005 Hz, reversed to a lag of 23 degs at 0.08 Hz, and reversed again to a lead of 5 degs at 0.4 Hz. The gain of the VCOR was reduced relative to the gain of the HCOR by a factor of 2-3. The HCOR and HVOR were measured separately and in combination (HCOR + HVOR) by rotation of the head about the fixed body. These reflexes combine linearly. The latency and pattern of horizontal eye movements evoked by horizontal step-angular (20 deg) displacements of the body were also studied. The initial compensatory eye movement evoked by step stimulation had a mean latency of 37 ±13 msec (N=9). This initial low velocity compensatory eye movement was usually followed by a fast compensatory eye movement at a mean latency of 300 ±70 msec. During conjoint neck proprioceptive and vestibular step stimulation (HCOR + HVOR) the fast compensatory eye movements evoked by neck proprioceptive stimulation (HCOR) effectively canceled the vestibularly-induced anticompensatory resetting eye movements (HVOR). This cancellation would enable maintenance of horizontally eccentric eye position during active head movements. The relative movements of the first three cervical vertebrae during stimulation of the HCOR and VCOR were measured. For the HCOR, the largest angular displacement (74%) occurs between C_1 and C_2. For the VCOR, the largest relative angular displacement (45%) occurs between C_2 and C_3. We conclude that the HCOR partially compensates for the absence of otolithic information concerning low frequencies of horizontal head movement. (Supported by NIH Grant EY00848 and the Oregon Lions Sight & Hearing Foundation.)

237.8 ELECTROPHYSIOLOGICAL IDENTIFICATION AND PHYSIOLOGICAL ACTIVITY OF LATERAL VESTIBULAR NEURONS IN THE ALERT CAT. H. Reisine, A. Strassman and S.M. Highstein. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Cats were implanted with electrodes for electric pulse stimulation in the IIIrd nucleus, on the VIth nerve, and over the bony ampulla of the horizontal semicircular canal (S.C.C.). Cats' heads were fixed to a rotating table (sinusoidal motion), eye movements monitored with E.O.G. electrodes and head velocity and position were also recorded. Glass microelectrodes were inserted into the brainstem of alert cats through an implanted chamber.

Some neurons recorded 2-3mm lateral to the VIth nucleus were activated antidromically from the IIIrd nucleus (ANTI.) and monosynaptically from the S.C.C. These neurons encode a head velocity signal (Type I, c.f. Reisine & Highstein, Brain Res. 1979). These same neurons also encode eye position with an on-direction correlated with activation of the ipsilateral medial rectus extraocular muscle (MR). Other neurons were ANTI., but disynaptically activated from the S.C.C. These neurons did not encode head velocity but encoded eye position with an on-direction as above. The majority of neurons were poly- or disynaptically activated from the S.C.C., were not ANTI., encoded Type II head velocity signals and were not related to eye position.

We conclude that some neurons in the lateral vestibular area projecting to the IIIrd nucleus are driven monosynaptically from the ipsilateral labyrinth, and encode a mixture of Type I head velocity and horizontal eye position signals. These neurons are presumed to be ventral lateral vestibular neurons projecting via the ascending tract of Deiters' to the MR. Signals carried over this pathway may account for the remaining V.O.R. after MLF lesion. Supported by NIH EY-01670, NIH K04-EY0003, NIH NS-07512, and NIH 1F32-NS-06019.

237.9 VESTIBULAR STIMULATION ALTERS THE NEURAL CONTROL OF PRIMATE FOVEAL PURSUIT IN THE FINAL 'COMMON' PATHWAY.

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It was recently shown that during foveal pursuit in Java monkeys the velocity component ($R\times\dot{\theta}$) of most oculomotor motoneurons is different in the agonist versus antagonist phase(Eckmiller and Mackeben, Pflügers Arch.377:15,1978). In the present study, single unit activity in the III. or VI.nerve nuclei, as well as eye movements(EOG), were recorded while the trained monkey pursued a small light spot(4 - 8 min.of arc in diameter) under three conditions: a) light spot moved horizontally on a screen; monkey head stationary. b) light spot stationary; primate chair rotated around vertical axis. c) light spot stationary; primate chair moved on a slide track parallel to the screen.

Quantitative analysis of the impulse rate time course of these neurons(with features characteristic of motoneurons) revealed: 1. A few neurons had exactly the same $R\times\dot{\theta}$ for agonist and antagonist phase, not only during condition a), but also during the other conditions(like monitors of eye movements). 2. Many neurons having different $R\times\dot{\theta}$ for agonist versus antagonist phase under condition a) exhibited another $R\times\dot{\theta}$ (without agonist-antagonist phase differences) under conditions b) and c). Further analysis of this class of neurons suggests that under condition a) they receive a velocity input only in one phase(agonist or antagonist). This velocity input could be provided by a newly discovered class of pre-motor neurons which are correlated with eye velocity in one direction during pursuit under condition a) but not b) (Eckmiller and Mackeben,Brain Res.184:210,1980).

Differences in $R\times\dot{\theta}$ for a given motoneuron during condition b)(semicircular canal stimulation) and condition c)(otolith organ stimulation) will be discussed.

One interesting conclusion is: although the visual stimulation is almost identical under all three conditions, the ocurrence of additional vestibular stimulation(b) and c)) leads to significant changes in the neural activity of most oculomotor motoneurons. This suggests that the phylogenetically oldest oculomotor subsystem, the VOR, dominates the youngest subsystem, i.e. the foveal pursuit system.

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237.10 SMOOTH PURSUIT EYE MOVEMENTS WITH THE SACCADIC SYSTEM RENDERED INEFFECTIVE. Harry J. Wyatt and Jordan Pola. State College of Optometry, State University of New York, New York, NY 10010.

It is our contention that not only target velocity, but also target offset from the fovea can elicit smooth pursuit eye movements. This view is based on experiments using the "open-loop" pursuit system (Vision Res. 20(6)). However, under normal (closed-loop) circumstances, it is difficult to observe pursuit responses to target offset, since saccadic eye movements quickly eliminate the offset. Therefore, we have developed a technique of "saccadic stabilization" -- that is, of defeating attempts by the saccadic system to achieve foveation. The basis for this technique is the detection of a saccade, and the consequent jumping of the target by an amount equal to saccade length, so that target offset is the same after as before the saccade.

When "saccadic stabilization" is used, subjects can achieve fixation of a stationary eccentric target by means of slow eye movements. We have also used "saccadic stabilization" when the stimulus is ramp target motion -- i.e., constant-velocity motion with sudden onset. In response to ordinary ramp targets, Robinson (1965) noted that subjects often make a saccade to reduce target offset, followed by a "catch-up" pursuit to eliminate any residual offset. (The catch-up pursuit is faster than the target motion.) With a "saccade-stabilized" ramp target, the saccadic attempt to reduce offset is ineffectual, and the post-saccadic offset of the target is greater than it would be for an ordinary ramp target. The post-saccadic offset can be further exaggerated by introducing target jumps that are larger than, instead of equal to, saccade length. This type of target elicits catch-up pursuit movements that are faster and more prolonged than is the case for the usual unstabilized ramp targets. These findings add support to the view that target offset is an important stimulus for the smooth pursuit eye movement system.

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238.1 THE INFLUENCE OF THE PERIVENTRICULAR TISSUE OF THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) ON THE RELEASE OF VASOPRESSIN (VP). A. K. Johnson, S. L. Bealer, J. R. McNeil, J. Schoun, & J. Möhring*. Department of Psychology, University of Iowa, Iowa City, IA 52242 and Merrell International Research Center, Strasbourg, France.

Small lesions that destroy the tissue surrounding the AV3V produce marked disruptions in body fluid balance. The acute effects of AV3V lesions are adipsia and impaired antidiuretic responses (Johnson & Buggy, Am. J. Physiol., 234, R122, 1978). Although animals with lesions may regain ad lib drinking and survive, they manifest signs of disrupted controls of water intake and retention. Chronically, drinking responses to systemic angiotensin II (AII) and hypertonic saline (HTS) treatment are significantly reduced (Buggy & Johnson, Am. J. Physiol., 233, R44, 1977) and antidiuretic responses to intracranial injections of AII and HTS are blunted (Johnson, Hoffman, & Buggy, Br. Res., 157, 161, 1978). The impaired antidiuretic responses in both the acute and chronic post-lesion states suggest that VP release may be impaired by AV3V lesions. The present studies investigated this hypothesis.

In the first experiment rats received either sham (SL) or AV3V lesions (AV3VX). One-half of the SL animals (SL-W) and all of the rats with AV3VX received water and food post surgery; the second half of the SL group (SL-NW) had only food. The next day rats were decapitated and trunk blood, the posterior pituitary (PP) and punches of the supraoptic nuclei (SON) were collected. As usual, AV3V destruction produced adipsia. Both hematocrit and plasma osmolality were significantly increased in the AV3VX and in SL-NW as compared to SL-W. However, plasma VP was elevated only in the SL-NW. There was no difference in plasma VP levels of SL-W or AV3VX. There were no differences among groups in PP and SON VP content.

In a second experiment rats were divided into two groups. One group received AV3VX and the second SL. Several weeks post-lesion and after ad lib drinking had recovered, SL and AV3VX were randomly assigned to one of four groups to receive s.c. injections (2 ml/100g body weight) of saline in concentrations of .15M, .3M, .45M, and .6M. One-half hour after the injection trunk blood was collected. The results indicated that HTS systematically increased the levels of circulating VP. However, this was not the case for AV3VX rats where VP levels were not significantly elevated. The results from experiments with animals in acute and chronic post-lesion states show that the integrity of the AV3V is necessary for the normal control of VP to systemic dehydration. Furthermore, the results from the first experiment suggest that this impairment occurs even when VP is present in the hypothalamo-hypophyseal system. (USPHS NIH HLP-14388 & 1 RO1-H124102; NIMH 1-KO2-MH00064)

238.2 FURTHER STUDIES ON THE ROLE OF ANGIOTENSIN IN THE OSMOTIC CONTROL OF VASOPRESSIN RELEASE BY THE ORGAN CULTURED RAT HYPOTHALAMO-NEUROHYPOPHYSEAL SYSTEM. Celia D. Sladek, Depts. Neurology and Anatomy, Univ. Rochester, Rochester, NY 14642.

Physiological increases in osmolality achieved by adding either NaCl or mannitol stimulate VP release by organ cultured hypothalamo-neurohypophyseal explants from rats (Endocrin. 101:1834, 1977). This response can be blocked by the angiotensin (AII) competitive antagonist, saralasin (Endocrin. 106:173, 1980). This observation suggests that AII may either mediate or modulate osmotically stimulated VP release by HNS explants, and also suggests that AII is generated in the culture system. These possibilites were evaluated using the converting enzyme inhibitor, SQ14,255.

Converting enzyme activity in HNS cultures was demonstrated in experiments evaluating the effectiveness of AI as a stimulus for VP release. AI (10^{-5}M) significantly increased VP release to 189 ± 31% of control release ($p < .005$) however, it was less potent than AII (AII, 10^{-5}M, yields a 390 ± 85% increase). The response to AI was blocked by the simultaneous addition of SQ14,255 (10^{-5}M) suggesting that AI must be converted to AII in order to stimulate VP release and that this conversion takes place in the culture system.

The role of culture system-generated AII in the osmotic stimulation of VP release was evaluated by assessing the osmotic response of explants exposed to SQ14,255 for various periods prior to the application of the osmotic stimulus. SQ14,255 (10^{-5}M) was added either 24 hours prior, 4 hours prior, or simultaneously with sufficient NaCl to increase the culture medium osmolality from 295 to 315 mosmol/kg H_2O. There was a significant increase in VP release by the explants exposed simultaneously to the osmotic stimulus and SQ14,255, but the explants with prior exposure to the converting enzyme inhibitor did not show a significant response to the osmotic stimulus. They did respond to a comparable osmotic stimulus on the subsequent day in culture following a 24 hour incubation in the absence of SQ14,255. Since SQ14,255 and saralasin do not alter unstimulated VP release, these observations suggest that AII is being generated in the culture system in amounts which potentiate the osmotic response, but which alone are insufficient to stimulate VP release. Since the culture medium contains fetal calf serum (20%), it is possible that some of the factors necessary for AII generation are furnished by the culture medium. The ability of AII to potentiate osmotic stimuli corresponds to observations in vivo of AII potentiation of drinking and VP release in response to osmotic stimuli.

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238.3 NALOXONE AND CORTICOSTERONE POTENTIATE RELEASE OF VASOPRESSIN TO HEMORRHAGE IN CONSCIOUS RATS. A.J. Baertschi*, M.J. Wicki* and M. Friedli* (SPON: B. Gähwiler). Dept. of Biology, University of Geneva, 1211 Geneva 4, Switzerland.

To test the hypothesis that endogenous opiates may be involved in blood pressure control and neurohypophysial hormone release, conscious rats with carotid catheters were subjected to a 1 ml/min hemorrhage (H) for 4 min and following a 6 min interval again for 2 min. Three blood samples (A,B,C) of 2 ml were collected and analyzed for plasma vasopressin (AVP) by a specific RIA, and arterial blood pressures were monitored before (Bef) and after (Aft) H in 4 groups of 5-8 rats (250-300g): CONTR=controls; NALOX=10 µg naloxone 30 min before H; CS 5'=10 µg corticosterone 5 min before H; CS 2h=10 µg corticosterone 2 hours before H; treatments were all given intra-arterially. Results are shown in Table:

GROUP	Mean pg AVP/ml plasma ± SE			Mean pressure, mm Hg, ± SE			
	A	B	C	Bef A	Aft B	Bef C	Aft C
CONTR	4.0±0.6	10.8±4.2	20± 5	111±4	106± 4	104± 3	46±6
NALOX	11.2±3.3	33.8±8.7	161±47	100±6	60±12	67±11	35±7
CS 5'	8.8±3.0	17.5±8.7	196±66	125±4	57±16	83±17	48±5
CS 2h	2.6±0.1	6.2±1.6	31±14	117±4	100± 4	92± 7	43±3

Thus AVP release to H was potentiated 7-fold by naloxone and acutely injected corticosterone. Plasma AVP was related to the integrated blood pressure decrease (IPD) by: $AVP = AVP' \cdot \exp(b \cdot IPD)$. For the NALOX and CS 5' groups combined, r=0.67 and AVP'=15.6 pg/ml. For the control and CS 2h groups combined, r=0.59 and AVP'= 4.5 pg/ml. The b factor was not significantly different ($p>0.4$) for these groups, demonstrating a similar blood pressure dependence of AVP release. However, AVP' was significantly ($p<0.005$) larger for the combined NALOX and CS 5' groups. The slope of the linear regression curve was two times larger ($p<0.01$) for the combined NALOX and CS 5' groups (r=0.80) than for the control and CS 2h groups (r=0.62). Thus even by taking into account the blood pressure effects, naloxone and acutely injected corticosterone potentiate AVP release to H by 350%. The results suggest that endogenous opiates participate in short-term regulation of arterial blood pressure and AVP release. The similarity of action between naloxone and acutely injected corticosterone may indicate that corticosterone transiently inhibits the release and/or inhibitory effect of endogenous opiates on the hypothalamo-neurohypophysial system. The site(s) of action of endogenous opiates, perhaps at the level of the hypothalamus or hypothalamo-hypophysial exons, remains to be investigated.

238.4

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238.6 CONCENTRATIONS OF DOPAMINE IN PITUITARY STALK PLASMA AND OF PROLACTIN IN SYSTEMIC PLASMA IN RATS GIVEN 5-HYDROXYTRYPTAMINE. N. S. Pilotte and J. C. Porter. Dept. of Ob-Gyn and Physiology, Univ. of Texas Health Sci. Ctr., Dallas, TX 75235.

The release of prolactin (PRL) from the pituitary gland is enhanced after the administration of 5-hydroxytryptamine (5HT) to rats. We tested the hypothesis that 5HT augments the secretion of PRL by modifying the release of dopamine (DA) from the hypothalamus into hypophysial portal blood by measuring the concentration of DA in the pituitary stalk plasma of male rats after the intracerebroventricular administration of 5HT or its solvent vehicle. A cannula was implanted into the lateral ventricle of each rat 5-7 days before use. The animals were anesthetized with urethane, and pituitary stalk blood was collected for 1 hr after the injection of 0.5 μg or 5 μg 5HT creatinine sulfate (free base) in 10 μl phosphate-buffered saline (PBS) or 10 μl PBS. DA was measured in acidified extracts of plasma using a radioenzymatic procedure. The concentration of DA was 0.80 ± 0.18 ng/ml (mean ± SE), 0.32 ± 0.06 ng/ml, and 0.176 ± 0.04 ng/ml in the pituitary stalk plasma of rats given PBS, 0.5 μg 5HT, and 5 μg 5HT, respectively. The concentrations of PRL in another group of similarly treated animals did not change 10 min after injection of the solvent vehicle (26 ± 4 ng/ml), but increased at least 10-fold 10 min after the injection of 5 μg 5HT (from 25 ± 5 to 347 ± 19 ng/ml). The administration of 0.5 μg 5HT was followed by a 4-fold increase in the peripheral concentration of PRL (from 30 ± 4 to 169 ± 32 ng/ml). Two additional groups of male rats were used to test the hypothesis that the replacement of DA in the portal plasma would be sufficient to block the effect of serotonin on PRL release. Polyethylene cannulas were placed in the femoral artery and in the right atrium of the heart via the right jugular vein. A solution of DA in 5% glucose or 5% glucose alone was infused into the jugular cannula for 45 min. Blood withdrawn through the femoral cannula was replaced with heparinized 0.15 M NaCl at 15-min intervals beginning 15 min before the commencement of the infusion. Fifteen min after the initiation of the DA or glucose infusion, 5 μg 5HT was injected into the lateral ventricle of each rat. The concentration of plasma PRL was increased from 68 ± 15 ng/ml to 723 ± 149 ng/ml in rats infused with glucose and from 81 ± 20 ng/ml to 460 ± 77 ng/ml in rats infused with DA. The concentration of DA in the peripheral serum was 2.54 ± 0.29 ng/ml or 0.08 ± 0.03 ng/ml 15 min after the initiation of the DA or glucose infusions, respectively. These results are suggestive that 5HT modulates the secretion of PRL (1) through a direct action on the release of DA into the pituitary stalk blood and (2) through a separate mechanism that may involve a PRL releasing factor.

238.7 THE DISTRIBUTION OF LHRH FIBERS FOLLOWING SAGITTAL OR CORONAL KNIFE CUTS. J.C.King, C.W.Scouten, A.A.Gerall and F.L.Snavely*. Dept. of Anatomy, Tufts Univ.Sch. of Med., Boston, Ma 02111; Dept. of Psychol., MUN, St. John's Newfoundland, A1B3X9; Dept. of Psychol., Tulane Univ., New Orleans, La. 70118. Knife cuts were made in adult female rats in: 1)sagittal plane 1mm lateral to the midline from the preoptic area extending 2mm caudally; 2)coronal plane just caudal to the preoptic area ± 1mm across the midline; and 3)coronal plane rostral to the arcuate nucleus ± 1mm across the midline. Smears were taken to assess vaginal cyclicity following the knife cuts. Prior to perfusion with Bouin's fixative, females were ovariectomized and given 3 daily injections of 6.6ug estradiol benzoate i.m. Fifty micron vibratome sections were incubated with LHRH antisera, Arimura's #743, Jackson's #29 or antisera absorbed with LHRH and processed according to the PAP technique. The only group of animals demonstrating persistent vaginal cornification were those with a coronal cut caudal to the preoptic area (2); the other groups demonstrated some irregularities but were ovulating. In control animals, LHRH fibers extended in horizontal sections from the rostral preoptic area to the median eminence (ME) in both a direct midline path in the rostral tuberoinfundibular pathway and in close association with ventricular elements to the ME and a curvilinear pathway diverging from the midline rostrally to enter the region of the medial forebrain bundle converging caudally to enter the ME from a lateral position. Sagittal cuts did not affect the midline pathway and often only transected a small proportion of the diffuse laterally directed fibers thus having a minimal effect on the number of fibers reaching the ME. Coronal cuts at the level of the rostral arcuate nucleus did not disturb lateral fibers and often did not extend basally to disturb the majority of midline fibers. The only cut to induce persistent vaginal estrus, the coronal cut caudal to the preoptic area, disturbed both the lateral fibers as they were diverging from the midline and basal midline fibers. Although there were fibers remaining in the ME after this coronal cut, these were evidently not adequate to induce ovulation. This study was a replication of identical knife cuts in which the LHRH system was studied in paraffin sections with similar results. This work was supported by NIH grant HD 14092-01 to J.C.K.; HD00867-15 to A.A.G. and MH 28440 to C.W.M.

238.8 Gonadotropin-Releasing Hormone (GnRH): Distribution in Human Hypothalamic Nuclei. M.J. Kubek, J.F. Wilber*, and J.M. George*. Dept. of Anatomy, Indiana Univ. Sch. Med., Indianapolis, IN 46223; Depts. of Medicine, L.S.U. Med. Ctr., New Orleans, LA 70112; and Ohio State U. Sch. Med., Columbus, OH 43210.

It is well established that the hypothalamus occupies a central role in the regulation of gonadotropin secretion. Radioimmunoassay (RIA) studies of GnRH activity in human hypothalamic tissue blocks previously have revealed a rather generalized distribution of GnRH. However, precise localization of GnRH within specific human hypothalamic nuclei is needed in order to define neuroendocrine and potential non-endocrine roles of GnRH in man. To examine this problem, the hypothalamus and infundibular stalk of 8 human brains obtained at autopsy were dissected and frozen. Mean time between death and tissue processing was 8.96 hrs. Serial alternating 100 um and 300 um sections were cut from hypothalamic blocks in the frontal plane and serial "micropunch" samples were extirpated from sections with 1000 um diameter stainless steel canulae. Pooled samples were homogenized in 0.1N HCl and sonicated. Aliquots were taken for protein determinations, and samples were then lyophilized and eluted in neutral buffer for GnRH determinations by specific and sensitive RIA. GnRH immunoreactivity was quantitated in all structures examined. Highest concentrations (ng/mg protein ± SE) were localized to the infundibular stalk (0.878 ± .514) and median eminence area (0.343 ± .231) as well as the arcuate nucleus (0.222 ± .091). Somewhat lower levels were found in the supraoptic (0.145 ± .082) and paraventricular nuclei (0.115 ± .039). Lowest GnRH concentrations were observed in the mammillary complex (0.038 ± .022), posterior (0.010 ± .004) and anterior (0.008 ± .004) nuclei. Potential instability of GnRH in intact tissues were assessed in representative structures of the rat under conditions simulating human postmortem variables of time and temperature. GnRH activity in both hypothalamus and cortex was found to be stable for 16 hrs. at 4°C. Conclusions: GnRH is localized in man to those hypothalamic loci which have been implicated in gonadotropin regulation experimentally (stalk-median eminence, and arcuate nuclei). The variations in concentrations of GnRH found in each region cannot be attributed to postmortem autolysis. The presence of GnRH in additional hypothalamic areas (mammillary complex, posterior nuclei, supraoptic and paraventricular nuclei) suggests that GnRH may subserve other hypothalamic functions as well in the role of a peptide neurotransmitter or neuromodulator. (Supported in part by NIH Grants AM-106699 and AM-14997 and the V.A., Columbus, OH).

238.9 EFFECTS OF ARGININE VASOTOCIN AND VASOPRESSIN ON THE LUTEINIZING HORMONE RELEASING HORMONE-INDUCED RELEASE OF LUTEINIZING HORMONE IN THE RHESUS MONKEY. L. Y. Johnson, R. H. Asch, R. J. Reiter and M. K. Vaughan. Dept. of Anatomy, The Univ. of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

Arginine vasotocin (AVT), a nonapeptide, has been tentatively identified in the human fetal neurohypophysis and pineal gland. The ability of pretreatment with AVT or arginine vasopressin (AVP), a neurohypophyseal hormone similar in structure to AVT, to alter the hormonal response to a single injection of luteinizing hormone-releasing hormone (LRH) was investigated in the monkey.

Four adult rhesus monkeys (6-8 kg) which had been bilaterally salpingoophorectomized at least 9 months prior to the experiment were maintained in an LD 14:10 lighting cycle. At 0900h, monkeys received a 0.5 ml injection of either AVT (12µg), AVP (12µg) or vehicle (0.5% bovine serum albumin in Ringer's lactate solution). Blood was drawn immediately prior to drug administration (0m) and at 10 and 20m. At 60m, blood was sampled immediately prior to the iv injection of LRH (150µg) in 0.5 ml vehicle. Additional blood samples were obtained at 70, 80, 90, 120 and 180m. Blood samples were drawn from and drugs administered via the femoral or saphenous veins after sedation with ketamine hydrochloride (7-10 mg/kg, im); serum was assayed for rhesus luteinizing hormone (LH) by radioimmunoassay. Each monkey received all three treatments with studies being separated by at least 7 days.

In the controls, an injection of vehicle at 0m was followed by no significant alteration of LH levels for 60m; LRH administration at 60m resulted in significantly elevated LH titers at 70 (3248±252 ng/ml), 80 (2587±303 ng/ml), 90 (2332±370 ng/ml) and 120m (2218±406 ng/ml) relative to the value at 60m (1411±202 ng/ml) ($p<0.001$, $p<0.01$, $p<0.02$ and $p<0.05$, respectively).

Serum LH values after AVT or AVP injection did not vary significantly from those in control studies at 0, 10, 20 or 60m. However, peptide administration did alter the serum LH response to LRH treatment. LH titers were significantly lower after AVT treatment relative to levels resulting from vehicle injection at 70m (1768±116 ng/ml vs. 3248±252 ng/ml, $p<0.01$); after AVP injection serum levels were significantly lower than those in control studies at 70 (1246±241 ng/ml vs. 3248±252 ng/ml, $p<0.001$) and 80m (1450±231 ng/ml vs. 2587±303 ng/ml, $p<0.05$). After AVT or AVP treatment, LRH injection produced no significant rise in serum LH relative to the preinjection 60m value.

The neuropeptides AVT and AVP appear to have the ability to alter the action of LRH at the level of the anterior pituitary and could be possible physiological modulators of LH release in the monkey. (Supported by NIH Biomedical Research Support Grant RR 05654, NSF Grant PCM 77-05734 and NIH Grant 5P30 HD10202.)

238.10 EFFECTS OF RAPHE NUCLEI LESIONS ON PROLACTIN SECRETION IN MALE RATS. D. Eljarmak*, G. Charpenet*, J.C. Jéquier* and R. Collu. Neuroendocrine Research Lab., Pediat. Res. Ctr., Ste-Justine Hosp. and Univ. of Montreal, Montreal, Que. H3T 1C5.

Brain serotonin (SER) appears to play a major role in the control of prolactin (PRL) secretion at least in rats. PRL release induced by stress, pentobarbital (PB) anesthesia and the opiate peptide ß-endorphin (END) has been reported to be regulated, at least partly, by serotonergic neuronal pathways. Major serotonergic inputs to mammalian forebrain originate from cell bodies located in dorsal and medial raphe nuclei of the mesencephalon. Several experiments were performed in adult (240-260 g), male Sprague-Dawley rats in order to verify whether either one or both raphe nuclei might be involved in basal, stress-, PB- or END-induced PRL release. In addition, since thyrotropin-releasing hormone (TRH) has been found to antagonize PB- and facilitate END-induced PRL release (Taché et al., Eur. J. Pharmacol. 45: 369, 1977; Taché et al., Life Sci. 21: 841, 1977) through a central monoaminergic mechanism, the role of raphe nuclei in these extrapituitary effects of TRH has been explored. For this purpose, radiofrequency lesions were made with a stainless steel electrode either in the dorsal or medial raphe nuclei under PB anesthesia. At the same time the rats were implanted with a chronic cannula in the right lateral brain ventricle, and with a permanent silastic catheter in the right atrium for blood sampling. Sham lesions were performed in some rats by simply lowering the electrode into the mesencephalon. Groups of rats either sham operated (S), lesioned in the dorsal (LD) or the medial (LM) raphe nuclei were either sampled every 15 min for 3h30 min without anesthesia, for determination of baseline plasma PRL values by radioimmunoassay, or submitted to various treatments: immobilization stress for 30 min; PB (50 mg/kg, ip) and saline (10 µl/rat, intraventricularly), PB and TRH (10 µg/rat, ivt); END (10 µg/rat, ivt) END and TRH. Blood was collected every 15 min for 120 min for PRL determination. At the end of the experiment the animals were killed by decapitation and the brains examined for localization and extent of the lesion. Data were statistically evaluated by factorial analysis of variance. The results obtained indicate that: 1) baseline values of PRL are not influenced by raphe nuclei lesions; 2) the rise of PRL values induced by immobilization stress is suppressed in LM rats; 3) the PB-induced PRL secretion is enhanced in LD rats, blocked by TRH in S but not in LD or LM rats; 4) the END-induced PRL secretion is greatly enhanced in LD rats, potentiated by TRH in S and ever more in LD but not in LM rats. These results indicate that raphe nuclei may intervene in induced PRL secretion either in a facilitatory (medial) or inhibitory (dorsal) role.

238.11 IN VIVO AUTOREGULATION OF RAT ADENOHYPOPHYSEAL THYROTROPIN-RELEASING HORMONE-RECEPTOR. A. Banerji* and C. Prasad. Section of Endocrinology, Department of Medicine, LSU Medical Center, New Orleans, LA. 70112.

Autoregulation of membrane receptor concentration appears to be one mechanism of target cell desensitization. To see if the adenohypophyseal thyrotropin-releasing hormone (TRH)-receptor undergoes autoregulation in vivo, we have studied the down-regulation of TRH-receptor in this tissue by TRH. Rats (male Sprague-Dawley, 200-300g) were treated subcutaneously (s/c) with 100 µg TRH or saline (twice daily) for 0 to 4 days. TRH-receptor concentration was determined as described elsewhere (Endocrinology 100: 1496, 1977) except that the homogenization buffer also contained 250 mM sucrose. TRH treatment led to a time dependent decrease in the TRH-receptor number (50 and 80% decrease at day 1 and 3 respectively). This decrease was not due to the receptor occupancy because the half-life for dissociation of TRH binding to pituitary membranes is < 10 minutes while the decrease in the receptor number persisted for at least one week after termination of TRH treatment. Scatchard analysis using ligand concentration of 4×10^{-8}M to 2×10^{-7}M showed a significant decrease in both the equilibrium dissociation constant (K_D) and the maximum binding capacity (B_{max}) following TRH treatment for 3 days (see table below).

Treatment	K_D (nM)	B_{max} (fmole/mg protein)
Saline	26.5	60.2
TRH	10.0	15.2

Since TRH treatment increases both thyrotropin (TSH) and triiodothyronine (T_3), we have studied the possible role of these hormones in the modulation of TRH-receptor in vivo. In euthyroid animals neither TSH nor T_3 treatments (500 µg/day for 5 days, s/c) had any effect on the TRH-receptor number (fmole TRH bound/mg protein, control = 51.2, TSH-treated = 51.3, and T_3-treated = 55.0). In thyroidectomized rats, like euthyroid, TRH was effective in reducing its receptor number.

These data show that TRH regulates the number of its own receptor in vivo. This regulatory role of TRH is not associated with its hypophysiotropic action and therefore independent of TSH and T_3 release.

238.12 REDUCTION IN CELL FIRING INDUCED BY OPIOIDS IN HYPOTHALAMIC SLICES. M. Mühlethaler* and J.J. Dreifuss. Dept. Physiol., Univ. of Geneva Med. Sch., Geneva, Switzerland.

Endogenous opiates have been implicated in a growing number of nervous mechanisms, including the control of pituitary hormone secretion. High amounts of opiate receptors and of enkephalin are found in the basal hypothalamus, including the paraventricular nucleus (PVN). In order to ascertain whether PVN neurones possess opiate receptors and could therefore respond to locally released enkephalins, we have bath-applied stable Met-enkephalin analogues while recording the firing of PVN neurones in coronal slices prepared from the hypothalamus of adult rats.

The two analogues FK 33-824 and FW 34-569 were tested on 26 PVN neurones in 23 slices. The cells displayed stable patterns of spontaneous activity with frequencies ranging from 1-20 Hz. Enkephalins inhibited the firing of 19 cells; 7 were unaffected, although they could be excited by glutamate (5.10^{-4} M) and were inhibited by an increase in external Mg concentration. The enkephalin effect was rapid in onset and reversible. No difference was noted when assessing the effect on phasic, beating and random cells or when comparing the two analogues. Concentrations of 10^{-8} M were usually effective and a 50% inhibition was seen at about 5.10^{-7} M. In 6 cells the enkephalin effect was tested for reversibility by the opiate antagonist, naloxone. In 4, the reduction in cell firing induced by 10^{-7} M enkephalin was completely reversed by 10^{-6} M naloxone. In one cell, the antagonism was only partial and in another, naloxone had no effect. Morphine, 10^{-6} to 10^{-4} M, was tried on 12 cells; seven were reversibly inhibited.

It has been recently shown that vasopressin and oxytocin release are reduced by exogenous opiates acting on the neurohypophysial secretory axons. Our results suggest that, in addition, opiates could also regulate neurohypophysial hormone release by an effect exerted on or near the hypothalamic perikarya.
(Supported by Swiss NSF grant 3.469.79).

239.1 ANIMAL ACTIVITY MONITOR FOR CHRONIC DRUG STUDIES. K. A. Stauderman,* D. M. Stoff and R. J. Wyatt (SPON: F. Vargas). Laboratory of Clinical Psychopharmacology, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Animal models of human movement and psychiatric disorders are based almost exclusively on changes caused by CNS stimulants on stereotyped and locomotor behaviors. However, the conditions and techniques of measurement to study motor behavior impose certain limitations: (1) preclude continuous and simultaneous measurement of different motor responses under chronic conditions, (2) relatively insensitive to progressive changes in spontaneous behavior patterns of normal and drug-treated animals, and (3) require separate observational methods and intrusive instruments to distinguish differences in response topography among CNS stimulants acting through different mechanisms.

We have developed a new computerized technique (using capacitance sensing fields) for the longitudinal measurement of motor activity in the animal's laboratory/residential environment. At a very high resolution rate and wide range of sensitivities this technique is able to continuously monitor rat behavior during chronic drug infusion. It tracks the path of movement of the animal or sets threshold limits to detect specific behavioral events (e.g., rearing, circling, gross movements) in both temporal and spatial dimensions through the use of computer programs for behavioral algorithms. This technique monitors behavior patterns around the clock for weeks or months, and traces the locomotor history of the rat by quantifying the various responses and ultradian and circadian changes in activity.

In this paper we (1) describe the technique, (2) present data on entrained and free-running activity cycles in normal rats (Fig. 1) and with chronic treatment of haloperidol or amphetamine, and (3) demonstrate different response profiles for indirect and direct dopamine agonists, amphetamine and apomorphine.

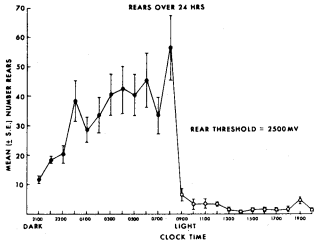
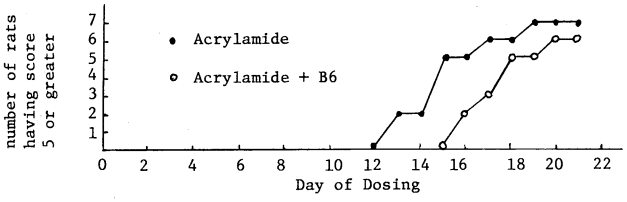


FIG. 1

239.2 VITAMIN B6 PROTECTION AGAINST ACRYLAMIDE NEUROTOXICITY. A.L. Loeb* and R.J. Anderson. Dept. Pharmacol., George Washington Univ., Washington, D.C. 20037.

The effect of vitamin B6 supplementation on the development and severity of chronic acrylamide neurotoxicity in rats was examined. Thirty rats divided into 4 groups were dosed daily for 21 days IP with: (1) saline (n=8); (2) B6 (5 mg) (n=8); (3) acrylamide (30 mg/kg) (n=7); and (4) acrylamide (30 mg/kg) + B6 (5 mg) (n=7). Development and severity of neurotoxicity were quantified by: (1) performance on an inclined screen using a quantal 7-point scoring system and (2) measurement of hind limb footspread upon landing from a 30 cm height. Development of motor dysfunction on the inclined screen was delayed and less severe in rats receiving acrylamide + B6 compared to those receiving acrylamide alone. Acrylamide only treated animals first showed toxicity on day 9 (score = 3) and severe toxicity (score = 5) on day 13, compared with day 11 and day 16 respectively in the acrylamide + B6 group. The figure below shows the number of rats with a score of 5 or greater as a function of time with chronic dosing. At the end of the study, all the acrylamide only treated animals, whereas 4 of the 7 rats in the acrylamide + B6 group, had scores of 6 or 7. Footspread was increased over controls in both acrylamide only and acrylamide + B6 groups by day 11 ($p < 0.05$). B6 did not affect the degree of footspread increase between the acrylamide groups. Weight loss was seen in both groups receiving acrylamide 48 hours after the first dose and continued to decrease. Water intake did not decrease until after day 12 and thus probably was not responsible for the early weight loss. The saline and B6 only groups gained weight over the entire experimental period.

In conclusion, B6 was shown to delay the onset and severity of acrylamide neurotoxicity in rats as measured by the inclined screen test. This suggests that acrylamide toxicity may be caused by an interaction of acrylamide with systems utilizing B6 as a cofactor or that acrylamide may be depleting nutritional factors, which if partly replaced by B6 treatment, slow the progression of neurotoxicity. (Supported by NIH grant #ES01951.)



239.3 EFFECTS OF ACUTE LITHIUM ADMINISTRATION ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT. S. Suda*, M. Miyaoka*, C. Kennedy, and L. Sokoloff. Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

Lithium salts have been reported to alter carbohydrate metabolism of brain in vitro, in particular to stimulate glucose utilization (Mickel et al., Biochem. Pharmacol. 27:799, 1977). The close coupling of energy metabolism and functional activity in brain suggested the possibility that such stimulation might be related to the mechanism of lithium's pharmacologic actions. To examine this question we have employed the [^{14}C]deoxyglucose method to measure local rates of cerebral glucose utilization in vivo. Normal male adult Sprague-Dawley rats were injected with 7 mmoles/kg of lithium chloride intraperitoneally. Three, 14 and 22 hours later, [^{14}C]deoxyglucose was administered as a pulse intravenously and followed by the procedure for measuring local cerebral glucose utilization as previously described. Local cerebral glucose was measured by the [^{14}C]deoxyglucose method in four animals at each of these post-injection times, and the values were compared with those obtained in an equal number of control animals which received 7 mmoles/kg of sodium chloride intraperitoneally. At 3 hours lithium was found to induce sluggish responses to stimuli, a reduction in spontaneous activity, and a moderate metabolic acidosis (arterial pH, 7.30). In these animals there was a widespread reduction in rates of cerebral glucose utilization which ranged from 21% to 37% in 18 out of 35 structures. A weighted average rate for the brain as a whole was 21% below that of control animals ($p < 0.05$). In animals in which measurements were made at 14 and 22 hours after lithium administration no behavioral depression was observed, nor was there evidence of acidosis (arterial pH 7.36 and 7.39 respectively). Although six structures in both groups of animals exhibited rates slightly but statistically significantly below those of control animals, the weighted averages for the brain as a whole were unchanged from controls. Previous studies have shown that lithium reaches its maximal concentration in brain about 22 hours following intravenous administration. In our studies at 22 hours after intraperitoneal injection no structure exhibited an augmented rate of glucose utilization. Thus, our results are in contrast to in vitro observations. The marked reduction in cerebral glucose utilization three hours after lithium administration occurred in the presence of a metabolic acidosis and is in keeping with similar reductions in local cerebral metabolic rates found in our laboratory during acidosis induced with ammonium chloride. The depressant action of lithium following acute administration, therefore, appears to be non-specific.

239.4 **ACTION OF LITHIUM CHLORIDE ON MOTOR COORDINATION IN THE MURICIDAL RAT.** **Patricia A. Broderick, O.P.† and Vincent de Paul Lynch*. St. John's Univ., Jamaica, N.Y. 11439.**

The history of lithium usage in psychiatry is well established (Schou, 1959). Yet, the role of motor effects in delineating the behavioral actions of lithium are not conclusive. We found a significant increase in the motor ability of isolated, male, Long Evans, muricidal (mouse killing) rats (300-400gms) as tested by standard rotarod apparatus. These effects were observed after acute intraperitoneal administration of lithium chloride, 1.0, 2.0 and 4.0 meq/kg, and after intraperitoneal lithium chloride administration, 0.5, 1.0 and 2.0 meq/kg††, for seven days, daily. Significance levels, which were determined statistically by paired student t test, were p<0. 05 and above. Corresponding plasma lithium was quantitated (Perkin Elmer, 360). Both rotarod testing and plasma lithium determinations were done one hour after acute injection. During chronic studies, testing was carried out 24 hours after final injection. Other lithium dosage regimens, both acute and chronic, did not significantly increase motor coordination. These results are of interest because these isolated, muricidal "aberrant" rats are hyperactive and have notable prehensile strength. These features are dampened and motor coordination is enhanced by lithium chloride. Lithium modified locomotor response to methamphetamine (Furukawa et al., 1975); decreased locomotor activity in hyperthyroid rats (Rastogi and Singhal, 1977) and decreased locomotor activity in albino mice (unpublished observation). Further research will elucidate whether this increase in motor ability is dependent on physiological or environmental conditions. It will also clarify strain, intrastrain or species differences. Muricidal behavior is used as a pragmatic model for the identification of antidepressant drugs (Horovitz et al., 1966). Perhaps, an aberrant animal model might be more applicable to clinical situations. Perhaps, too, such a model might be more applicable to the relationship between motor effects and behavior in the clinic.

††twice a day

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239.5 CHLORPROMAZINE AND BRAIN-STIMULATION REWARD: POTENTIATION OF EFFECTS BY NALOXONE. Ralph U. Esposito, William Perry* and Conan Kornetsky (SPON: J.H. Mendelson). Laboratory of Behavioral Pharmacology, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118.

Rats were implanted with electrodes aimed at either the medial forebrain bundle or the ventral tegmentum, and intracranial self-stimulation thresholds were determined by means of a modification of the classical psychophysical method of limits. Chlorpromazine (CPZ) (.25-2.0 mg/kg, i.p.) produced dose-dependent threshold increases, while naloxone (NAX) (4 mg/kg, i.p.) was devoid of effect. However, NAX administered concurrently with CPZ produced substantial potentiation of the threshold increases. In addition, behavioral "seizures" (i.e., body tremors, twitching, head bobbing and rhythmic exophthalmos, gnawing and chewing) were observed when NAX was combined with the higher doses of CPZ. These results are discussed within the context of a catecholamine-enkephalinergic involvement in the regulation of central reward processes.
(Supported by NIMH Grant MH 12568, NIDA Grant DA 02326 and Research Scientist Awardee MH 1759 - CK).

239.6 INTRACRANIAL SELF-ADMINISTRATION OF ACETALDEHYDE BY SPRAGUE-DAWLEY RATS. B.R. Smith* (SPON: Z.W. Brown). Center for Research on Drug Dependence, Concordia University, Montreal, Quebec, Canada H3G 1M8.

It has been demonstrated that rats will self-administer acetaldehyde directly into the brain and it was suggested that acetaldehyde may mediate some of the psychopharmacological effects of ethanol. However, this finding was reported only in Wistar rats and it may be specific to the strain used. The present paper reports on preliminary data examining the rate of self-administration of acetaldehyde in Sprague-Dawley rats. Naive rats were placed in operant chambers for 10 consecutive days during which they could lever press for acetaldehyde (.5% v/v) or Ringer's solution. These compounds were delivered directly into the left lateral ventricle via a chronically implanted cannula. Analysis of the data revealed that from day 4, animals receiving acetaldehyde lever pressed at higher rates than control animals receiving Ringer's solution. It is interesting to note that in previous studies the lowest dose which would be self-administered by Wistars was 1% v/v, yet, in this experiment Sprague-Dawleys self-administered at half this dose. This resembles the differences in each strain's preference for ethanol as Wistars have been shown to drink higher quantities of ethanol than Sprague-Dawleys. This data may support other research which suggests that acetaldehyde may be involved in ethanol consumption in rats.

239.7 THE EFFECTS OF ENVIRONMENTAL TEMPERATURE AND ATMOSPHERIC PRESSURE ON ETHANOL NARCOSIS IN MICE. R.L. Alkana and R.D. Malcolm. School of Pharmacy, University of Southern California, Los Angeles, CA. 90033

Recent studies show that hyperbaric helium-oxygen (He-Ox) environments from 4-12 atmospheres absolute (ATA) antagonize ethanol narcosis (3.2-4.0 g/kg) in a pressure and dose related manner. Experiments using hyperbaric chambers heated to 30°C demonstrated that the antagonism from hyperbaric He-Ox was not due to the hypothermic effects of helium. Interestingly, the 1 ATA air control mice slept significantly longer in the 30°C environment than controls kept at room temperature (21-25°C). Two experiments are presented here which further investigated the relationship between ethanol sleep-time, environmental temperature and atmospheric pressure. The first experiment tested the effect of ethanol and hyperbaric He-Ox on sleep-time, wake-up blood ethanol (BEC) and brain ethanol (BrEC) concentrations under temperature conditions of 33.5°C (air) and 34.5°C (He-Ox) which eliminated the hypothermic effects of ethanol and helium. Drug-naive, male C57 mice were injected i.p. with 3.6 g/kg ethanol (20%w/v). Upon loss of their righting reflex, mice were placed in a hyperbaric chamber. Atmospheric pressure was taken to 1 ATA air or to 1,6, 8,10 or 12 ATA He-Ox. During He-Ox treatments, the chambers were kept at 34.5°C by immersion in a water bath. Air controls were kept at room temperature or 33.5°C. Rectal temperatures were monitored in separate groups of animals exposed to the same treatments and conditions. Upon wake-up, the chamber was rapidly decompressed. Blood and brain samples were taken for later gas chromatographic ethanol analysis. Hyperbaric He-Ox significantly reduced sleep-time and increased wake-up BECs and BrECs at 8,10 and 12 ATA in non-hypothermic mice (t-test). Sleep-time was significantly increased and wake-up BrEcs decreased in non-hypothermic versus hypothermic 1 ATA air controls. The second experiment investigated the effect of a range of temperature environments on rectal temperature, sleep-time and wake-up BECs and BrECs in 1 ATA air. Mice were injected with 3.6 g/kg ethanol and placed on sleep racks at room temperature (22-26°C) or at 12,14,16,30,34 or 37°C. The results suggest that sleep-time and wake-up BrECs vary in a statistically significant, temperature related manner (supported in part by NIAAA Grant AA-03972, NIH BRSG RR-05792 and the USC Pharmacy Alumni Association).

239.8 Effects of Lisuride on the behavior of cats: Implications for the limb flick model of drug-induced hallucinogenesis. James L. Marini*, Michael H. Sheard, Barry L. Jacobs and Michael E. Trulson, Dept. of Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508 and Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

Lisuride is a non-hallucinogenic ergoline derivative which is structurally similar to d-lysergic acid diethylamide (LSD), and, like LSD, reduces brain serotonin turnover (e.g., Nature 272: 278-280, 1978) and has a powerful depressant action on 5HT-containing raphe neurons (Life Sci. 24: 1289-1298, 1979). Indeed, lisuride is 5-10 times as potent as LSD in depressing raphe neuronal activity. Given the potency of lisuride on the 5HT system, we tested its behavioral effects in cats. Previous studies showed that hallucinogenic drugs which depress the central 5HT system elicit specific behaviors such as limb flicking and abortive grooming in cats (Comm. Psychopharm. 1: 243-253, 1977). Adult male and female cats were given saline or lisuride hydrogen maleate (6.25, 12.5, 25, 50 and 100 ug/kg, ip) and then observed for one hr by a rater blind to dose. Lisuride produced a dose-dependent increase in the rate of limb flicking, from a near-zero saline baseline to a maximum of 16/hr at a dose of 50 ug/kg. The limb flick rate was less (9/hr) at the 100 ug/kg dose. Abortive grooming showed a similar response: From a saline baseline of 0, there was a dose-dependent increase to a maximum of 7/hr at 50 ug/kg, and a decrease to 6/hr at 100 ug/kg. Grooming was also similar: From a saline baseline of 17/hr, there was a dose-dependent increase to a maximum of 47/hr at 50 ug/kg, and a decrease to 34/hr at the 100 ug/kg dose. A time-course study with the most effective dose (50 ug/kg) revealed that the frequencies of the behaviors reached a maximum during the first 2 hrs post-injection, declined rapidly, and were comparable to saline frequencies by 6 hrs post-injection. To determine whether tolerance develops to its repeated administration, lisuride (50 ug/kg) was administered to cats, followed by a test dose of 50 ug/kg at 6, 24 or 72 hrs after the initial dose. These studies revealed no significant tolerance effect. Finally, to test for cross tolerance with LSD, additional cats received an initial dose of 50 ug/kg of either LSD or lisuride, followed by a 50 ug/kg dose of lisuride or LSD 24 hrs later. These studies revealed no significant cross tolerance. The present data question the validity of the limb flick model for hallucinogenesis, since lisuride is very effective in eliciting limb flicks, but is not hallucinogenic in man. The model can be revised to include the condition that LSD-like tolerance must develop to repeated drug administration, since all hallucinogens tested produce a dramatic tolerance effect on limb flicks, while lisuride does not.

239.9 RX 336-M AND ACTH (1-24): STIMULANTS OF GROOMING AND SHAKING IN RATS. D.E. Gmerek* and A. Cowan (SPON: J.J. O'Neill). Dept. of Pharmacology, Temple Univ. Sch. of Med., Philadelphia, PA 19140.

RX 336-M (7,8-dihydro-5',6'-dimethylcyclohex-5'-eno-1',2', 8',14 codeinone) induces a remarkable, dose-related behavioral syndrome of excessive grooming and 'wet-dog' shakes (WDS) when administered peripherally (1.5-12 mg/kg), but not centrally, to rats. In the present work, we have studied the pharmacological bases of the excessive grooming induced by both RX 336-M and ACTH (1-24), the reference compound that causes excessive grooming when given icv to rats (Gispen et al, Life Sci. 17:645, 1975)[b]. Note that, in our hands, ACTH (1-24) (3-12 µg, icv) elicits WDS in male Sprague-Dawley rats (200-220 g) in addition to the excessive grooming.

We used groups of 8 male, S.D. albino rats (120-140 g); during test sessions they were housed individually in Plexiglas observation boxes (22 cm long; 18 cm wide; 25 cm high). The standard doses of RX 336-M and ACTH (1-24) were 6 mg/kg, i.p. and 3 µg (in 3 µl) icv, respectively. Grooming was monitored for 5 sec every 15 sec over 30 min with the help of a computer. Scoring started immediately after the injection of RX 336-M and 15 min after the administration of ACTH (1-24).

When rats were pretreated (at -15 min) with either haloperidol (0.25-1 mg/kg, s.c.) or morphine (0.3-3 mg/kg, s.c. or 1-10 µg, icv), the excessive grooming associated with both RX 336-M and ACTH (1-24) was attenuated in a dose-related manner. Naloxone (1-10 mg/kg, s.c. at -15 min) had no marked influence on RX 336-M - induced grooming. Interestingly, Gispen and Wiegent (Neurosci. Lett. 2: 159, 1976)[a] have reported that naloxone (1 mg/kg, s.c.) attenuates the excessive grooming elicited by ACTH (1-24).

Tolerance developed to excessive grooming after twice-daily injections (at 0900 h and 1800 h) of RX 336-M (6 mg/kg, i.p.) for 7 days. There was no cross-tolerance to ACTH (1-24) in the RX 336-M - tolerant rats.

Comparative data for RX 336-M and ACTH (1-24) are summarized in the Table.

	RX 336-M	ACTH (1-24)
Active route	peripheral	central
'Wet-dog' shakes	+	+
Excessive grooming	+	+
Antag. by haloperidol	+	+
Antag. by morphine	+	+
Antag. by naloxone	-	+ (a)
Antag. by adrenalectomy	-	- (b)
Tolerance	+	+ (?) (c)
Cross-tolerance		-

(c) Jolles et al, Neurosci. Lett. 9: 261, 78

Excessive grooming may be a way whereby a rat's state of arousal [raised by RX 336-M and ACTH (1-24)] is lowered and homeostasis is maintained. If so, then there are at least two pathways that can be triggered to cause the phenomenon of excessive grooming.

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239.10 EFFECTS OF APOMORPHINE ON SELF STIMULATION (SS) RESPONDING: DOES THE DRUG MIMIC THE CURRENT? Nancy J. Leith. Dept. Pharmacol., Vanderbilt Med. Sch., Nashville, TN 37232

A substantial body of data implicates DA as important in mediating (SS) responding (see Wise, Brain Res., 1978, p. 215; Fibiger, Ann. Rev. Pharmacol. Toxicol., 1978, p. 37 for reviews). However, when apomorphine, a direct DA receptor agonist, is administered to SS animals, there is sometimes an increase and sometimes a decrease in responding reported (Wauquier & Niemegers, Psychopharm., 1973, p. 163; Broekkamp & Van Rossum, Psychopharm., 1974, p. 71), presumably because the direct receptor activation disrupts the contingency relationship between the animal's behavior and the reward produced. The present study evaluated the effects of the drug at a variety of current intensities since it was postulated that at threshold current values, the addition of low doses of the drug should produce receptor activation normally associated with suprathreshold intensities and consequently be reflected as increased responding. Animals with electrodes implanted in the MFB were repeatedly tested at 15 current intensities throughout a 30 min session. During the 1st 15 minutes, baseline responding was assessed. Then various doses of apomorphine (0.02 - 0.2 mg/kg) were injected and 5 minutes later testing was resumed for an additional 15 min. At the lowest dose, there was a significant elevation of the reward threshold in all animals presumably reflecting the stimulation of presynaptic receptors which predominates at this low dose and results in an inhibition of the firing of the DA neuron. As the dose was increased the effect shifted to a flattening of the response rate-current intensity function. That is responding was decreased at the high intensities and increased at the low, as though the animal was responding independent of the current. However, if the current was reduced to zero, the animals showed a typical extinction pattern of responding. If, on the other hand, there was current available at the first step of the 15 step sequence, the animal would respond and then persist throughout the remaining 14 steps without current. Thus, these data clearly indicate an interaction of apomorphine with the stimulating current and suggest that apomorphine may be mimicking only one aspect of the stimulation--perhaps the rewarding but not the priming or motivating.

239.11 PLASMA LEVELS OF TRICYCLIC ANTIDEPRESSANTS IN PANIC DISORDER. D.R. Sweeney, M.S. Gold, A.L.C. Pottash and D. Martin*. (SPON: Carl Boast) CIBA-GEIGY Pharmaceutical Corp., Summit, NJ 07901 Research Facilities, Fair Oaks Hospital, Summit, NJ 07901.

Spontaneous panic attacks associated with agoraphobia have been noted to respond to treatment with antidepressants such as imipramine or phenelzine. Some individuals with panic disorder respond to doses of tricyclic antidepressants that are much smaller than the typical doses employed to treat depressive disorders. Either of these patients achieve relatively high plasma levels of tricyclic antidepressants (TCA) despite the small doses employed or the plasma levels achieved correspond to the low dosages, thereby implying that the neurochemical mechanisms of response are different in these patients with panic disorder than those with depressive disorder.

Five female patients with the DSM III diagnosis of agoraphobia with panic attacks were studied. After three weeks on a fixed low dose of imipramine (10-30 mg/day) all patients had experienced a significant alleviation and the frequency and intensity of panic attacks. Plasma levels of imipramine + desipramine were assayed at 800 h on $\geq$ weekly basis by the HPLC method of Martin (Neurosci. Abstr. 5:409, 1979). All patients demonstrated a significant decrease from pretreatment behavior and self-ratings at $\geq$ 21 days. At the time of clinical response all of the plasma TCA levels corresponded to the drug dosages, i.e., they were significantly lower than those defining the typical therapeutic range for depressed patients.

This finding, along with the finding that patients with panic disorder tend to have a positive response to imipramine within the first several days, implies that the psychobiological mechanism underlying panic disorder are different than those underlying depression. A possible mechanism which may explain this difference is a TCA-induced decrease in noradrenergic locus coeruleus activity by stimulation of inhibitory autoreceptors or another mechanism. This noradrenergic inhibition hypothesis for the therapeutic efficacy of TCA in panic states is also supported by the efficacy of clonidine in naturally-occurring and spontaneous panic (Gold, et. al, Biomedicine 30:1-4, 1979), the phenomenological similarities between opiate withdrawal and panic states, and other human and primate data. A noradrenergic hyperactivity hypothesis for panic will be discussed.

240.1 AUTORADIOGRAPHIC LOCALIZATION OF SITES OF OCTOPAMINE SYNTHESIS IN LIMULUS VENTRAL EYE. B-A Battelle* and S.C. Chamberlain* (SPON: H.H. Hess) LVR, National Eye Institute, NIH, Bethesda, MD and Institute for Sensory Research, Syracuse University, Syracuse,NY.

Ventral eyes of Limulus polyphemus appear to contain two neuronal elements: photoreceptor cells with their axons and small fibers, believed to be efferents, which contain dense-core granules and ramify extensively to surround photoreceptor cell bodies. Biochemical studies have shown that the biogenic amine octopamine is specifically associated with a photoreceptor cell body-rich fraction (P-fraction) of ventral eyes. P-fractions contain an average 2.9±0.55 times more endogenous octopamine and synthesize 7.4±1.6 times more octopamine from tyramine than nerve-rich fractions. Autoradiographic studies have been carried out to determine whether it is the photoreceptor cells themselves or the surrounding efferents which synthesize and accumulate octopamine.

Ventral eyes were incubated overnight *in vitro* in saline containing a low concentration of ^{3}H-tyramine (6×10^{-8}M), then fixed, embedded in plastic and cut in 2μm sections. The ^{3}H-octopamine present in ventral eyes incubated under these conditions accounted for 37% of radioactivity in the preparation as a whole and 58% of radioactivity in the P-fraction. Autoradiographic analysis showed silver grains concentrated over a few small (1-2 μm) fibers in the optic nerve, in punctate clusters located close around the periphery of photoreceptor cell bodies and occasionally within glial folds that penetrate photoreceptor cell bodies. This pattern of labeling is reminiscent of the distribution of efferent fibers in the ventral eyes. Only background levels of silver grains were present over photoreceptor cell bodies and the large photoreceptor cell axons. On the basis of these results we suggest that octopamine in Limulus ventral eyes is associated with efferent fibers and not with the photoreceptor cells themselves. Attempts to verify this hypothesis at the ultrastructural level are in progress. We are also using the autoradiographic localization of sites of octopamine synthesis and accumulation to search for the cell bodies of efferent fibers within the Limulus protocerebrum.

240.2 ERGOT ALKALOIDS ANTAGONIZE DOPAMINE INHIBITION OF BURST-FIRING IN CELL R 15 OF APLYSIA CALIFORNICA. Sidney M. Gospe, Jr., and Wilkie A. Wilson, Jr., Department of Pharmacology, Duke University Medical Center, and Epilepsy Center, V.A. Medical Center, Durham, NC 27710.

Dopamine (DA) inhibits burst-firing in cell R 15 by reducing inward currents associated with bursting. This effect can be seen as a reduction of the region of negative slope conductance (NSC) in the current-voltage (I-V) relationship curve generated under voltage clamp conditions. Using bath application of DA (50-500 μM) we have established a dose-response relationship for this process. We have previously reported that several phenethylamines (e.g. (-) NE, (+) NE, epinine, (-) phenylephrine) have full agonist activity in the same concentration range as DA. The effects of these agonists are not blocked by neuroleptics and adrenergic blocking agents.

Ergot alkaloids do, however, antagonize DA inhibition of burst-firing. Ergotamine, methysergide, LSD, dihydroergotamine, and dihydroergocryptine antagonize DA action in a noncompetitive manner. The DA dose-response curve is shifted to the right and the maximum effect is reduced. This antagonism develops slowly, and the response to a test dose of DA may continue to decrease even after four hours of exposure to the ergot.

Ergonovine and methylergonovine antagonize DA in a different manner. These ergot drugs are partial agonists, and over the course of a three hour exposure their agonist effect decays. Upon initial application, these drugs reduce the region of NSC in the I-V curve, but the maximum effect produced by these ergots is less than the maximum DA effect. As the length of exposure to the ergot is increased, its partial agonist effect decays and antagonism to DA develops.

These data are supported by a previous study showing that ergonovine and methylergonovine are specific antagonists for inhibitory DA receptors on molluscan neurons. In certain cells of Aplysia (Ascher, J. Physiol. 225:173, 1972) a direct membrane hyperpolarization has been observed to accompany this DA antagonism. Ergonovine and methylergonovine may be partial agonists at these neurons as well as at R 15.

240.3 THE ACTION OF PROCTOLIN, A PEPTIDE, ON A LOBSTER MUSCLE. T. L. Schwarz*, R. Harris-Warrick, S. Glusman, M. Goy*, and E. A. Kravitz. Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115.

Proctolin, a neuropeptide first isolated from insects (Brown & Starratt, 1975), causes sustained contractions of the opener muscle of the dactyl of the lobster (Homarus americanus) walking leg at concentrations as low as 10^{-10}M. Higher doses (5×10^{-8}M) cause larger contractures that persist for up to an hour after the peptide is washed from the bath. Proctolin appears to act directly on the muscle. Intracellular recordings were made in individual muscle fibers while stimulating the single excitatory and inhibitory axons that innervate the muscle. No presynaptic action of proctolin was found; excitatory and inhibitory junctional potential sizes and the frequency of spontaneous miniature excitatory junctional potentials are unaffected by proctolin.

We have begun to study the mechanism of proctolin action on the muscle and have found that extracellular Ca^{++} and the level of the membrane potential are both important. In a Ca^{++}-free medium, proctolin fails to evoke a response and Ca^{++} blockers (20mM Co^{++} or 5mM Mn^{++}) will prevent a contracture. The peptide elicits the contracture without significantly depolarizing the muscle; no more than a 1mV depolarization is seen, at concentrations of proctolin up to 10^{-7}M. The proctolin-induced contracture, however, can be abolished by hyperpolarizing preparations with low K^{+} (12mM) or GABA (10^{-5}M). These observations suggest that the peptide's action involves a voltage sensitive component, perhaps the Ca^{++} channel itself (see also Glusman, Moore & Kravitz, these proceedings, 1980).

Proctolin is probably not the transmitter of the excitatory nerve that innervates this preparation. Unlike the excitatory transmitter, the peptide-induced contracture is long lasting and is accompanied by neither a depolarization nor a desensitization to the excitatory transmitter. Proctolin, or a proctolin-like substance, may be functioning as a neurohormone in lobsters.

Clearly, a complex control system exists superimposed on the excitatory and inhibitory nerves that govern the activity of the opener muscle. Serotonin and octopamine circulate in the lobster haemolymph and act on the opener muscle in a manner resembling that of proctolin. Dopamine, also found in lobsters, relaxes the muscle. Thus, proctolin is the fourth agent found to have long lasting effects on this preparation.

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240.4 THE MODULATORY ACTION OF SEROTONIN AT LOBSTER NEUROMUSCULAR JUNCTIONS. S. Glusman, J. W. Moore and E. A. Kravitz. Harvard Med. Sch., Boston, MA 02115 and Duke Univ., Durham, N.C. 27710.

Serotonin seems to function, at least in part, as a circulating neurohormone in lobsters. At exoskeletal muscles, serotonin is reported to increase the release of the excitatory transmitter compound, and to cause a contracture of the muscle (Dudel, 1965; Battelle & Kravitz, 1978). In this communication we discuss ionic mechanisms which may underlie the serotonin induced increase in contractility of exoskeletal muscles. Furthermore, we show that serotonin enhances the effectiveness of inhibitory as well as excitatory neurotransmission.

Serotonin (2×10^{-7}M), applied to the opener muscle of the dactyl of the walking leg, induces a prolonged contracture, a small depolarization of muscle membrane potential (1-2 mv), a slight increase in muscle fiber input resistance (10-15%), and the appearance of large propagating action potentials in muscle fibers where none were seen before. A voltage clamp analysis was performed to study these effects further. We were able to demonstrate the existence of the following currents in these muscle fibers: 1) a voltage dependent inward Ca^{++} current (blocked by Co^{++}); 2) a voltage dependent outward current [blocked by tetraethylammonium (TEA)]; 3) an outward current dependent on the inward Ca^{++} current; 4) a residual voltage dependent outward current that remains in the presence of TEA (100mM) and Co^{++} (30mM). With serotonin treatment there is a marked increase in the inward Ca^{++} current while the TEA sensitive and the residual outward currents are unaffected. The same results were obtained if the Ca^{++} in the bathing medium was replaced with Sr^{++} or Ba^{++}, both of which pass through the Ca^{++} channel but do not appear to activate the Ca^{++} sensitive outward conductance. We have not yet tested whether serotonin affects the outward current dependent on Ca^{++} entry as well. However, the demonstration that serotonin enhances Ca^{++} entry through Ca^{++} channels could account for the appearance of action potentials in these muscle fibers.

In a second series of experiments the effects of serotonin on inhibitory junctional potentials (IJPs) and inhibitory junctional currents (IJCs) were measured at different levels of membrane potential. Serotonin caused a 2-3 fold increase in the size of both IJPs and IJCs without changes in the level of the equilibrium potential for inhibition. At present we are examining whether this is a pre- or postsynaptic action of serotonin. Serotonin thus elevates the level of activity of all the components of this neuromuscular preparation. Supported by NIH grant #NS-07848.

240.5 A NEUROACTIVE FACTOR FROM THE LOBSTER SINUS GLAND MODULATES THE SPONTANEOUS ACTIVITY OF IDENTIFIED NEURAL NETWORKS. Jorge R. Mancillas*, S. Leff* and A. Selverston (SPON: T. Melnechuk)

The sinus gland, a neurohemal organ located in the eyestalk of decapod crustaceans, is known to contain a variety of hormones. We report the existence, in extracts of the lobster sinus gland, of a neuroactive substance(s) that can modulate the activity of two identified neuronal pools in the lobster and crayfish.

Sinus glands were removed from the eyestalks of spiny lobsters and boiled at 90°C for 5 min. The tissue was homogenized, treated with chloroform and acetone, and centrifuged. The supernatant (crude extract) was stored at -70°C. The crude extract was tested for its effect on the spontaneous rhythmic activity of the stomatogastric nervous system, which was prepared for recording as described previously (Mulloney and Selverston, J. Comp. Physiol. 91:1, 1974). Different concentrations of extract were applied to the whole, non-desheathed ganglion, which was isolated from the rest of the preparation by a small vaseline pool. The main effect observed was a disruption of the coordinated rhythmic output of both the pyloric and gastric subsystems, and eventually, a cessation of the spontaneous firing of action potentials by all units monitored. An exception were some PY units, which went into a tonic firing pattern. The crude extract was also assayed on the slow flexor motoneurons of the lobster abdominal ganglia. The spontaneous tonic firing pattern was monitored and found to be consistently reduced upon application of the extract. The intensity and duration of the response was proportional to the concentration and length of exposure. The same effects were obtained when the crude extract was applied to the stomatogastric and slow flexor motoneuron system of the crayfish (Procambarus Clarkii).

A preliminary analysis of the molecular characteristics of the neuroactive substance(s) was undertaken. The crude extract was subjected to gel filtration using Sephadex G-25 columns. Using the systems mentioned as an assay, the inhibitory activity was recovered in a narrow band eluting with a Ve/Vt of 0.92 (±0.04). A crude MW estimate based on that observation yields an approximate figure of 1200. The activity is thermostable, surviving temperatures as low as -70°C and as high as 110°C. The sensitivity of the neuroactive factor to proteolytic enzymes is currently under investigation.

Some of the properties of this neuroactive factor are similar to those of Neurodepressing Hormone (NDH). NDH is a peptide neurohormone isolated from the crayfish sinus gland that has been postulated as a mediator of circadian rhythmicity in crustaceans (Huberman et al., Eur. J. Biochem 99:203 1979). Further studies are in progress to establish the identity of the neuroactive substance(s) from the lobster sinus gland and its mechanism of action. -Supported by NSF grant BNS 79-29182.

240.6 DOPAMINE AND SEROTONIN AS PUTATIVE NEUROTRANSMITTERS IN THE OPALINE GLAND OF APLYSIA CALIFORNICA. S.H. Tritt*, M. Zigmond and J.H. Byrne Depts. of Biological Sciences and Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

We have previously identified three motor neurons which mediate the contraction of the opaline gland (Tritt and Byrne, 1980). In order to further characterize the motor component of this behavior we have attempted to identify the neurotransmitter released by the motor neurons. Neurotransmitters were screened for their effect on opaline gland contraction by infusing the substances into the gland via an arterial cannula. Infusion of 0.2-0.5 ml of glycine, GABA, glutamate, histamine or octopamine at concentrations of 10^{-4}M was without effect. In contrast, infusion of dopamine at concentrations as low as 10^{-6}M produced gland contractions similar to the response obtained by firing the opaline gland motor neurons or by electrical stimulation of nerve P5 in which the motor neurons send their axons. The size of the gland response, as measured by the change in pressure within the gland, was increased by raising the dopamine concentration from 10^{-6}M to 10^{-3}M. The response to dopamine could be blocked by infusing a mixture of dopamine and the dopamine antagonist fluphenazine. Infusion of fluphenazine alone also produced a graded block of neurally mediated gland contraction over a concentration range of 10^{-4} to 10^{-3}M. The block was reversed by washing out the fluphenazine. In contrast to the effects of fluphenazine, curare and hexamethonium at the same concentrations had no effect on either the dopamine response or the contraction of the gland elicited by stimulation of nerve P5. These results suggest that dopamine is involved in mediating opaline gland contraction and may be the neurotransmitter released by the identified motor neurons. Infusion of the ACh at concentrations between 10^{-6}M and 10^{-3}M caused a graded decrease in gland pressure, however, no neural pathways have so far been identified which mediate a gland response similar to this pharmacologic effect.

We have also obtained evidence that serotonin may play a role in the motor component of opaline secretion. Infusion of serotonin at concentrations greater than 10^{-5}M produced no detectable gland response, however, it enhanced subsequent gland contractions elicited by either dopamine infusion or stimulation of neural pathways. Even after washing out the transmitter, the size of the gland contraction remained greater than control for over 10 min. This modulation of the opaline gland contraction by serotonin may be similar to the serotonergic modulation of buccal muscle in Aplysia (Weiss et al., 1978).

The opaline motor neurons are large (250 μm) and readily identifiable, and may thus serve as a model system to study dopamine metabolism and axonal transport in a single identified neuron with a known behavioral function. Supported by NIH grants NS 00200 and NS 13511.

240.7 THE EFFECTS OF CHOLINE ON A CHOLINERGIC SYNAPSE. Susan R. Feinstein* and Alan Gelperin. Dept. Biology, Princeton University, Princeton, New Jersey 08544.

An important determinant of acetylcholine synthesis is the level of choline in the blood. Cholinergic neurons possess a specialized high affinity uptake system that transports choline from the extracellular fluid into the axons and nerve terminals where it is converted to acetylcholine. As circulating levels of choline vary, choline and acetylcholine stores in the neuron may fluctuate, and the acetylcholine output at the synapse may change. In order to study the dependence of transmitter output on changes in transmitter stores, we used the cholinergic synapse between the salivary burster neuron and the salivary duct muscle in the slug, Limax maximus.

The salivary burster (SB) is an autoactive motoneuron located in the buccal ganglion. Its axon traverses the salivary nerve to innervate the salivary duct musculature. Each SB spike is followed by a junction potential (JP) recorded from the salivary duct. The amplitude of these JPs was used as a measure of the amount of transmitter released by the SB. If transmitter output increases with elevated transmitter stores, then an increase in the amplitude of JPs recorded from the salivary duct should be seen.

When acetylcholine levels in the SB were elevated by incubation of the buccal ganglia and attached salivary ducts in choline-enriched saline, the amplitude of JPs increased. Changing the choline concentration from 5 to 15μM increased JP size by 69%. This effect of choline occured only after several hours and persisted for at least one hour after choline was removed from the bath. Hemicholinium-3, an inhibitor of the high affinity uptake process for choline, blocked the effect of choline on JP amplitude.

The increase in JP amplitude seen in the presence of choline is not due to a direct effect of choline on the muscle. The increase occurs only after several hours, persists even after choline is removed from the bath, and is blocked by the choline uptake inhibitor, hemicholinium-3. More likely, choline in the bath is taken up by the axon and nerve terminals of the SB where it is converted to acetylcholine. As acetylcholine stores in the cell grow, transmitter output also increases. A change as small as 10μM in choline concentration in the slug's haemolymph could affect transmitter levels in the SB and the amount of transmitter released at the synapse.

Supported by NSF Graduate Fellowship (S.R.F.) and NIH grant MH15698 (A.G.).

240.8 MODULATION OF ARTERIAL MUSCLE CONTRACTION BY GLYCINE AND NEURON R14 IN APLYSIA. D.J. McAdoo, M. Sawada*, J.E. Blankenship and C.H. Price. Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, TX. 77550.

We here report that exogenous glycine and electrical activity in neuron R14 both enhance the contractility of muscle in the wall of the anterior aorta of the gastropod Aplysia. We have previously shown that Aplysia neurons R3-R14 have high glycine concentrations, a glycine uptake system, a glycine axonal transport system and that glycine is associated with characteristic vesicles in those neurons. Their morphology indicates that R3-R14 innervate circulatory vessel musculature. Simultaneous recording from the soma of R14 and a varicosity in a branch of R14 in the aorta muscle confirmed the innervation of the muscle by R14. The parietovisceral ganglion, the innervated section of the anterior aorta, and the vulvar nerve connecting them were dissected from the animal as a unit. R14 was stimulated to fire with histidine or an intracellular electrode. Intracellular records were obtained from R14 and the muscle fibers while simultaneously recording muscle tension with a strain gage. Neither firing of R14 nor exogenous glycine resulted in any electrophysiological effect on the muscle or on junctional potentials produced in muscle fibers by identified motoneurons (Blankenship et al., abstract, this meeting). However, both bath applied glycine and firing of R14 enhanced muscle contractions induced by stimulation of the vulvar nerve or application of 5-HT. Thus glycine appears to modulate Aplysia muscle contraction. Alanine, serine, taurine and histidine were without effect. Glycine has now met the "identity of action" criterion for establishing that it is utilized by R14 as a neurochemical messenger. Release must yet be demonstrated. Supported by NS 13311, NSF-PCM 79 12175, and NS 11255.

240.9 IDENTIFIED PEPTIDERGIC NEURONS ACTIVATE THE FEEDING MOTOR PROGRAM IN TRITONIA. P.E. Lloyd* and A.O.D. Willows. Dept. of Zoology and Friday Harbor Lab., Univ. of Washington, Seattle, WA 98195.

In the gastropod, Tritonia, the paired buccal ganglia are largely responsible for generating the feeding motor program. Each ganglion contains a large dorsal white cell (LDWC). These neurons have been shown to contain a bioactive peptide, termed small cardioactive peptide (SCP). The LDWCs have been shown to modulate the spontaneous activity of the gut (Lloyd, P.E., Soc. Neurosci. Abst. 5, 248).

In addition to the peripheral responses, intracellular stimulation of the LDWCs also caused a change in the patterned output of the feeding motor neurons located in the buccal ganglia. If a motor neuron demonstrated ongoing patterned activity, LDWC stimulation caused an increase in both the frequency and intensity of bursting. In those preparations that were not active, LDWC stimulation often produced patterned activity. This activation of the feeding motor program occurred when the LDWCs were stimulated to produce a pattern of bursting activity similar to that observed for these neurons in semi-intact 'feeding' preparations. This suggests that the central actions of the LDWCs described above are physiological, There was no evidence for direct synaptic interactions between LDWCs and motor neurons. Indeed, even when the LDWCs were stimulated to fire at high frequency for long durations, the responses observed in the motor neurons varied considerably in latency and intensity. Thus, the LDWCs do not play a 'command' role, but rather appear to be involved in more subtle modulation of the feeding motor program.

The bioactive peptide (SCP) was derived by either gel filtration of isolated LDWC somata, or sequential gel filtration, ion exchange, and aromatic affinity chromatography of whole ganglia. The application of this peptide to isolated buccal ganglia produced responses in motor neurons similar to those observed during LDWC stimulation. The threshold for these effects was the peptide content of 0.01 LDWC/µl. These responses were abolished by previous incubation of the applied samples with trypsin or Pronase.

Thus the LDWCs provide a model system in which an identified peptidergic neuron modulates both peripherally and centrally an animal's behavior. (Supported by NSF BNS-7906280)

240.10 PROTEIN KINASE INHIBITOR:EFFECTS ON PROTEIN PHOSPHORYLATION AND PHYSIOLOGICAL RESPONSES TO NEUROTRANSMITTERS IN APLYSIA NERVOUS SYSTEM. W.B.Adams and I.B. Levitan, Friedrich Miescher-Institut, POB 273, 4002 Basel, Switzerl

Protein kinase inhibitor (PKI) purified from rabbit skeletal muscle (Demaille, Peters & Fischer, Biochemistry 16 (1977) 3080-3086) was tested for its ability to modulate protein phosphorylation in Aplysia nervous system. In homogenates of Aplysia ganglia, PKI had no effect on basal incorporation of ^{32}P from γ-[^{32}P]ATP into Aplysia proteins, but completely inhibited cyclic AMP-dependent phosphorylation. This is similar to the effect of PKI in mammalian systems. To test the hypothesis that protein phosphorylation might mediate certain physiological responses, we injected PKI into Aplysia neuron R15, to give estimated intracellular concentrations between 50 and 300 µg/ml (5 to 30 µM). Three responses were examined: 1) a serotonin-induced increase in K^+ conductance which is mediated by cyclic AMP (Drummond, Benson & Levitan, PNAS (1980) in press); 2) a dopamine-induced decrease in inward ion conductance (Wilson & Wachtel, Science 202 (1978) 772-775) which does not appear to involve cyclic AMP; and 3) a long-lasting synaptic inhibition consisting of an increase in K^+ conductance together with a decrease in inward ion conductance (Adams, Parnas & Levitan, J. Neurophysiol. (1980) in press). In one experiment PKI completely inhibited the K^+ conductance increase produced by serotonin and had no effect on the response to dopamine. The response of the cell to serotonin returned to normal by 7 hours after injection. In other experiments PKI partially inhibited the response of R15 to serotonin and had little effect on the response to dopamine. In addition, PKI injection partially inhibited the K^+ conductance increase, but had little effect on the decrease in inward ion conductance, produced by synaptic stimulation. The results are consistent with the possibility that protein phosphorylation plays a role in the cyclic AMP mediated K^+ conductance increase evoked by serotonin.

We are grateful to Dr. E. Fischer for his generous gift of homogeneous PKI.

240.11 IDENTIFICATION OF GIANT PEPTIDERGIC (PROCTOLIN CONTAINING?)NEURONS IN INSECTS. M. O'Shea* and M. Adams* (SPON: A. Heller). Dept. of Pharmacol., Physiol., Univ. Chicago, Illinois, 60637 and Abteilung Huber, MPIV, Seewiesen, W. Germany.

In addition to revealing monoamine-containing dorsal mid-line neurons, the dye Neutral Red also stains a single, lateral pair of giant cell bodies in each thoracic and abdominal ganglion of several insect species (locusts, grasshoppers, crickets and cockroaches). These lateral somata are visible as white cells when live, unstained ganglia are illuminated against a dark background. We have identified the lateral Neutral Red staining somata as a system of homologous, segmentally organized, giant peptidergic neurons and we call them the Lateral Giant Peptide Neurons (LGPNs). They are accessible to functional studies by intracellular techniques (Adams & O'Shea, this volume) and can be readily isolated for single-cell biochemistry and assay of cell content.

A remarkable component of the soma is an enormous vacuole-like inclusion which forms a complex network of invaginations throughout the cell body. The cytoplasm is extremely granular when viewed with light microscopy. Ultrastructural studies show a high density of large osmiophilic granules. These granules and associated Golgi apparatus are seen bordering the vacuole. The giant vacuole is a most singular structure and we speculate it is a storage structure for the LGPN's transmitter.

Following dissection and isolation of the LGPN's soma, the soluble content is extracted in 2N acetic acid, lyophilized and then redissolved in buffer for assay of biological activity and biochemical nature. In tests for biological activity, the cell body extract is applied to a group of locust striated muscle fibers which show a myogenic, heart-like rhythm of contractions. The extract causes a marked increase in the frequency of contraction. This physiological action of the extract resembles that of Proctolin, a pentapeptide isolated from cockroach CNS. Biological activity is lost when extract or proctolin is preincubated with peptidase enzymes (Protease and Pronase). Enzymes like trypsin for example, which do not affect proctolin, do not reduce the biological activity of the LGPN extract either. This and preliminary results of high pressure liquid chromatography analysis are consistent with the idea that proctolin or a similar peptide is the active soluble component in the LGPN cell bodies. Currently we are investigating the physiology and function of the LGPN's (Adams and O'Shea, this volume) and attempting further to characterize the transmitter by immunological and chromatographic techniques. Purification of the biologically active component(s) of the cell body will enable us more precisely to characterize the transmitter(s) of the LGPNs.

240.12 ANATOMY, ELECTROPHYSIOLOGY AND FUNCTION OF AN IDENTIFIED PEPTIDERGIC NEURON IN INSECTS. M. Adams* and M. O'Shea* (SPON: J.W. Crayton). Abt. Huber, MPIV, Seewiesen, W. Germany and Dept. of Pharmacol., Physiol., Univ. Chicago, Illinois, 60637.

The abdominal and thoracic ganglia of crickets, cockroaches and grasshoppers each contain a single pair of giant lateral peptidergic neurons. We call the cells the Lateral Giant Peptide Neurons (LGPNs). They have been individually characterized by intracellular recording and dye injection techniques and are shown to form a system of serially homologous identified neurons. The somata of the LGPNs are visible in dark-field illuminated ganglia and analysis of their content has demonstrated the presence of a biologically active peptide, possibly proctolin (O'Shea and Adams, this volume). Intracellular injection of the LGPN's soma with Lucifer yellow, cobalt and horseradish peroxidase (HRP) have revealed the following anatomical features. The cell body gives rise to a single primary neurite which enters the neuropile, crosses the midline and forms a major axon which descends in the contralateral nerve cord to the next ganglion. This axon leaves the CNS via the first segmental nerve of that ganglion and passes dorsally to end at or near the myocardium. Arborizations from the primary neurite are extensive and occurs in both the neuropile and in axon tracts. The anatomy of the neuropile arborizations are typical of output or presynaptic terminations and suggests that the LGPN has widespread effects within the CNS. Arborizations also make contact with axons of sensory neurons and intersegmental interneurons contained in discrete fiber tracts. Long finger-like processes extend at least 100µm into the ascending and descending intersegmental connectives and there they interdigitate between axons of intersegmental neurons. The function of this most unusual association with axons is currently being investigated.

The LGPN soma supports overshooting, long duration action potentials, a feature untypical of insect motoneurons and interneurons. A steady volley of EPSP is shared by members of a pair of LGPNs and by LGPNs in different ganglia. Both ascending and descending interneurons contribute to the common presynaptic input. The pattern of inputs suggest that the LGPNs are activated together as a functional unit, and is suggestive of an arousal or modulatory role. We are currently investigating the function peripherally and in the CNS. Preliminary results show that the LGPNs increase the amplitude of the heartbeat an effect which is consistent with a role in general arousal. Furthermore, the effect of LGPN stimulation on the heart is similar to the effect of bath applied proctolin, the pentapeptide which we suspect may be the neurotransmitter.

240.13 EFFECTS OF TEMPERATURE ACCLIMATION ON SEROTONIN INDUCED CILIO-EXCITATION OF THE LATERAL CILIATED GILL EPITHELIUM OF MYTILUS EDULIS. Edward J. Catapane, Joyleen A. Thomas*, George B. Stefano and Denis F. Paul* Division of Natural Sciences, Medgar Evers College, C.U.N.Y., Brooklyn, N.Y. 11229.

Lateral ciliary activity of the gill of the bivalve mollusc Mytilus edulis is controlled in part by excitatory serotonergic fibers of the branchial nerve which originates in the CNS and innervates the gill epithelium. In nature and in the lab the ciliary activity of the lateral cells is modified by the temperature of the water in which the animals are placed. Warmer temperatures increase the activity. In order to determine the site of this effect of temperature the following study was performed. Animals were maintained for 2 months at 6°C or 18°C under otherwise identical conditions prior to studying ciliary activity. Other sets of animals were maintained in flowing sea water at ambient temperatures of 20 - 25°C at MBL, Woods Hole, Ma. and their ciliary activity measured at different temperatures. Ciliary activity from sections of isolated-denervated gills were studied for their response to the superfusion of serotonin at different bath temperatures by stroboscopic microscopy. The study illustrated that the ciliated cells displayed a temperature dependent activity, that the site of the temperature sensitive mechanism was in the gill, presumedly within the lateral cells themselves, and that prolonged temperature changes of 2 months, but not short-term changes of 5 hours resulted in temperature acclimation of this response. The effects of temperature may be due in part to changes in the affinity or number of postjunctional serotonin receptors in the lateral cells.

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241.1 EFFECTS OF DROSOPHILA PHOTORECEPTOR MUTATIONS ON THE CIRCADIAN ACTIVITY RHYTHM. Ronald J. Konopka. Biol. Div., California Institute of Technology, Pasadena, CA 91125.

Locomotor activity rhythms of adults of the following Drosophila strains were determined by means of an infrared actigraph: Canton-S and Oregon-R wild types, ocelliless, which lacks the three ocelli, sine oculis, a mutant which lacks the compound eyes, ocelli, and at least the first optic lobe, and norp A (allele p12), a mutant which has eyes but whose receptor potential measured by the electroretinogram is greatly reduced.

The activity rhythm of wild type flies in a light-dark cycle is usually bimodal, with morning and evening peaks. Ocelliless flies also have a bimodal activity rhythm, but sine oculis flies have a prominent evening peak and a poor or absent morning peak. The activity rhythm in constant temperature and constant infrared is usually unimodal, with the peak deriving from the evening peak expressed in a light-dark cycle. Flies carrying either the ocelliless or sine oculis mutation have an activity rhythm that persists in constant conditions. The circadian pacemaker of sine oculis flies can be reset by light pulses to the same extent as wild type. Thus the Drosophila circadian pacemaker possesses an extra-retinal photoreceptor.

Constant dim white light lengthens the period of the circadian pacemaker relative to that in constant infrared. The lengthening produced in the norp A mutant is similar to that produced in the Oregon-R wild type. Therefore, the lengthening effect of light on the activity rhythm is apparently not mediated by the retinula cells in the compound eye.

241.2 A CIRCADIAN RHYTHM RECORDED IN THE ISOLATED EYE OF BURSATELLA. G.D. Block and M.H. Roberts, Department of Biology, University of Virginia, Charlottesville, Virginia 22903.

The eyes of two marine molluscs, Aplysia californica and Navanax inermis, express circadian rhythms in the frequency of optic nerve potentials (Jacklet, Science, 164: 562, 1969; Eskin & Harbombe, Comp. Biochem. Physiol., 57: 443, 1977). In Aplysia the role of the eye in controlling locomotor behavior has been explored and it appears that the ocular pacemakers are involved in timing the locomotor rhythm (Strumwasser, Physiologist, 16: 9, 1973; Lickey et al., J. Comp. Physiol., 118: 121, 1977).

We currently report that Bursatella leachi plei, an anaspid opisthobranch, also possesses an ocular circadian pacemaker which will free run in vitro for several cycles. Similar to Aplysia, Bursatella eyes respond to illumination with compound action potentials which also occur spontaneously in darkness and express a circadian rhythm in their ongoing frequency. This ocular rhythm differs from the Aplysia rhythm in several respects: 1) The mean peak frequency of impulses on the first day in vitro is 96 (S.D.=36) for Bursatella compared with 247 (S.D.=61) for Aplysia eyes. 2) Unlike the circadian waveform of Aplysia eyes which contains a characteristic hump on the falling phase, the Bursatella waveform shows both a rapid rise and fall from peak values. 3) Evaluated simultaneously, the average free running period of Bursatella eyes in artificial seawater is 21.2 hr (S.D.=.6) compared to 24.3 hr (S.D.=.9) for Aplysia eyes. 4) The steady-state phase angle for entrainment differs by 4 hr from Aplysia, with Bursatella eyes showing activity peaks in the late subjective night.

We have also investigated the role of the eyes in controlling the locomotor rhythm. On L:D,12:12 Bursatella, like Aplysia, are diurnal with locomotor activity beginning near dawn. When the photoperiod is shortened (L:D, 9:15), locomotor onsets anticipate dawn by 1-2 hours. Following eye removal, locomotor movements continue to anticipate light onset with only minor changes in locomotor patterning. This is in contrast to Aplysia where there is a marked loss of anticipatory locomotor behavior following eye removal (Lickey et al., Photochem. & Photobiol., 23: 253, 1976).

Apparently the ocular pacemakers in Bursatella do not play a prominent role in timing locomotor activity when light cycles are present. We are currently investigating the role of the ocular pacemakers during free runs in continual darkness. NS15264.

241.3 PHASE RELATIONS BETWEEN THE CIRCADIAN EYE RHYTHMS AND THE LOCOMOTOR RHYTHM IN APLYSIA WITH ONE OR BOTH OPTIC NERVES CUT. M. E. Lickey, D. J. Hudson* and S. O. Hiaasen*. Dept. of Psychology, Univ. of Ore., Eugene, OR 97403.

The Aplysia eye shows a circadian rhythm of neural activity in vitro, and locomotor activity has a circadian rhythm in the whole animal. We have conducted experiments to determine the phase relation between these 2 rhythms and to determine if the optic nerve mediates coupling between them. METHODS: After entraining Aplysia to LD 12:12 we cut either one (1-X group) or both (2-X group) optic nerves. Control animals were left intact. Two to four days after surgery we released the animals into constant conditions (DD, either constant darkness or very dim constant light) and recorded their locomotor rhythms on an actograph. After 14 or more days in DD we removed the eyes and recorded their rhythms in vitro in constant darkness. RESULTS: (1) Intact animals' locomotor rhythms varied from vigorously rhythmic to nearly arrhythmic. Their eyes were nearly always found to be strongly rhythmic, but in many cases they were quite out of phase with each other. In animals that showed strong locomotor rhythms the eyes were nearly always synchronized and the rising phase of the eye rhythms corresponded to the onset of locomotion. In animals with weak locomotor rhythms the eyes were often, though not always, out of phase. (2) 1-X animals' locomotor and eye rhythms were comparable to the intact controls. In some animals, however, there was a strong locomotor rhythm even though the eyes were out of phase. In such cases the rising phase of the intact eye's rhythm nearly always corresponded to the onset of the locomotor rhythm; the cut eye's phase was not correlated with locomotor phase. (3) In most 2-X animals, locomotion was arrhythmic or nearly so, even though both eyes were nearly always rhythmic and often in phase. DISCUSSION: Coupling between the eyes and locomotion is obviously mediated by neural activity in the optic nerve. Our results give no evidence that ocular hormones are involved as suggested by the morphology of the eye(Jacklet et al., 1972) and the occurrence of calcium dependent peptide release from the eye in vitro (Harf et al., 1976; Strumwasser et al., 1979). It is also apparent that strong locomotor rhythmicity depends on proper internal synchronization of multiple oscillators and that behavioral arrhythmicity, when it occurs in intact animals, may often be due to internal desynchronization. Since there is a phase lock between the eye rhythms and the locomotor rhythm in animals with strong behavioral freerunning, the period of the locomotor rhythm is a good estimate of the period of the eye rhythm in situ. Our results on the relation between locomotor phase and eye phase agree with the results of Block (1979). (Supported by PHS NS 12374 and NSF 77-28251)

241.4 CEREBRO-PEDAL CONNECTIVES: CRITICAL PATHWAYS IN THE APLYSIA CIRCADIAN SYSTEM. M.H. Roberts and G.D. Block, Department of Biology, University of Virginia, Charlottesville, Virginia 22903.

Aplysia express a circadian rhythm in locomotor behavior (Strumwasser, In: The Neurosciences: An Intensive Study Program, p. 516, 1967). When exposed to light cycles (Light:Dark, 12hr:12hr), they are predominantly diurnal and begin locomotor activity near dawn. On shorter photoperiods (L:D, 9:15), locomotor movements typically "anticipate" the onset of light by several hours. When the eyes are surgically removed, anticipatory activity ceases. This, along with the fact that the eyes contain circadian oscillators (Jacklet, Science 164: 562, 1969) has led to the hypothesis that the ocular pacemakers are responsible for timing pre-dawn locomotor activity (Strumwasser, Physiologist 16: 9, 1973); Lickey et al., J. Comp. Physiol. 118: 121, 1977). An attempt was made to identify central pathways by which phase information travels from the ocular oscillators to the pedal ganglia, whose nerves serve the foot. Aplysia were placed in locomotor monitors and exposed to light cycles (L:D, 9:15) in order to confirm the presence of a clear locomotor rhythm. Following this, animals were removed from their tanks and the cerebro-pleural (n=8) or cerebro-pedal (n=17) connectives were bilaterally severed. After recovery from surgery, the Aplysia were returned to their monitors (L:D, 9:15) and locomotor activity was evaluated.

The results indicate that only the cerebro-pedal connectives are critical pathways in the circadian system. Bilateral section of the cerebro-pleural connectives resulted in only minor changes in locomotor patterning: anticipatory activity was not affected. In contrast, bilateral section of the cerebro-pedal connectives resulted in two reproducible effects. First, locomotor activity was markedly reduced from average pre-operative levels. This value however, was still above night time levels observed pre-operatively. Secondly, the animals failed to respond to light and locomotor behavior appeared arrythmic. These results lead us to believe that the cerebro-pedal pathways not only carry phase information from the ocular oscillators, but also carry signals from extraocular photoreceptors, which are capable of maintaining a diurnal locomotor rhythm in eyeless Aplysia (Block and Lickey, J. Comp. Physiol. 84: 367, 1974).

Whether phase information from the extraocular and ocular systems is carried separately in the cerebro-pedal pathways or first integrated in the cerebral ganglion is currently being investigated. NS15264.

241.5 LITHIUM INCREASES THE PERIOD OF A NEURONAL CIRCADIAN OSCILLATOR. Felix Strumwasser and Daniel P. Viele.* Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Halberg (1968) has suggested that circular manic-depressive cycles in patients, with severe mood disorders, could occur from a beat frequency resulting from the interaction of two endogenous oscillators only one of which remains entrained to the environment while the other free-runs.

Lithium is the present treatment of choice for patients with manic-depressive symptoms. The mechanisms of action of Li on the nervous system remain unclear but there is accumulating evidence that one of its important actions is on the circadian oscillator system. Manic-depressive patients turn out to have a fast circadian clock as evidenced by measurements of their sleep-waking or body temperature cycle (Pflug et al., 1976; Kripke et al., 1978) and Li appears to delay the circadian sleep rhythm of normal subjects in a normal social environment (Kripke et al., 1979).

We have investigated the effects of sustained Li on the free-running period of a neuronal circadian oscillator system in the eye of the opisthobranch mollusc, Aplysia. This preparation is an ideal model system because Li effects can be measured on the isolated eye without the complication of coupling factors and other oscillators present in the totally intact animal. The circadian rhythm is continuously recorded in darkness by monitoring the output of the optic nerve. The spikes in the optic nerve are compound action potentials (CAPs) due to synchronization of the output neurons located in the eye. In an artificial sea water medium (mM: NaCl, 465; KCl, 10.4; $MgSO_4$, 28; $MgCl_2$, 27; $CaCl_2$, 11; $NaHCO_3$, 13; HEPES, 15; glucose, 5) the period (τ) of the CAP frequency rhythm is 24.1 hours as measured over 4 to 6 cycles. When Li is substituted for Na we observe minimal effects on τ at 10 mM but remarkable lengthening of τ ($\sim$28 hours) at 30 mM. Reduction of Na (replacement by Mg) does not significantly alter τ. This is the first demonstration that Li has direct effects on τ of a neuronal circadian oscillator system studied in vitro and that it does slow the circadian clock as has been suggested from work on intact animals and plants (Engelman, 1973; Kripke and Wyborney, 1980). [Supported by NIH grant, NS 07071].

241.6 MAXIMUM ENTROPY SPECTRAL ANALYSIS OF JELLYFISH SWIMMING BEHAVIOR. J. L. Leonard. Dept. of Zoology, University of Wisconsin-Madison, 53706 and Friday Harbor Laboratories, Friday Harbor, Washington 98250.

A new technique (maximum entropy spectral analysis) for detecting rhythms in time-series data has been used to describe complex temporal patterns of swimming behavior in a hydrozoan jellyfish. This technique (MESA) is superior to more conventional methods of spectral analysis, such as the Fast Fourier Transform, in several respects (Childers, D. Modern Spectrum Analysis. IEEE Press, 1978). MESA is mathematically equivalent to fitting a n^{th} order Markov process to the data by the maximum likelihood procedure (Van den Bos, A. IEEE Trans. Inform. Theory vol. IT-17, 1971). MESA has two important advantages for biological work. The first is that it makes fewer assumptions about the data than does the FFT. This makes MESA more appropriate than the FFT for descriptive work. The second advantage is that MESA provides better resolution at the low frequency end of the power spectrum than does the FFT. This means that for the same length of data MESA will allow detection of low-frequency rhythms that would not be detectable with the FFT.

MESA was found to eliminate ambiguity present in FFT spectra as to which data records showed rhythms. The improved resolution at the low frequency end of the power spectra obtained by using MESA resulted in two important findings:

1. The swimming behavior of some animals showed more than one rhythm within a data record.
2. Much of the variability in the swimming behavior of an individual jellyfish is due to switching between rhythms of different frequency.

241.7 ABERRATIONS OF CIRCADIAN RHYTHMS OF BODY TEMPERATURE IN RATS WITH MEDIAL PREOPTIC LESIONS. Joseph Liran*, Evelyn Satinoff and Ronald M. Clapman*. Psychology Department, University of Illinois, Champaign, IL. 61820.

Evidence suggests that after lesions in the suprachiasmatic nucleus of the hypothalamus the circadian rhythm of body temperature (Tb) may be abolished, although the daily amplitude of the rhythm remains the same or is attenuated. We report here that lesions in the medial preoptic area (MPO), the area of the brain concerned with Tb regulation, produces severe aberrations in several aspects of the circadian rhythm of Tb.

Male rats were maintained at an ambient temperature (Ta) of 25°C in a 12:12 light:dark cycle with food and water available ad libitum. Lesions of the MPO were made through previously implanted stainless steel electrodes (2mA anodal current, 10-20 sec) in unanesthetized animals. Within 4 days the rats were tested for their ability to maintain normal Tb at a Ta of 5°C. Those that were deficient were implanted intraperitoneally with a miniature temperature transmitter and returned to their cages. Tb's were recorded every 5 min for several months.

Normal rats' Tb's fluctuated between 36.1 and 37.9°C with a mean daily Tb of 37.0 $\pm$ .06°C.(n=5). Rats with MPO lesions showed two major types of abnormalities. 1. Exaggerated amplitude of the rhythm. Tb of these rats (n=5) fluctuated between 36.0 and 41.5°C with a mean daily Tb of 38.8 $\pm$ .03°C. As the animals recovered the troughs remained essentially the same and the peaks became gradually lower although to date, four months postoperatively in several of the rats, no animal has returned to normal. 2. Exaggerated amplitudes of ultradian and the appearance of supradian components in some rats (n=3). Peaks and troughs of Tb declined successively over 4-5 days, often reaching lows of 31-33°C before drifting up again.

We interpret these results in terms of mutually inhibitory and facilitatory components of the daily Tb rhythm that together contribute to normal, small amplitude circadian oscillations. If some of the inhibitory components are preferentially damaged by MPO lesions, the resultant excessive influence of the less damaged components result in exaggerated swings of Tb and the emergence of noncircadian contributions to the overall fluctuations of Tb.

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241.8 DIURNAL CHANGES IN EFFECTS OF HALOPERIDOL IN RAT. A. Campbell,* M. Herschel,* J. R. Madsen,* R. J. Baldessarini. Mailman Research Center, Harvard Medical School, McLean/Massachusetts General Hospital, Belmont, MA 02178.

To investigate diurnal variations in the behavioral effects of haloperidol, electronically monitored locomotion, catelepsy and ptosis were measured at 4h intervals over 24-h periods in rats kept under a controlled 12-h light-dark cycle (lights on, 7 a.m.). Following administration of haloperidol (1 mg/kg, i.p.) the cataleptic response was maximal at 4 p.m. and minimal at 4 a.m. The sedative responses (ptosis and inhibition of motor activity) showed a similar peak and nadir, or just the opposite of the normal diurnal pattern of spontaneous activity. After one month of reversed lighting conditions, during which time continuous electronic monitoring showed a complete reversal of day-night variations in spontaneous activity, the timing of the peak and nadir of the cataleptic response was unchanged while the sedative responses underwent a phase-shift of about 4 hours.

In addition, preliminary assays of haloperidol levels in the rat brain after injections of haloperidol (using a radioreceptor assay) also suggested the presence of corresponding diurnal variation, with levels showing a peak corresponding to the maximal cataleptic responses.

These results suggest that the cyclic cataleptic response to haloperidol is an endogenous rhythm, persistent even after the lighting changes that reversed the spontaneous activity and altered the peaks of haloperidol-induced sedation.

241.9 CHOLINERGIC STIMULATION OF PONTINE BRAINSTEM CELLS BY MINI-PUMP INFUSION AUGMENTS PARADOXICAL SLEEP FOR FIVE DAYS. P. Shiromani and W. Fishbein. Psychobiology Lab., Dept. of Psychology, The City College, CUNY, New York, N.Y. 10031.

In 1961, Jouvet (In P.G. Waser (Ed), Cholinergic Mechanisms, Raven, 1975) proposed that paradoxical sleep (PS) could depend upon brainstem cholinergic mechanisms. Much converging evidence has favored this hypothesis. Local injection of cholinergic agonists in the dorsolateral part of the pontine reticular formation trigger PS and antagonists inhibit it. Other research implicates the same cholinergic mechanisms in affective psychopathological (Sitaram et al., Science, 208, 200-1, 1980), and memory disorders (Wurtman, Trends in Neurosci., 3, VII-X, 1980). Such links imply the need to chart long-term sleep-wake alterations induced by cholinergic agonists and antagonists. In the present work we employ the implantable Alzet mini-pump to infuse drugs at a controlled rate (1 ul/hr) over 7 days.

Nine rats are implanted with chronic indwelling EEG and EMG electrodes, and a cannula in the gigantocellular tegmental field (FTG) (carbachol, n=3, .5 ug/hr; scopolamine, n=2, 9 ug/hr) or the nucleus reticularis pontis caudalis (RPC) (carbachol, n=4, .5 ug/hr). A 24 hr baseline recording is obtained one week later. The pump is then implanted and 9 days of continuous recordings follow (7 days drug, 2 days post-drug). One week after the pump is exhausted a 24 hr post-experimental record is obtained. All results are compared to pooled baseline data. The drugs are effective during the first 5 days of pump operation. In the FTG, the major increase in PS is in the night cycle (PM) (FTG=+93%, RPC=+50%). During the day cycle (AM), the FTG and RPC show similar increases (FTG=+18%, RPC=+19%). Scopolamine induces a 41% decrease in the day and no change in the night cycle. No change in slow wave sleep in any drug condition is observed. The change in PS is due to alteration in PS frequency, without any change in mean duration of PS periods. The augmenting effects of carbachol are seen only during the PM cycle (normally awake) and not in the AM cycle. This suggests that during the AM cycle, carbachol does not alter PS mechanisms beyond already existing, genetically programmed, peak levels. Conversely, scopolamine depresses PS levels in the AM but has no effect during the PM cycle.

The experiment supports the view that (1) PS is modulated by brainstem cholinergic mechanisms, and (2) PS can be neuropharmacologically augmented for a considerable time period. Indeed, this study is the first of its kind to show such a protracted augmentation.

241.10 STRUCTURE AND FUNCTION OF SUPRACHIASMATIC NUCLEI (SCN) IN HUMAN AND NON-HUMAN PRIMATES. M.C. Moore-Ede, R. Lydic, C.A. Czeisler, C.A. Fuller and H.E. Albers*. Department of Physiology, Harvard Medical School, Boston, MA 02115.

In rodents, the SCN of the hypothalamus have been demonstrated to act as a key pacemaker of the circadian timing system. Entrainment by the 24 hr light-dark (LD) cycle is mediated via a direct retinohypothalamic tract (RHT). We have extended studies of SCN structure and function to primates including man. In the New World primate, the squirrel monkey (*Saimiri sciureus*), the SCN are located on either side of the tip of the optic recess of ventricle III and have a complex three-dimensional structure with distinct anterior and posterior poles (Lydic and Moore-Ede, *Neurosci. Letts.*, In Press, 1980). Retrograde transport studies demonstrate an RHT innervating the caudal SCN of squirrel monkeys. With the broadening of the base of ventricle III in an Old World primate, the rhesus monkey (*Macaca mulatta*), the SCN are more laterally placed and comprise a more diffuse neuronal cluster. The chimpanzee (*Pan troglodytes*) also possesses a diffusely organized and more laterally placed SCN, innervated by an RHT (Tigges, J. *et al., J. Comp. Neur. 172:* 367, 1977). In human fetal, child and adult brains we found a neuronal cluster homologous to SCN bordering ventricle III. However in human brains SCN were still more laterally placed and more diffusely organized than in non-human primates (Lydic *et al., Sleep*, In Press, 1980). Although current retrograde transport techniques for demonstrating RHT are not applicable to human studies, LD cycles can entrain humans.

Physiological studies of human subjects isolated from time cues (Czeisler *et al., Sleep Res.*, In Press, 1980) demonstrate that the circadian sleep-wake cycle is generated by a separate pacemaker from the body temperature rhythm, because these two rhythms can show distinctly different free-running periods. To determine if the SCN in primates are responsible for both the rest-activity and temperature rhythms, we have placed total bilateral SCN lesions in squirrel monkeys. These lesions result in complete loss of circadian rhythmicity in the rest-activity, feeding and drinking rhythms, but a clear body temperature rhythm persists as confirmed by spectral analysis. We conclude that while the SCN acts as the pacemaker of the rest-activity cycle in primates, a separate oscillator, located outside the SCN, generates the body temperature rhythm.

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241.11 LIGHT-DARK CYCLE ENTRAINMENT OF THE PERSISTING CIRCADIAN RHYTHM OF CORE BODY TEMPERATURE IN SCN-LESIONED PRIMATES. Albers, H.E.*, Lydic, R. and Moore-Ede, M.C. (SPON: A.A. Gerall.) Dept. of Physiology, Harvard Medical School, Boston, MA 02115.

The suprachiasmatic nuclei of the hypothalamus (SCN) are an important pacemaker in the mammalian circadian system, generating the circadian rhythms of feeding, drinking and locomotor activity. Entrainment of these rhythms to the environmental light-dark (LD) cycle appears to be mediated via the retinohypothalamic tract (RHT), a direct projection from the retina to the SCN. The existence of a second circadian pacemaker has been confirmed by the persistence of the free-running circadian rhythm of core body temperature in SCN-lesioned monkeys (Moore-Ede *et al., Neurosci. Abstr.*, 1980).

To determine whether this second circadian pacemaker can be entrained to LD cycles, in the absence of the SCN-RHT complex, SCN-lesioned adult male squirrel monkeys were studied under LD 12:12 (period {T}=24 hr). These animals were prepared with bilateral radio frequency lesions (60°C; 1 min duration) stereotaxically placed in the SCN under x-ray visualization. SCN-lesioned monkeys displayed no significant circadian periodicity in drinking patterns in constant illumination. Following at least 2 wks of exposure to LD 12:12, each animal, previously trained to accept chair restraint, was placed in a metabolism chair, with a rectal probe to measure core body temperature.

A robust 24-hr rhythm of core body temperature was observed in all animals, which anticipated lights-on and remained elevated throughout the 12 hr light phase. To verify entrainment of the temperature rhythm to light-dark cycles in SCN-lesioned animals, a LD 1:22 cycle (T=23 hrs) was then imposed. The temperature rhythm rapidly adopted a 23 hr period as confirmed by sequential acrophase fits and spectral analyses in both SCN-lesioned and control animals. Again the initial rise in temperature anticipated lights-on but remained elevated for several hrs after the daily 1 hr light pulse.

We conclude that the circadian rhythm of core body temperature is not generated by the SCN. Furthermore, the SCN are not essential for entrainment of the pacemaker responsible for this temperature rhythm.

(Supported by NIH-NS13921 and AFOSR 78-3560.)

242.1 SPINAL CORD INFARCTION: A NEW APPROACH IN THE EXPERIMENTAL INVESTIGATION OF STROKE. J.A. Zivin, U. DeGirolami*. Department of Neurology, University of Massachusetts Medical Center, Worcester, Massachusetts 01605.

A major obstacle to the experimental investigation of stroke has been the lack of a reproducible model of focal ischemia. To overcome this problem we have developed a method which employs infarction of the rabbit spinal cord. We believe it is reasonable to assume that the changes that occur in the spinal cord are qualatatively similar to the changes that occur in most regions of the brain during ischemia. We have found that it is possible to produce focal ischemia in 100% of the animals in a very stereotyped anatomical distribution and the lesion can be produced while the animal is fully conscious. Therefore, this model is ideally suited for biochemical and morphological correlative studies.

Snare ligatures were placed around the abdominal aortas of rabbits below the renal arteries and after recovery from anesthesia the aortas were occluded for varying periods of up to one hour. The ligatures were then removed. In 48 animals, the average time to produce any clinical signs of neurological dysfunction was 20.6 $\pm$ 2.5 min. (mean $\pm$ s.e.). The average time to produce complete paraplegia as 32.9 $\pm$ 1.8 min. Microscopic examination one week after the insult showed gray matter damage in all animals that had signs of clinical deficits but no abnormalities were observed in the rabbits that regained normal neurological function.

We believe this method is the most reproducible CNS focal ischemia model yet devised.

This study is supported in part by NIH Grants NS00456 and NS15827.

242.2 CHANGES IN RELATIVE TISSUE OXYGEN CONSUMPTION, TISSUE OXYGEN CONTENT AND BLOOD FLOW AFTER SPINAL CORD INJURY. N. Hayashi*, J. C. de la Torre, B. A. Green. Dept. of Neurosurgery, Univ. of Miami, Sch. of Med., Miami, FL 33101.

We have previously reported gray and white matter local spinal cord blood flow (lSCBF) in the rat using a multiple micro-electrode hydrogen washout technique (Hayashi et al. Neurology 30, 406, 1980). In the present study, lSCBF, tissue oxygen content (TOC) and relative tissue oxygen consumption (RTOC) were measured simultaneously using the micro-electrode technique before and after 100 g/cm force spinal cord compression injury. This corresponds to a moderately severe injury resulting in permanent paralysis of the animal.

Male Sprague-Dawley rats underwent laminectomy from T_{10}-L_1 and microelectrodes were inserted in gray and white matter in the following regions: i) at the lesion site; ii) proximal to the lesion site; and iii) distal to the lesion site.

The placement of the distal and proximal recording electrodes extended to about 2 spinal cord segments away from the lesion site.

Results show that at the lesion site lSCBF in gray matter gradually decreased but, RTOC was found to fall faster than lSCBF and did not recover for 4 hours. TOC was slightly increased during the first hour after injury, but began to decrease during the next 3 hours.

In the regions proximal to the injury site, the pattern of lSCBF reduction resembled that seen at the injury site but was less pronounced. Similarly, RTOC initially decreased 30 min after injury but then, gradually increased for the next 3 hours even as lSCBF decreased. One hour after injury this dissociation of blood flow and RTOC value was found highest at the proximal site, while TOC levels showed lower than normal levels.

Distal to the lesion site, lSCBF and RTOC were less decreased than at the site of injury.

These results suggest that inside the immediate perimeter of the lesion site, distortion of the neuronal tissue may be the most critical factor in the eventual pathophysiology that develops after trauma. However, outside the lesion site, particularly in the proximal region, blood flow and metabolic factors (such as RTOC) are probably more important in accounting for the secondary changes seen after cord injury.

242.3 SUPERSENSITIVITY TO 5-HT AFTER SPINAL CORD TRANSSECTION IN THE RAT. H. Barbeau and P. Bédard. Lab. de neurobiologie, Univ. Laval, Québec, Canada, G1K 7P4.

After spinal cord transsection in the rat, the level of spontaneous EMG activity in response to 5-hydroxytryptophan (5-HTP) measured in the extensor muscles of the hinblimbs increases with the time elapsed after the section. The response is maximal 20 to 30 days after transsection. It is blocked completely by cyproheptadine 10 mg/kg and does not appear to be affected by drugs acting on other neurotransmitters.

In parallel, during the same period of 20 to 30 days there is a ten fold increase in the strength of the nociceptive flexor reflex elicited by an electric stimulation of the paw (duration 3 ms, frequency 10 Hz, intensity 2 MA). The tension of the nociceptive reflex is measured in grams with the help of a transducer. In another set of experiments, 5-7 dihydroxytryptamine (5-7DHT) 200 mg was injected into the left cerebral ventricle. All animals were pretreated with desipramine 65 mg/kg. Twenty days after the injection of this neurotoxin all animals underwent a spinal cord transsection. The day after the transsection they were tested for spontaneous EMG activity in the hindlimbs in response to 100 mg/kg of 5-HTP and for nociceptive response after stimulation of the paw. The level of spontaneous EMG activity in response to 5-HTP is much higher than in acutely spinalized rats not pretreated with 5-7DHT and of the same order as in chronic spinal rats. On the contrary the nociceptive reflexes remained similar to those obtained in acute spinal rats untreated with 5-7DHT. Our results suggest that lumbar motoneurones in the rat become supersensitive to 5-HT after treatment with a neurotoxin. The same probably occurs after section of the descending 5-HT pathways in chronically spinalized animals. On the other hand, treatement with 5-7DHT does not appear to increase the sensitivity of lumbar neuron to noniceptive stimuli. The same reflexes are however increased in chronically spinalized rats. In the latter situation we must therefore infer that two mechanisms probably coexist namely a certain degree of supersensitivity of the 5-HT receptors which is specific to that neurotransmitter and second a certain degree of increased responsiveness of the lumbar neurons due to other mechanisms possibibly disinhibition or synaptic rearrangement.

242.4 THERAPEUTIC DIALYSIS OF CEREBROSPINAL FLUID BY EPIDURAL COOLING IN SPINAL CORD INJURIES. C. Romero-Sierra, R. Ethier*, R.N. Henriksen* and R.B. Marina*. Queen's University, Kingston, Ontario, Canada K7L 3N6.

Paraplegias of traumatic origin may be classified in primary and secondary. It is accepted knowledge that secondary traumatic paraplegia (S.T.P.) is due to an autodestructive process.

Of the various factors suggested as causal agents in the secondary process, none of them alone or combined gives a complete picture. Furthermore, different authors have published results supporting or contradicting the therapeutic effects of durotomy alone or associated with exposed spinal cord with a solution at normal temperature or by irrigation with a cold solution. It appears that, while decompression and open dialysis might be beneficial, the surgical trauma over the injured region is detrimental.

A method of local epidural spinal cord cooling has been developed and found very successful in the treatment of S.T.P. With this method no surgical injury or damage is imposed on the dura, cerebrospinal fluid (C.S.F.) or spinal cord. Furthermore, several of the beneficial effects attributed to hypothermia in a traumatized area are evident, e.g., reduction in metabolic demands, edema, swelling, vasospasm and blood pressure.

Aware of the benefits that dialysis may have in S.T.P., as well as of the very good results obtainable with local epidural spinal cord cooling, it was hypothesized that this method of hypothermia may in some way trigger C.S.F. dialysis. Based on this hypothesis, a model approximating the behaviour of the C.S.F. was developed for the situation in which a cold source is applied to the dura.

Using dimensionless analysis techniques, it was shown that the C.S.F. under the cooled region of the dura experiences convective motion, even in adverse situations where the spinal cord has swollen. In the steady state, the moving fluid forms into several Bénard cells directly under the cold source.

The steady state temperature distribution within the C.S.F., when the cold probe is applied, was then solved for. Further, a mathematical expression describing the axial flow field of the C.S.F. when fluid formation is just beginning, was derived from the approximate equations of motion. This expression indicates that there will initially be effective axial fluid transport near the cold source. This fact, along with the knowledge that steady state convection will occur, allows us to conclude that the cold source in local epidural cooling is an effective mechanism for generating C.S.F. flow.

242.5 MONOAMINE METABOLITE CONCENTRATIONS IN SPINAL GRAY AND WHITE MATTER, PLASMA, AND CISTERNAL CSF SUBSEQUENT TO THORACIC (T_5) CONTUSION OF EXPOSED CAT SPINAL CORD. Robert H. Roth, Donald Kay Riker, Dean Lohse* and William F. Collins, Jr. Depts. Pharmacology, Psychiatry and Neurosurgery, Yale Univ. Sch. Med., New Haven, CT 06510.

Monoamines present in spinal tissues as neurotransmitters or blood-borne hormones could be vasoactive when released from tissue by spinal injury or taken up at the injured site. For example, the presence of vasoconstrictive amines at the site of injury could cause local spreading ischemia and a worsened prognosis. We examined the distribution and spread of dopamine (homovanillic acid, HVA) and serotonin (5-hydroxyindoleacetic acid, 5-HIAA) metabolites by gas chromatography-mass spectrometry in cat gray and white matter after contusion of T_5. Following anesthesia and laminectomy a reproducible paraplegia-producing blow (500 g/cm) was delivered to the dorsal surface of T_5 and corroborated by transient hypertension. Cisternal CSF (cCSF) and venuous plasma samples were taken from in-dwelling catheters just before and 1, 2 and 3 hours after injury. Cats were sacrificed by pentobarbital overdose at 3 hours post-injury. The spinal cord was removed rapidly and transferred to dry ice. Frozen segments T_4, T_5, T_6, and T_{12} were dissected and gray matter punched out with a 15 g blunt needle.

Increases in HVA and 5-HIAA did not occur in any white matter surrounding the site of injury (N=5; -4 to -27%) compared to sham-operated cats run in parallel. Significant changes in HVA (-44%) and 5-HIAA (-25%) in gray matter occurred at T_5 but not in T_4 or T_6. These data support the hypothesis that local catabolism may be altered by cellular injury but no evidence for significant spread of dopamine or serotonin into surrounding tissue was obtained.

Plasma HVA (r=.49) and dihydroxyphenylacetic acid (DOPAC, r= .86) were correlated with cCSF levels of HVA and DOPAC, respectively; plasma 5-HIAA did not correlate with cCSF values. In control cats cCSF HVA consistently increased from 100 ng/ml to a plateau of 160 ng/ml at +1 hr. Three of four injured cats had a dramatic rise in HVA 1-3 hrs after trauma (200-300 ng/ml). cCSF 5-HIAA showed a similar but more variable pattern. Only 2 of 5 injured cats reflected these changes by increases in plasma metabolites. These results suggest that increased central dopamine and serotonin metabolism follows within 2 hrs of spinal injury. Clearly increased levels of HVA and 5-HIAA in CSF within 2-4 hrs of injury might be predictive of significant spinal injury. (Supported in part by a grant from USPHS, NS-10174).

242.6 THE EFFECT OF DURATION OF COMPRESSION IN ACUTE SPINAL CORD TRAUMA. A.C.Nacimiento,H.-D.Herrmann* and F.Loew* Dept.of Physiology I and Dept. of Neurosurgery, Saarland University, School of Medicine, 6650 Homburg FRG

Time course and intensity of functional losses developing acutely in a suddenly compressed L7 segment in cat under experimental conditions previously described (Soc.f.Neurosci. 1978,4,1828; ibid 1979,5,2455) are clearly influenced by the duration of compression. Polysynaptic reflex discharges (PRD) and conduction through the injured segment before, during and for 5 hrs following injury. Pathophysiological changes develop along a regular pattern in which three postraumatic phases can be discerned as a function of time: an early one, within 1-5 min after compression, followed by an intermediate one for the next 5-10 min and a late phase, generally lasting until termination of the experiment. A compression of 3 mm was maintained in 3 experimental series for 50 msec, 500 msec and 1 sec, respectively. The pattern for a duration of 50 msec was as follows: within 1 min there was a sharp decrease in PRD of about 60 % and conduction block through the injured region. The following intermediate phase was characterized by stabilization of the reduced PRD and by a partial, progressive recovery of conduction. The late phase showed no further major changes neither in recovery nor in severity in the reduced PRD, whereas conduction recovered to about 40 % of the control values. With a duration of 500 msec all changes greatly increased in severity: PRD fell by 80-90 % and conduction was totally blocked within 1 min following compression. The intermediate phase showed very little recovery of PRD and none in conduction. In the last period, reflex activity remained very low and conduction returned at the most to about 10-15 % of pre-injury level. A compression lasting 1 sec produced changes which were not significantly different from those observed at 500 msec. It is concluded that (a) functional changes occurring very early after compression do contribute to the setting of the subsequent pattern of functional recovery and (b) no further increment in severity of functional losses occurs upon reaching a duration of compression with "threshold" qualities in terms of plasticity of recovery.
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242.7 EFFECTS OF METHYLPREDNISOLONE ON INJURED SPINAL CORD MICROVASCULAR PERFUSION & METABOLISM. D.K. Anderson, E.D. Means, T.R. Waters* & C.J. Spears*. VA Medical Center, Cincinnati, OH. 45220.

We have demonstrated (Neurosci. Absts. 7:519, 1979) the efficacy of prolonged, high dose methylprednisolone (MP) treatment in enhancing neurologic recovery & reducing the degree of tissue damage in experimentally injured feline spinal cords(SC). The purpose of this study was to determine the effect of MP on SC microvascular perfusion (MVP), carbohydrate & energy metabolism. For this study a total of 53 female mongrel cats, ranging in wt from 2.6 to 4.1kg were anesthetized with Na pentobarbital (30mg/kg i.p) immobilized in a stereotaxic frame & a 1 segment laminectomy was performed at L_2. To study SC MVP, cats were sacrificed at 8hrs by intracardiac perfusion of 10% formalin succeeded by a mixture of colloidal carbon & gelatin following either laminectomy only (n= 4), SC compression (SCC) with a 170gm wt/5 min (n=4) or SCC plus 30mg/kg MP i.v. 1hr postinjury (n=4). The number of colloidal carbon filled vessels were counted in gray & white matter using an eye piece reticule in a photomicroscope at 20 or 40X on the H&E stained sections. Concentrations of carbohydrate & energy metabolites were measured in SC tissue frozen in situ with liquid nitrogen at 8 (n=5) and 24 (n=6) hrs following SCC (170gm/5min) or at 8 (n=10) & 24 (n=6) hrs following SCC plus 30mg/kg MP i.v. 1hr postinjury. Controls for this study were 14 laminectomized but uninjured cats. Frozen SC segments were extracted in $HClO_4$ & analyzed for ATP, ADP, AMP, P-creatine, glu, lact & pyr using the enzymatic fluorometric methods of Lowery & Passonneau. Control SC's averaged 231 & 57 filled vessels/mm^2 of gray & white matter, respectively. Injured, untreated SC's averaged 60 & 14 vessels/mm^2 & MP treated SC's averaged 191 & 39 vessels/mm^2. In both injured treated & untreated SC's at both 8 & 24hrs, levels of ATP, ADP, AMP & P-creatine were significantly subnormal, whereas lact levels were significantly elevated. However, there were no differences in the levels of these metabolites in SC's from injured untreated & MP treated cats at either 8 or 24hrs. The only metabolite differences in the SC's of untreated vs MP treated cats were higher glu levels in the treated cats & higher pyr levels in the untreated cats at 8hr. Our data demonstrates preservation of SC MVP at 8hrs postinjury in MP treated cats perhaps due to the anti-edemic action of MP and/or some as yet unidentified direct vascular effect(s) of this steroid. However, this maintenance of MVP was not reflected in a concommittant recovery of energy metabolites in the SC's of MP treated cats at 8 or 24 hrs. Perhaps preservation of MVP occurred primarily in nonnutrient vessels or MP treatment depressed SC carbohydrate metabolism for the 24hr postinjury period. Additional studies are required to determine the basis for MP protection of the feline SC from SCC. (Supported in part by the VA & the Upjohn Corporation.)

242.8 MORPHOMETRIC STUDY OF SPINAL CORD BLOOD VESSELS IN NORMAL AND CONTUSED FELINE SPINAL CORD. E.D. Means, D.K. Anderson, L. Kalaf* & T.R. Waters* VA Medical Center, Cincinnati, Ohio 45220.

Ischemia appears to play an important role in the pathogenesis of paraplegia following experimental spinal cord injury. The proposed effects of trauma on the spinal cord vasculature include thrombosis, rupture with hemorrhage, spasm & extrinsic pressure on the microcirculation by surrounding edema. To test the hypothesis that extrinsic pressure leads to compression of spinal cord blood vessels following blunt injury, the diameters of the vessels in gray matter were calculated on the normal feline spinal cord (n=4) & at 8 (n=4) & 24 (n=4) hours following compression injury.

The cats were immobilized in a stereotaxic frame following anesthesia with sodium pentobarbital (30mg/kg). The spinal cord was compressed using a 170gm weight for 5 minutes following a one segment laminectomy at the L_2 level. At 8 & 24 hours following injury the animals were sacrificed by intraortic perfusion fixation using a combination of paraformaldehyde-gluteraldehyde. The T_5, L_2 & L_5 segments of the spinal cord were removed in normal animals or the injured segment (usually L_2) in the experimental group. The spinal cord was divided into 2-3mm transverse segments & then processed for electron microscopy. The diameter distribution of spinal cord blood vessels in gray matter was calculated from 1μ toluidine blue stained transverse sections using a Leitz camera lucida attachment adapted to a Nikon photomicroscope. A MOP-3 Kontron (Zeiss) semiautomatic image analyzer interfaced with a PDP-12 computer produced a histogram plot of vessel diameters. In injured animals, histogram plots were calculated for tissue at the center of the lesion & for nonhemorrhagic-edematous tissue at the "pole" of the injury.

Histogram plots (diameter distribution) of blood vessels in normal spinal cord at T_5, L_2 & L_5 displayed a range from 2μ to 65μ with a majority of vessels in the 4-16μ range. The density & diameter distribution of vessels at the T_5, L_2, & L_5 spinal cord segments in normal cats were virtually identical (average density = 212 vessels/mm^2). Injured spinal cord at the L_2 segment through the center & the nonhemorrhagic "poles" of the lesion showed a significant increase in the number of vessels (vessels/mm^2) in the 4-8μ range while there was a decrease of 8-14μ sized vessels.

Preliminary ultrastructural & ultrastructural morphometric data suggest that venules (8-14μ) are diminished in number due to increased transmural pressure as a result of edema formation. We speculate that the post-injury increase in the number of small diameter vessels (4-8μ) is due to opening of previously closed capillary beds secondary to venular collapse & stasis. (Supported in part by the VA & the Upjohn Corporation.)

242.9 GAIT CHANGES IN CATS FOLLOWING CORTICOSPINAL TRACT LESIONS. E. Eidelberg and Jen Yu. Audie L. Murphy VA Hosp. and Depts. of Surgery and PM&R, Univ. of Texas Health Sci. Cent., San Anton. TX 78284.

Eight cats were trained to walk upon a moving treadmill. Subsequently in four of them the motor cortex was ablated bilaterally, and in the other two unilaterally. They were retested on treadmill serially after surgery. Gait analysis was carried out in all four limbs by footfall diagrams, joint angle trajectory measurements and interlimb coordination measurements, using Super 8 movie film data (54 frames per second). We found that all the animals rapidly recovered the ability to locomote, without significant loss of interlimb coordination. Joint angle studies revealed a tendency to increased duration and excursion of joint extension phases. This diminished in the course of time. The sharp and quick wrist flexion in the swing phase, which resembles part of the placing response, was not affected significantly by any of the lesions, although these lesions abolished tactile placing permanently. These results indicate that the corticospinal system does influence locomotor activity, but its role is relatively minor and can be compensated for after lesions.

242.10 ACTIVATION OF MOTOR UNITS IN MAN BY STIMULATION OF ANTERIOR AND POSTERIOR STRUCTURES OF THE SPINAL CORD. M.R. Dimitrijevic, J. Faganel*, J.A.R. Lenman* and P.C. Sharkey*. Dept. of Clinical Neurophysiology, The Institute for Rehabilitation and Research, Houston, TX 77030.

Stimulation of spinal cord structures was accomplished via epidural electrodes in 11 patients, two with the electrodes laterally, two with one electrode on the anterior aspect, and seven with both electrodes near the midline of the dorsal column. (Studies were conducted as a part of the evaluation of continuous spinal cord stimulation for modification of motor control.) Stimuli were applied at a 1 Hz rate, at a motor threshold level, in the form of 0.2 to 0.4 ms pulses, with an amplitude ranging from 6 to 15 mA. Other studies were conducted with stimulus rates up to 150 Hz. EMG responses were recorded using recessed silver-silver chloride electrodes placed 3 cm apart over the muscle belly under study, and in some instances, with a single fiber EMG electrode for estimation of the range of latency variation.

Stimuli applied through the dorsal column will evoke only localized motor responses, confined to those muscles innervated by the stimulated segment. These localized responses are unresponsive to high rates of stimulation, with the one-to-one stimulus-response ratio beginning to fail at frequencies above 10 Hz.

Stimuli to the anterior or lateral portions of the cord evoked responses both at the segmental level of stimulation, and in some cases, at caudal levels. This motor response has a shorter refractory period than does the posterior response, and is responsive to high rates of stimulation, consistent with direct activation of motor neurons.

243.1 GLUCOCORTICOID EFFECTS ON CAT LUMBOSACRAL MOTOR NEURON ACTION POTENTIAL CHARACTERISTICS. E.D. Hall Prog. in Pharmacol. Northeastern Ohio Univ. College of Medicine, Rootstown, Ohio 44272

Previous work has demonstrated that an intensive short-term glucocorticoid regimen in cats enhances certain excitatory parameters of lumbar spinal monosynaptic reflex transmission at least in part through a direct action on the Ia afferent terminals (Hall et al., J Pharmacol. Exp. Ther. 206: 361-370, 1978; Hall and Baker J. Pharmacol. Exp. Ther. 210: 112-115, 1979).

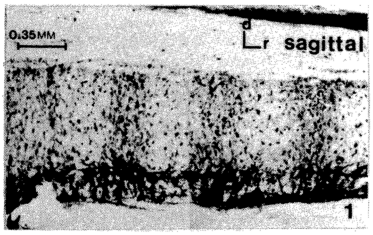
In the present study the actions of an intensive short-term methylprednisolone acetate regimen (MPA, 8 mg/kg i.m. once daily for 7 days) were examined on the characteristics of antidromically-evoked lumbosacral alpha motor neuron action potentials. All experiments were conducted in acute spinal (C-1) cats (1.8-3.5 kg). Supramaximal stimuli (0.2 msec) were applied with bipolar platinum electrodes to the proximal end of the sectioned L7 and S1 ventral roots once per sec. Glass microelectrodes (5-10 megohms) filled with 2M potassium acetate were used for recording. Data was obtained from a total of 37 antidromically activated motor neurons from 11 MPA treated cats vs. 34 cells from 12 untreated animals. Cells with resting potentials less than 50mV were discarded.

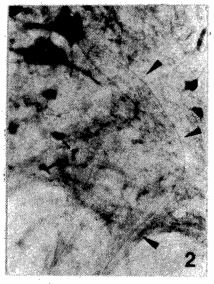
As a result of the 7 day MPA pretreatment the mean resting membrane potential was 4.2 mV more negative (-68.1) than in the untreated animals (-63.9), $p<0.002$ by t-test. Secondly, the latency from the antidromic ventral root stimulus to the beginning of conduction of the action potential through the initial axon segment was shortened from 0.67 to 0.59 msec ($p<0.03$) in the MPA treated animals. However, the conduction of the action potential through the initial segment was prolonged from 0.26 to 0.36 msec ($p<0.0002$) after MPA treatment. The only other significant change in the motor neuron action potential was a decrease in the zero overshoot of the soma-dendritic portion from 14.6 mV in the untreated neurons to 8.8 mV ($p<0.0001$) in the treated ones. This effect, however, appears to be due to the MPA-induced resting hyperpolarization since the absolute amplitude of the action potential spike was not significantly different, 78.4 mV in the untreated and 77.0 mV in the treated ($p<0.2$). The threshold and duration of the soma-dendritic spike and the after potential amplitudes were not affected by MPA.

These results demonstrate that the motor neuron is also a site of glucocorticoid action. The resting hyperpolarization, the increased myelinated axon conduction velocity and the slowed conduction through the unmyelinated initial segment undoubtedly reflect a direct glucocorticoid effect to produce a complex alteration in specific neuronal ionic conductance mechanisms. (Supported by the Amyotrophic Lateral Sclerosis Society of America and by NIMH 34111.

243.2 ALTERNATING TRANSVERSE AChE RICH AND AChE POOR BANDS IN LAMINAE VI AND VII IN THE LUMBOSACRAL CORD OF THE RAT. R. Marchand and H. Barbeau. Centre de neurobiologie, Hôpital de l'Enfant-Jésus, Québec, Qué. G1J 1Z4.

The pharmaco-histochemical method for the demonstration of acetylcholinesterase (AChE) has been applied. Ten rats were treated with di-isopropylfluorophosphate at various time intervals before sacrifice and their lumbosacral cord sectioned in either the sagittal, horizontal or transverse plane. Following this treatment AChE activity is greatly reduced in the neuropile of all regions including the dorsal horn where the activity of the enzyme is normally very diffuse. The fairly large cells of lamina I display an intense AChE activity. Lamina II is characterized by an AChE positive neuropile where tightly packed small cells displaya moderate AChE activity. Lamina III contains less densely packed neurons which display a light to moderate AChE activity in an unstained neuropile. In horizontal and sagittal sections the lateral zone of lamina VI and lamina VII show alternate AChE rich and AChE poor bands giving to this region the typical aspect seen in figure I. The AChE rich bands contain more AChE positive neurons which are predominantly orientated in the transverse plane. Long dendrites originating from the ventrolateral motor column are also present at the same level. Large AChE neurons which may correspond to the proprioneurons also occupy lamina VII. The cell bodies and processes of the neurons of the nucleus of Clark, the nucleusof the lateral funiculus and of the intermedio-lateral and medial columns also display AChE activity. In lamina IX, intensely stained large and medium sized neurons are grouped in well-defined columns inside which long dendrites are mainly orientated rostrocaudally and arranged in dendritic bundles. Some dendrites originating from the ventromedial and ventrolateral columns also converge inside laminae VIII and VII to form dendrites bundles. Other dendrites also extend dorsally along the lateral funiculus in the AChE rich bands toward the nucleus reticularis or far across the lateral funiculus. Three types of neurons may be easily identified in lamina IX. Large and medium intensely reactive cells compose most of the motor cell columns and likely correspond to the α and γ motoneurons. The third type possesses a small cell body with moderate AChE activity and occupies the ventromedial neuropile of the lamina IX near the area radiculares ventralis (fig. 2). They may possibly correspond to Renshaw cells.





243.3 DISTRIBUTION OF MONOSYNAPTIC SEGMENTAL INPUT TO CAT SPLENIUS AND BIVENTER MOTONEURONS. E. E. BRINK*, K. Jinnai*, and V. J. Wilson. Lab. of Neurophysiology, The Rockefeller University, N.Y.C. 10021.

Splenius (Sp) and biventer cervicis (Biv) are both considered single muscles but they are subdivided into compartments by tendinous inscriptions and receive innervation from several upper cervical segments (1). Do Sp and Biv motoneurons receive monosynaptic (presumably Ia) spindle input from the whole muscle or only from the compartment innervated by their axon? We have studied this problem by recording intracellularly from motoneurons in C2-C4 (with axons in C2-C4 nerves) in cats under nembutal anesthesia and stimulating branches of C1-C5 muscle nerves. Results from Sp and Biv are similar. Although the origin of monosynaptic input is restricted it does not correspond to compartments and has a rostro-caudal asymmetry. Table 1 shows the distribution of inputs judged monosynaptic to Sp motoneurons.

Muscle compartment	(1)	(1)	(2)	(3)
Nerve stimulated	C1	C2	C3	C4
segment recorded				
C2	0/4	12/12 (850)	12/12 (285)	4/9 (190)
C3	0/3	1/8 (80)	10/10 (680)	10/10 (215)
C4	0/2	1/10 (70)	2/9 (130)	11/11 (1270)

Numbers in brackets are mean EPSPs (in μV) of cases where response was present. Homonymous EPSPs were often not maximal because of antidromic firing.

Motoneurons typically receive monosynaptic EPSPs from afferents in the same nerve and from more caudal nerves. As stimulated nerves get more distant EPSP amplitude decreases and latency increases. There is usually little input from more rostral nerves except that in Biv, stimulation of the most caudal C3 branch evokes EPSPs in C4 motoneurons (6/8). In contrast to the situation in the hindlimb, therefore, motoneurons do not receive monosynaptic input from spindles scattered throughout the muscle. Instead they are influenced by change of length in regions of muscle innervated by their own axons and in more caudal regions, but only occasionally in more rostral regions. This asymmetry, whose functional meaning is not clear at this point, may be due to anatomical factors: ascending Ia collaterals may be longer than descending ones. Supported by N.I.H. grant NS02619.

1. Richmond, F. J. R. and Abrahams, V. C. J. Neurophysiol., 1975, 38, 1312.

243.4 RECURRENT INHIBITION OF Ia INHIBITORY INTERNEURONS AND RENSHAW CELLS DURING FICTIVE LOCOMOTION. Carol A. Pratt and Larry M. Jordan. Dept. of Physiology, Univ. of Manitoba, Winnipeg, Manitoba, Canada.

In non-moving cats, Renshaw cells (RCs) have been shown to inhibit homonymous and heteronomous motoneurons (MNs), Ia inhibitory interneurons (IaINs) projecting to antagonist MNs, and other RCs. Evidence that the recurrent inhibition of IaINs and MNs was diminished during locomotion led to the suggestion that RCs are inhibited during locomotion. It has now been shown, however, that RCs are rhythmically active during fictive locomotion (McCrea, et al., J. Neurophysiol., in press). Evidence that segmental afferents do not inhibit RC activity during normal walking was provided by the demonstration that stimulation of cut ventral roots (VRs) produced a mean reduction in rhythmic MN activity of 69% during controlled treadmill locomotion (Pratt and Jordan, J. Neurophysiol., in press). The present experiments examined whether the activity of IaINs and RCs could also be inhibited by RCs during locomotion.

The activity of IaINs and RCs was recorded during fictive locomotion in postmammillary cats which had been paralyzed with Flaxedil. Fictive locomotion was produced by stimulation of the mesencephalic locomotor region and monitored by recording rhythmic MN activity in an L6 VR filament. Recurrent inhibition was produced by stimulation of the central portion of cut ipsilateral L5-S1 VRs.

In the 6 quadriceps-coupled IaINs studied, VR stimulation resulted in a mean reduction in spontaneous IaIN activity recorded at rest of 93%. During fictive locomotion, rhythmic IaIN activity was inhibited by a mean of 35% during periods of VR stimulation. In all 6 IaINs, the difference between the initial discharge frequency (Fi) and the discharge frequency during VR stimulation (Fs) was found to be significant ($p<.05$). A positive linear relationship was found to exist between the efficacy of the recurrent inhibition of IaINs and Fi ($r=0.97$). These data, plus the finding thatdecreases in IaIN discharge during fictive locomotion tended to occur concurrently with increases in RC activity,suggest that RCs may contribute to the control of the phasing of IaIN activity during locomotion.

VR stimulation was also effective in inhibiting the rhythmic activity of RCs during fictive locomotion. The duration of RC-RC inhibition appears to be about 200 msec.
(Supported by M.R.C. of Canada)

243.5 FUNCTIONAL PROPERTIES OF Ib INTERNEURONS IN SPINALIZED CATS. Corey L. Cleland, W. Zev Rymer and Frank R. Edwards*. Neuroscience Program and Depts. of Physiology and Neurology, Northwestern University Medical School, Chicago, IL 60611.

Because early investigations of spinal interneurons relied primarily on neuronal responses to electrical stimulation of muscle afferents, they did not provide any information about the interneuron's response to natural proprioceptive input. Particularly important is the inability of electrical stimulation to selectively activate golgi tendon organ and secondary spindle afferents without also activating low threshold primary spindle afferents. In order to avoid these difficulties, we have employed natural stimulation which can selectively activate Ia or Ib afferents. One of the initial goals of our study is to systematically describe the response of Ib interneurons to naturally induced activation of tendon organ receptors.

The discharge of 67 interneurons in the intermediate nucleus (depth 1.7-2.8 mm from cord dorsum) of L6-S1 has been extracellularly recorded using tungsten electrodes in decerebrate cats with spinal transection at T12. Interneurons were distinguished from tract neurons by their failure to be antidromically driven by tract stimulation at T12 and from motoneurons by their lack of response to ventral root stimulation. Interneurons were classified according to their responses to natural stimulation of muscle receptors in the lateral and medial gastrocnemius and soleus muscles. Ia interneurons were identified by their selective response to longitudinal tendon vibration at 120-200 Hz at an amplitude of 40-90 um, which specifically stimulates primary spindle afferents; Ib interneurons by their selective response to increases in isometric force induced by tetanic stimulation of either the peripheral nerve or ventral root at 8-20 Hz; secondary interneurons by their response to muscle stretch but lack of response to vibration and isometric force. Convergence from more than one major muscle afferent (i.e. Ia, Ib or II) was not observed.

Thirty-one of 67 interneurons were identified as Ib interneurons. The discharge of all Ib interneurons was substantially increased by isometric force and showed a time course which closely paralleled that of the whole muscle including small fluctuations in force. They were activated at latencies ranging from 0.7 to 8.0 ms from the incoming dorsal root volley, which corresponds to disynaptic or polysynaptic Ib input. Some of those with longer latencies to electrical stimulation showed a prolonged afterdischarge lasting in excess of 1 sec, and excitatory flexor reflex afferent (FRA) convergence from group III and IV muscle afferents.

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243.6 TOPOGRAPHIC ORGANIZATION OF MONOSYNAPTIC REFLEXES IN THE CAT SPINAL CORD. Ronald C. Kramis* and Marc D. Binder (SPON: H.D. Patton). Dept. of Physiol. & Biophys., Univ. of Wash., Seattle, Washington 98195.

It has recently been shown that Ia afferents entering a given spinal segment generate larger postsynaptic population potentials in motoneurons located within that segment than in those of an adjacent segment (Lüscher, Ruenzel and Henneman, J. Neurophysiol. 43: 968-985, 1980). These results suggest that topographic factors as well as neuronal "species recognition" are important in the establishment of Ia afferent-motoneuronal connections. In the experiments reported here analogous results were obtained from comparisons of the amplitude of monosynaptic reflex discharges recorded from a ventral root when different dorsal roots were stimulated. The L_5-S_1 dorsal roots of decapitate spinal cats were placed onto separate stimulating electrodes. Potentiated, monosynaptic reflex discharges were recorded from a ventral root consequent to stimulation of each of the dorsal root filaments individually, and to stimulation of all of them together. The sequence was repeated for each ventral root filament. Subsequently, the individual dorsal and ventral root filaments were split into 2 or 3 sub-divisions, maintaining their rostral-caudal orientation and the protocol was repeated for these subdivisions. The results indicate that up to 80% of the potentiated monosynaptic reflex discharge recorded in the L_5, L_6, or L_7 ventral roots can be attributed to synaptic input entering the cord at the same segment. The S_1 ventral root is different in that it appears to receive nearly equal input from the L_7 and S_1 dorsal roots. However, for all roots including S_1, the rostral portion of a dorsal root generally exerted a greater effect on the rostral portion of the corresponding ventral root than on a caudal portion of the same ventral root. The converse was generally found for a caudal portion of the same dorsal root. These results indicate that topographic factors are prominent in the organization of spinal reflexes. (Supported by NINCDS Grants NS 15404 and NS 00345 and BRS Grant RR 05432)

243.7 ANATOMY OF MONOSYNAPTIC CONTACTS FROM GROUP Ia AFFERENTS TO DEFINED TYPES OF EXTENSOR α MOTONEURONS IN THE CAT. R.E.Burke, M.J.Pinter*, A.Lev Tov* and M.J.O'Donovan*. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205

Horseradish peroxidase (HRP, Type VI, Sigma) was injected by iontophoresis into a functionally identified group Ia afferent (criteria: conduction velocity, stretch response, vibration sensitivity, pause during isometric muscle twitch) from one of the triceps surae heads or from plantaris, and then into 2 - 6 homonymous or synergist α motoneurons, identified as to motor unit type (see Brain Res. 160:347, 1979 for methods). Complete trajectories of Ia collaterals and motoneuron (MN) dendritic trees were reconstructed from serial sections and the numbers and spatial arrangement of presumed synaptic contacts (light microscopy only; op. cit.) onto labeled MNs noted. Although the number of complete reconstructions is thus far limited (4 cats; 4 group Ia afferents; 10 α MNs - 4 type FF, 3 type FR, 3 type S), several points can be made: (1) The number of contacts from a given afferent to a given MN (a "contact system") has varied from 3 to 30 (median 7 for 7 homonymous systems; 4 for 3 heteronymous systems). The material at hand is too limited to determine whether there are systematic differences in contact numbers or spatial positions in relation to motor unit type (see J. Physiol. 196:605, 1968). (2) Labeled contacts on a given MN can occur singly or in local groups of a few (a "contact region") but the whole contact system usually involves multiple contact regions that are widespread on different branches of a given dendrite or entirely different dendrites. (3) A given MN can receive contacts from as many as 3 adjacent collaterals from the same Ia fiber, with contact regions on dendrites both rostral and caudal to the MN cell body. (4) In only 1/10 cases were contact regions confined to the MN soma and basal dendrites; most examples have had contact regions dispersed widely at various anatomical (and presumably electrotonic) distances from the soma, including fine terminal branches. (5) Most of the studied contact systems involve profuse branching in each Ia collateral arborization and the first branch points of importance have usually been those in the base of the dorsal horn (in Rexed's laminae V - VI), where Ia collaterals first form the major branches that eventually reach the ventral horn. If failure of action potential invasion occurs at branch points in Ia collateral arborizations, this process could contribute to amplitude fluctuations seen in single-fiber Ia EPSPs. The available material confirms the hypothesis that Ia contacts are dispersed over the entire receptive surface of α MNs, although many contact systems do not appear to be confined to limited electrotonic loci as has been inferred from electrophysiological evidence.

243.8 SPATIAL DISTRIBUTION OF TRICEPS SURAE SYNERGISTIC MOTOR NUCLEI IN THE LUMBAR SPINAL CORD OF SQUIRREL MONKEY (SAIMIRI). William D. Letbetter, J. Tigges, M. Tigges, N. A. Cross*, and R. L. McBride. Department of Anatomy and Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322.

Retrograde labeling with horseradish peroxidase (HRP) was used to identify all the motoneurons belonging to members of the triceps surae muscle complex in squirrel monkeys. The nerves to medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (S) muscles of the ipsi- and contralateral hindlimbs in each anesthetized (Nembutal) preparation were soaked either singly or in various combinations in a 10% HRP-1.6 mg% hyaluronidase solution for 3 to 4 hours. After a 3-day survival period, the spinal cord segments L5 to S1 were serially sectioned into either 10- or 20-μ sections so that a detailed analysis of the spatial organization of the labeled motor nuclei could be made from 3-dimensional serial reconstructions.

It was established that bilateral symmetry obtains in these motor nuclei for both the number of cells labeled and their spatial distribution. The positions of MG, LG, and S motor nuclei, both relative to each other and relative to the whole triceps surae distribution, were determined to be constant from animal to animal, even though the rostro-caudal position of any one of these nuclei was seen to vary as much as one whole segment (4-5 mm) as determined by dorsal root entry zone borders. Thus, the 8.5 mm-long column of triceps cells always can be found someplace within the 13.5 mm length of spinal cord between the caudal end of S1 segment and the caudal 2 mm of L5 segment. Within the triceps column (which dorso-ventrally and medio-laterally is positioned similarly to its homologue in other species), cells of the MG and S motor nuclei are distributed coextensively in the caudalmost 5 mm of the rostro-caudal dimension. However, the S cells clearly are collected in a ventral tier of their own. The LG cells occupy the rostralmost 6 mm of the triceps column. Of the approximately 650 cells in the triceps column, 200 belong to the MG nucleus, 225 to the LG nucleus, and 225 to the S nucleus. Cell sizes ranged from approximately 20μ to 45μ ("equivalent diameters" calculated from maximum cross-sectional areas), although the major diameter value of some of the cells was as large as 70μ. The distribution of cell sizes appears to be less clearly bimodal than has been demonstrated in other species.

The clearly different distribution of the S motor nucleus in this species as compared to cat may provide a substrate for which experiments can be designed specifically to study principles governing the distribution of monosynaptic spindle afferent input to homonymous motor nuclei.

(Supported by NIH Grant RR-00165 to the Yerkes Regional Primate Research Center, NIH Grant EY-00638, and by NS 11949 from NINCDS)

243.9 VENTRAL ROOT RECORDINGS FOLLOWING MAUTHNER CELL ACTIVATION IN AMPHIBIANS. D. Les Brown and John T. Hackett. Dept. of Physiology, Univ. of Virginia Sch. of Medicine, Charlottesville, VA 22908

The Mauthner cell (MC), its activation and the resultant rapid tail flip are well studied. Less is known about MC-motoneuron interaction. We have examined the spinal component of the rapid tail flip in *Rana catesbeiana* tadpoles *in situ*, cooled to 10° C. Animals used were characterized by hindlimb development ranging from limb bud to functional leg with degenerating tail.

Cranial nerve VIII, in particular the saccular nerve, was stimulated electrically; electrode placement was critical for producing the tail flip. Ventral root (VR) records were obtained from VR III through XIII using suction electrodes. VR records elicited by ipsilateral saccular nerve stimulation showed a small, long latency (5-10 ms) activity which correlated in time with the later phase of the contralateral VR record. The early phase of the contralateral VR record had a definite threshold, shown by its appearance with an increase in stimulus amplitude of 0.4%. The waveform was compound, showing quantal variations in its peaks in response to repeated stimulation. The latency was variable, depending on stimulus amplitude and the VR used; the shortest latency recorded was 3.5 ms. Estimation of MC conduction velocity based on simultaneous records from a pair of contralateral VR is 18 m/s, suggesting the greater part of the delay between stimulation and VR activity occurs prior to MC activation. A similar compound wave was recorded with the suction electrode attached to the ventral surface of the spinal cord distal to VR XIII. The evoked activity was distinctly different from spontaneous activity or the patterned activity associated with swimming. In addition, no muscle contraction was seen in tadpoles with intact limbs nor could electrical responses be recorded in the limb nerves. Similar experiments on an adult bullfrog showed only long latency (20 ms) activity in ipsilateral VR records.

In conclusion, we have shown all-or-none responses evoked in the contralateral spinal cord by saccular nerve stimulation. This is explained by activation of the MC. The earliest response could arise from the MC axon or from a population of cells coupled to the MC. Quantal variation in form of the response is explained by a small pool of spinal neurons which may not always reach threshold for firing. Inability to detect activity in peripheral muscles or nerves implies specificity of MC-motoneuron connectivity. These results will enable us to study MC-motoneuron interactions during metamorphosis.

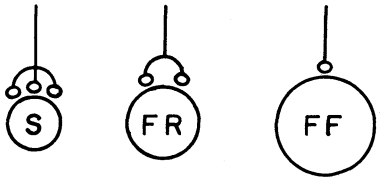
Research supported by NSF and NIDA.

243.10 SINGLE FIBER EPSP AMPLITUDE, CELL SIZE AND MOTOR UNIT TYPE. J.W. Fleshman, G.W. Sypert and J.B. Munson. Depts. of Neuroscience and Neurosurgery, U. of Fla. Coll. of Med. and VA Med. Ctr., Gainesville, FL 32610.

EPSPs elicited in cat medial gastrocnemius (MG) motoneurons by single MG group Ia afferents were recorded using the spike-triggered averaging technique. Input resistance was determined from bridge unbalance during small current pulses. Motoneurons were then classified into motor unit types based on muscle unit responses, using a modification of the criteria of Burke et al. (J. Physiol. 234, 1973): fast twitch, fatigue-sensitive (FF), fast twitch, fatigue-resistant (FR) and slow twitch, fatigue-resistant (S). As shown in the table, single fiber EPSP amplitude decreased in the order S>FR>FF. All paired comparisons were significant ($p<.01$). When FR and S units were combined, it was found that more fatigue-resistant units (FR+S) received group Ia connections than did fatigue-sensitive units (FF) (96% vs. 87%, $p<.03$).

	FF	FR	S
EPSP amplitude (μv)	71	118	179
Input resistance (MΩ)	0.7	1.0	1.5
Conduction velocity (m/s)	101	102	86

All three unit types were found to have different input resistances (R_N), even though the conduction velocity of FF and FR units were nearly identical. To see whether R_N could account for the EPSP amplitude differences, an analysis of covariance was performed using unit type as the variate and R_N as the covariate. After adjusting for the effect of R_N, there was still a strong effect of unit type on EPSP amplitude ($F=9.20$, $p<.0001$). From these results, we propose that the density of group Ia synaptic current increases in the order FF<FR<S, and that this increase is not explained by cell size. (See also Burke and Edgerton, Exer. Sport Sci. Rev. 3, 1975).



This work was supported by the RERDS and MRS of the Veterans Administration and by grant 1 RO1 NS 15913-01.

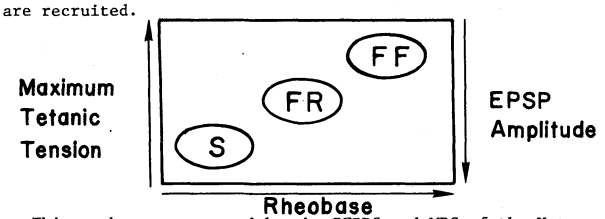
243.11 EXCITABILITY OF TYPE-IDENTIFIED MOTONEURONS. J.B. Munson, G.W. Sypert and J.W. Fleshman. Depts. of Neuroscience and Neurosurgery U. of Fla. Coll. of Med. and VA Med. Ctr., Gainesville, FL 32610.

Cat medial gastrocnemius motoneurons were impaled with micropipette electrodes. Input resistance (R_N) was determined from the bridge unbalance produced by small current pulses. Rheobase (I_{Rh}) was defined as the least current (50 ms pulse) capable of producing an action potential. EPSPs were generated by supramaximal group I stimulation of the lateral gastrocnemius-soleus (LGS) nerve. Motoneurons were identified as type S, FR, FI or FF based on the motor unit's mechanical response to intracellular stimulation, using a modification of the criteria of Burke et al. (J. Physiol. 234, 1973). Maximum tetanic tension (MTT) was determined for each motor unit.

	FF	FI	FR	S
R_N (mΩ)	0.6	0.8	0.9	1.7
I_{Rh} (na)	19.7	17.5	12.8	5.1
LGS EPSP (mv)	1.1	1.5	1.9	2.4
MTT (gm)	77	29	17	7

A very clear relation emerged between rheobase current and motor unit type. I_{Rh} was also highly correlated with R_N ($r=-.70$, $p < .0001$). However an analysis of covariance using R_N as the covariate indicated a strong relation between motor unit type and I_{Rh} (adjusted $F=10.62$, $p < .0001$), regardless of R_N.

I_{Rh} was also highly correlated with LGS EPSP amplitude ($r=-.58$, $p < .007$) and with MTT ($r=.60$, $p < .0001$). These data imply a coherent picture of motor unit recruitment by activation of group Ia synapses. Cells with the lowest rheobase thresholds (most excitable) receive the greatest group I excitation and generate the smallest tetanic tensions. Across the motor unit population, as synaptic drive is increased, motor units with higher rheobase thresholds and greater tetanic tensions are recruited.



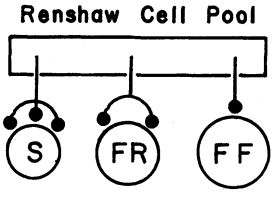
This work was supported by the RERDS and MRS of the Veterans Administration and by grant 1 RO1 N5 15913-01.

243.12 RECURRENT INHIBITION OF TYPE-IDENTIFIED MOTONEURONS. W.A. Friedman*, G.W. Sypert, J.B. Munson, J.W. Fleshman, Depts. of Neurosurgery and Neuroscience, U. of Fla. Coll. of Med. and VA Med. Ctr., Gainesville, FL 32610.

Cat medial gastrocnemius motoneurons were impaled with microelectrodes filled with 3M potassium acetate. Input resistance (R_N) was determined from the bridge unbalance produced by small current pulses. Recurrent IPSPs were generated antidromically, by stimulation of the lateral gastrocnemius-soleus (LGS) nerve, after dorsal rhizotomy of L_6, L_7, and S_1. Motoneurons were identified as type S, FR, FI or FF based on the motor unit's mechanical response to intracellular current injection, using a modification of the criteria of Burke et al. (J. Physiol. 234, 1973). As shown in the table, preliminary data indicate that both recurrent IPSP amplitude and input resistance decreased in the order S > (FR or FI) > FF.

	FF	FI	FR	S
IPSP Amplitude (μv)	101	388	389	1236
Input Resistance (MΩ)	.65	.89	.78	1.34

There was a 12-fold difference in IPSP amplitude between FF and S units, whereas the difference in R_N was approximately 2-fold. An analysis of covariance, with unit type as the variate and R_N as the covariate, was performed. After adjusting for the effect of R_N, there was still a strong effect of unit type on recurrent IPSP amplitude ($F=25.9$, $p < .0001$). These results indicate that the density of recurrent inhibitory synapses increases in the order FF < (FR or FI) < S. This conclusion is in agreement with earlier physiological studies indicating more powerful recurrent inhibition on tonic, as opposed to phasic alpha motoneurons (Kuno, J. Physiol. 149, 1959).



This work was supported by the RERDS and MRS of the Veterans Administration and by grants 1 RO1 NS 15913-01 and 1 F32 NS 06279-01.

243.13 INTERLIMB COORDINATION DURING IN-PHASE STEPPING IN THE CAT, AND TRANSITIONS BETWEEN ALTERNATE AND IN-PHASE. A. W. English* (SPON: R. Fricke) Dept. Anatomy, Emory Univ., Atlanta, GA 30322.

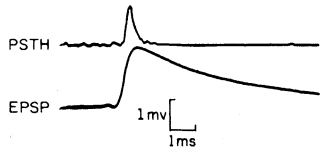
The coordination of step cycles of all four limbs during in-phase stepping and during transitions between alternate and in-phase was studied in 12 adult cats, during repeated overground stepping trials. The temporal spacing of step cycles of the different limbs was determined from analysis of electromyographic activity of single-joint extensor muscles of each limb. Patterns of coordination of the different limbs were established on the basis of the frequency with which the phase separating step cycles was encountered. For the analysis of in-phase stepping, only steps in which the phasing of hindlimb step cycles was closer to true in-phase coupling (0^0) than to alternation (180^0), and where similar phase relationships were present in both the preceeding and succeeding steps were analyzed. Distinct patterns of coordination of step cycles of the two forelimbs and of the forelimbs and hindlimbs were noted under these steady-state phase conditions. Forelimb step cycles were coordinated ca. 90^0 out of phase. Step cycles of the ipsilateral forelimb and hindlimb are coordinated about trotting (180^0), diagonal couplet ($\pm 120^0$) or lateral couplet ($\pm 60^0$) forms of coordination. Transitions between alternate and in-phase stepping were studied by analysis of the phase relationships of step cycles of the different limbs during consecutive steps in which a transition between the two main forms of hindlimb-hindlimb coordination occurred. Phasing of the step cycles of the two hindlimbs, the two forelimbs and of the ipsilateral forelimb and hindlimb all were observed to undergo a shift from one pattern to another during such transitions. Abrupt changes between alternate and in-phase patterns were encountered considerably less frequently. It is concluded that the step cycles of the four limbs are coordinated during in-phase stepping in the cat according to a few basic patterns. Phase angles are clearly grouped about a few preferred patterns. However, the variability about these patterns makes their association with any simple neural circuitry rather speculative. The graded transition between alternate and in-phase stepping is consistent with the notion that such transitions are dependent upon the particular phase relationships of the different limbs at the onset and termination of the transition and that they are not merely abrupt shifts from one motor program to another.

Supported by grant # 1R01 NS15452-01 from the USPHS.

243.14 RELATION BETWEEN SHAPES OF POST-SYNAPTIC POTENTIALS AND CROSS-CORRELOGRAM PEAKS IN CAT MOTONEURONS E.E. Fetz and B.G. Gustafsson*. Dept of Physiol, Univ of Göteborg, Göteborg, Sweden

The effect of postsynaptic potentials on the firing probability of active motoneurons was investigated to determine the relation between the time course of the PSP's and the shape of the resulting cross-correlogram peaks. Several possible relations between EPSP and correlogram shapes have been proposed on theoretical grounds: that the correlogram peak resembles the EPSP itself[1], or its temporal derivitive[2], or some combination of the two[3]. To investigate this relation empirically, we evoked EPSP's and IPSP's in cat lumbar motoneurons by electrical stimulation of peripheral nerve filaments—first leaving the motoneuron quiescent, to document the average PSP shape, and then making the motoneuron fire repetitively with intracellular current injection, to document the post-stimulus time histogram (PSTH) of motoneuron action potentials (i.e., the cross-correlogram between stimulus and spike trains). As illustrated below, EPSP's produced briefer correlogram peaks, typically occurring near the summit of the EPSP. On the average, the PSTH peak began about .53 ms. after onset of the EPSP (n=44). The EPSP's studied ranged in amplitude from .15 - 5 mV. (mean ± S.D. = 0.93 ± 0.74 mV.); the larger EPSP's produced larger and narrower correlogram peaks. The temporal derivative of each EPSP was calculated and its positive component (corresponding to the EPSP rise) was compared with the correlogram peak. In most cases the correlogram peaks resembled the EPSP derivatives, but were displaced by a mean temporal delay of .65 ± .26 ms. The half-widths of the correlogram peaks (.53 ± .37 ms) correlated positively with the half-widths of the EPSP derivatives (.60 ±.25 ms). Following the correlogram peak was a compensatory trough, representing the spikes whose occurrence had been advanced by the EPSP; this trough coincided with the decay (i.e., negative slope) of the EPSP. IPSP's also produced correlogram features which qualitatively resembled their temporal derivatives: a drop in firing probability with the rise and peak of the IPSP, followed by a compensatory increase during their decay. Preliminary results with unitary EPSP's, using spike-triggered averaging from single muscle afferents, suggest a similar relation, although appreciable synaptic noise may broaden the correlogram peaks.

[1]Moore et al., Biophys. J. 10:876; Watt et al., J. N. 39:1375. [2]Knox, Biophys. J. 14:567; Knox & Poppelle, J. N. 40:616. [3]Kirkwood & Sears, J. P. 275:103.



243.15 INTERACTIONS BETWEEN A CENTRAL MOTOR PROGRAM AND SENSORY INPUT IN BULLFROG TADPOLES. Donald J. Stehouwer* and Paul B. Farel (SPON: R.A. King) Dept. Physiol., Sch. Med., Univ. N. Carolina, Chapel Hill, NC 27514.

Spinal motoneurons of the in vitro nervous system of bullfrog larvae exhibit spontaneous episodes of patterned burst discharges which have been shown to underlie swimming. Although the CNS produces the pattern autonomously, the rostrocaudal delay in motoneuronal activation occurring in the normal animal appears to arise from an interaction between the CNS and peripheral influences arriving via the dorsal roots (Stehouwer and Farel, Brain Res., in press).

The present study demonstrates that there are phasic inputs related to tail movements, that dorsal root volleys initiate and modify episodes of patterned burst activity, and that the central motor program regulates transmission from primary afferent terminals.

In the first experiments, the central nervous system was completely removed, leaving long distal stumps of the spinal roots projecting into the vacant vertebral canal. Records were obtained from the dorsal stumps while the tail was manipulated. Rapid oscillation of the tadpole's tail with a pair of forceps or jets of water resulted in bilateral bursts of afferent activity with each movement of the tail. Slowly moving the tail to a fixed, bent position produced afferent activity which ceased when tail movement stopped, regardless of its position. If the tail was held stationary, water jets produced only slight afferent activity. Skinning the preparation did not prevent the response to tail movement. These experiments suggest that proprioceptors in the tadpole tail respond to movement but not static position and that many but perhaps not all of the proprioceptors are found in the muscle rather than in the skin.

The next experiments were conducted in vitro using the isolated nervous system of the larva. Stimulation of the dorsal roots during spontaneous episodes of bursting was found to alter the rate of bursting, usually increasing its frequency. A stimulus presented during one of the silent periods between burst episodes often elicited a burst episode indistinguishable from spontaneous episodes. In no case was a rostrocaudal lag produced. Dorsal root terminals became depolarized at the onset of a burst episode and showed subsequent peaks of depolarization with each ventral root burst. These dorsal root potentials suggest a presynaptic regulation of transmission of movement-induced sensory activity. Supported by NSF grant BNS 24528, NIH grant NS 14899 and NIMH postdoctoral fellowship MH 07409 (DJS).

243.16 NEURONAL RESPONSES TO PASSIVE LIMB DISPLACEMENT IN THE PRECENTRAL MOTOR CORTEX (MI) AND AREA 3a OF UNANESTHETIZED MACAQUES. S. P. Wise and J. Tanji. Lab. of Neurophysiol., NIMH, Bethesda, MD 20205.

Responses to passive foot displacements were examined in 250 single units in the MI and area 3a hindlimb representations of cynomolgus monkeys conditioned to tolerate imposed dorsiflexion and plantarflexion of 20 degrees. A servo-controlled torque motor displaced a pedal attached to the monkey's foot. "Trapezoidal" foot displacements in both directions, at four ramp velocities, and to five amplitudes of maintained displacement demonstrated the direction, velocity, and position sensitivity of cortical neurons. The study employed standard transdural recording techniques with platinum-iridium electrodes. EMG activity monitored during the displacements demonstrated that position related unit activity did not depend upon changes in the tonic activity levels of hindlimb muscles. The units were also tested for responses to tactile stimuli. Those units responsive to such stimuli were termed cutaneous; those unresponsive non-cutaneous.

MI and area 3a responses fell into two categories. One group of units responded both to ramps and maintained displacements and thus resembled the muscle spindle afferent response to muscle stretch. The other group responded only to the ramp phase of passive foot movements. Most (79%) non-cutaneous units, which were concentrated in both area 3a and the rostral part of MI (MI/r), were excited either in relation to passive dorsi- or plantarflexion of the foot, but not both and therefore displayed a direction sensitivity. Cutaneous units, which were concentrated in the caudal 3-4 mm of MI and in areas 3b and 1, rarely (<10%) showed such direction sensitivity. Area 3a unit activity showed greater sensitivity to a given velocity of foot displacement than that of MI/r units and correlated better with foot velocity, as judged by the mean peak firing frequency in response to the ramps. Conversely, MI/r units showed greater sensitivity to foot position than area 3a units. The data support the hypothesis that input corresponding to velocity and position of a limb segment plays a role in cortically mediated regulation of movement and posture and that both area 3a and MI may be involved in this regulation.

The part of the hindlimb sensorimotor cortex which we consider, on electrophysiological grounds, to be area 3a is a region containing a thin internal granular layer (layer IV) which can be distinguished from the caudally adjacent koniocortex (area 3b) and the rostrally adjacent agranular neocortex (area 4).

243.17 RED NUCLEUS NEURON (RNN) DISCHARGE WITH MOVEMENT TERMINATION. Y. Shinoda*, C. Fromm*, E. V. Evarts and J. Kröller* (SPON: J. C. Eberhart). Lab. of Neurophysiology, NIMH, Bethesda, MD 20205.

RNN discharge was recorded in a monkey rewarded for maintaining a handle in one of three positions (15° pronation, neutral, 15° supination) on the basis of a visual display. A shift of the visual display triggered active 15° pronation or supination movements between these three positions. A torque motor coupled to the handle allowed variation of steady state load in each position and provided a means of delivering supinating or pronating ramp displacements to test afferent responsiveness of RNNs.

160 of 229 RNNs recorded from the magnocellular and posterior parvocellular RN showed relations with some aspect of the task, and 34 of these 160 were highly related, i.e., their frequency changed even with the very small corrective supinations or pronations (1.5° or less in amplitude) performed in this paradigm during steady holding in a given position. For most RNNs, changes in frequency failed to precede EMG changes of arm muscles discharging in advance of large, high velocity movements, with RNN discharge starting at movement onset or during movement. The typical time course of RNN discharge (observed for 24 of the 34 highly related units) during the 15° movements was a gradual build-up of activity with peaking near termination. In contrast to this terminal peaking of RNN discharge, the activity of the muscles usually peaked around movement onset, and early peaking was also seen for both precentral and postcentral pyramidal tract neurons (PTNs). Unlike the majority of sensorimotor cortex PTNs, RNN discharge did not vary with different levels of muscle activity in the steady state due to different steady state loads or different steady state holding positions. Another difference between RNNs and PTNs was the relatively low incidence of short-latency afferent responses to passive ramp displacements in RNNs, especially RNNs related to small active movements. Only 8 of the 34 units related to small movements had response latencies less than 50 msec, whereas 20/34 of these units had long-latency responses (>100 ms) to the passive ramp displacements.

Many RNNs failing to discharge prior to large, high velocity movements nevertheless discharged prior to small corrective movements where the starting point of the movement was already close to the terminal point. In view of the findings of previous investigators concerning the action of RN on dynamic gamma motoneurons, it seems possible that accurate termination of a movement involves an increased bias upon the dynamic gamma motoneurons, while the initial acceleration is accompanied by a relative decrease of the dependence on the velocity parameter in the agonist myotatic loop. RNN discharge patterns fit nicely with this putative role in differential regulation of reflex excitability with different phases of movement.

243,18 A "STATE PREDICTOR" MODEL TO DESCRIBE MOTOR CONTROL. Lloyd D. Partridge and Laurel A. Benton*. Univ. Tennessee, Memphis, TN 38163 & Rancho los Amigos Hosp., Downey, CA 90242.

Increasing difficulty is experienced as one attempts to incorporate a feedback model into progressively more complete representations of motor control. Most successful motor actions involve multi-muscle coordination with details which must vary with both starting and ambient conditions within and outside of the muscles. Whether we describe the effector units as pure muscles or as systems with muscle properties modified by "reflex feedback", their coordinated control was found long ago to be adjusted by both response dependent and non-response dependent sensory signals. Presumably, drives to the peripheral systems originate by action of nervous system rules on information distributed centrally following sensory input plus any new central signals. In effect, sensory signals are a continuous but imperfect report on recent states of a vaguely bounded system overlapping the individual and environment. Likewise, new central signals become a command. The rules are described as subject to modification on criteria including their success in driving desired state trajectories. From this viewpoint, to relegate proprioception to a feedback role produces a more complex description than a predictor which uses all sensory signals to directly drive motor units, a form that also easily deals with variation of the dimensions controlled. We propose that both research and current teaching will be better served by use of diagrams of a state predictor model than by continued use and development of the more complex forms needed to describe the same data, based on models of motor control which represent the peripheral elements as separable feedback systems.

244.1 EXPERIMENTAL TREATMENT OF SCHIZOPHRENIA BY HEMODIALYSIS. L.W. Brown*, H. Wagemaker, Jr.*, and R.S. Levy. Laboratory Biological Psychiatry and Depts. of Biochemistry and Psychiatry, Univ. of Louisville School of Medicine, Louisville, KY 40292.

Fifteen chronically-ill schizophrenic patients were hemodialyzed for 5 hours twice a week for 8 weeks (16 sessions). Ten patients became relatively symptom-free; two showed moderate improvement; three were not affected. We examined the dialysates to determine if removal of specific compounds could be linked to remission of symptoms in these patients. For controls we used dialysates from renal patients who were not schizophrenic. We measured creatinine, dopamine, epinephrine, norepinephrine, uric acid, and β-endorphin levels in the dialysates. There were no significant differences in the quantitative removal of these compounds by hemodialysis when schizophrenic patients and controls were compared.

We found a characteristic absorbancy peak with an early retention time when high pressure liquid chromatography was used to analyze the dialysates from some patients. Applying a dialysate sample of 10 µl with a flow rate of 2 ml per minute, the material appeared just after a met-enkephalin reference standard when eluted with an acetonitrile-phosphate solution from a C_{18} reverse phase column using a Varian high pressure liquid chromatography with the UV detector set at 210 nm. This material increased in amount in dialysates from schizophrenics as opposed to those from non-schizophrenics. The characterization of this material will be reported.

244.2 METRIZAMIDE COMPETITIVELY INHIBITS HEXOKINASE. J. M. Bertoni* and G. M. Alexander* (SPON: J. Yu). Lab. of Neurology, The University of Texas Health Science Center, San Antonio, Texas 78284.

Although it is regarded as a safe radiological contrast agent for use in the human cerebrospinal fluid, metrizamide has been increasingly associated with a variety of adverse reactions of unknown mechanism. Because it is a 2-deoxyglucose (2DG) analog, we compared the effects of metrizamide on hexokinase (HK) activity to those of 2DG and 2-amino-2-deoxyglucose (glucosamine). The initial rates of HK were determined at 30° in a reaction coupled with glucose 6 phosphate dehydrogenase (G6PDH) using (final concentration) 0.05 M Tris buffer (pH 7.6), 0.54 mM ATP, 0.58 mM nicotinamide adenine dinucleotide phosphate (NADP), 7.3 mM $MgCl_2$, and from 0 to 0.16 mM glucose. NADPH formation was then followed spectrophotometrically at 340 nm:

$$\text{GLUCOSE} + \text{ATP} \xrightarrow{\text{HK}} \text{G6P} + \text{ADP}$$

$$\text{G6P} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} \text{6-PHOSPHOGLUCONATE} + \text{NADPH} + \text{H}^+$$

HK is rate limiting in this system.

The Km for glucose of HK is 0.12 mM. At 0.4, 1.0, and 2.0 mM inhibitor concentrations, the Km changes to 0.24, 0.33 and 0.78 mM for metrizamide; 0.17, 0.36 and 0.06 mM for 2DG; and 0.14, 0.23 and 0.49 for glucosamine. The Ki for each inhibitor is between 1 and 2 mM. These agents do not affect the Vmax of HK to a significant degree. 2.0 mM mannitol and metrizoic acid have no effect on either the Km for glucose or Vmax of HK. 2.0 mM metrizamide, 2DG and glucosamine have no effect on the Vmax or Km for glucose of G6PDH. 2DG is an effective substrate in this system, although the maximal rate of NADPH formation with 2DG is less than that with glucose. We conclude that metrizamide is a competitive inhibitor of HK by virtue of its similarity to 2DG, and that its presence in cerebrospinal fluid may be detrimental to glucose metabolism. Furthermore, the possibility that 2DG6P is metabolized through the pentose phosphate shunt has implications regarding the fate of 2DG used in quantifying metabolic rates. These studies utilizing microbial enzymes should be confirmed using enzymes purified from mammalian brain.

244.3 SELECTIVE ACTIVATION OF PIGEON nBOR WITH VERTICAL WHOLE-FIELD MOVEMENT AS REVEALED BY ^{14}C-2DG AUTORADIOGRAPHY. B.J. Frost, P. Ramm*, and B. Morgan*. Dept. of Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Anatomical evidence shows that the nucleus of the basal optic root (nBOR) in pigeons receives input directly from displaced ganglion cells and projects to the oculomotor complex and vestibulocerebellum (Brecha and Karten, 1979, Brecha, Karten, and Hunt, in press). Recent physiological investigations have shown that cells in this nucleus in pigeons (Morgan and Frost, 1979) and chickens (Burns and Wallman, 1979) and in the medial terminal nucleus in rabbits (Simpson, Soodak and Hess, 1979) have very large receptive fields and in general prefer large textured patterns moved slowly in upward directions.

In this experiment lightly anesthetized pigeons viewed a multifaceted tangent screen that allowed their right monocular visual field to be completely filled with a random noise pattern. The left eye was occluded. After injection of 25 µci of ^{14}2-2DG the pattern was moved at 2° sec^{-1} in either an upward or downward direction. Autoradiographs of coronal sections in the region of nBOR reveal marked increases in density in the contralteral nBOR for birds receiving upward movement. Downward movement produced only slight increases in density. The increased density produced by upward movement was not found throughout the entire nBOR but was confined to a medial and ventral band. The slight increases in density produced by downward movement were somewhat more diffuse with a tendency to be more laterally located. These results complement and confirm electrophysiological findings which suggest nBOR processes vertical pursuit slip required for optokinetic stabilization.

244.4 RABBIT NICTITATING MEMBRANE CONDITIONING STUDIED WITH ^{14}C 2-DEOXYGLUCOSE. L. Sargent Jones and J.F. Disterhoft. Department of Anatomy, Northwestern Univ. Med. Sch., Chicago, IL., 60611.

The ^{14}C 2-DG method can be used to study the rabbit auditory pathway (Jones and Disterhoft, Neurosci. Abst., 5, 1979, 23). The present study examined brain regions in rabbit thought to be involved in tone signalled nictitating membrane (NM) conditioning with this technique.

The 5 groups were conditioned, pseudoconditioned, tone alone, shock alone, unstimulated control. Four conditioned Ss received 2 days of a training paradigm which paired a 400 msec, 2 KHz, 80 dB tone with a 7 V shock delivered at the 250th msec of the tone. The shock pulse produced the NM reflex and a blink attempt but did not elicit any other overt behavioral response. One trial was given per min at ITIs between 20 and 100 sec; 100 trials were presented per session. The Ss showed conditioned responses about the 130th trial and were overtrained by 200 trials. On day 3 Ss were primed with 20 trials and then injected i.v. with the 2-DG. After 45 trials the Ss were decapitated and the brains processed. Four pseudoconditioned Ss received unpaired random tones and shocks; 3 tone alone and 4 shock alone Ss received just tones or shocks; 3 controls neither.

X-rays from all 18 rabbits were similar on inspection. Structures from all Ss were analyzed by scanning densitometry to see if density differences existed. No statistically significant differences were present between brain regions. The right inferior colliculus (IC), the left dorsal cochlear nucleus and the left medial nucleus of the trapezoid body had the highest density; the VIIth nucleus was lower; and the VIth, IIIrd, the primary sensory portion of the Vth nucleus on the left and the right dentate gyrus of the hippocampus had the lowest density. Failure to show differences led to the densitometric analysis of data reported previously (Jones and Disterhoft, '79). These rabbits had received a 50 msec tone once per sec for 45 min. Data from the right IC of this group was significantly different from that of the present work. Once per sec Ss showed an average increase of 50% in right IC density over any group in the learning study.

The lack of a measurable change in the overall pattern of 2-DG uptake after NM conditioning appears to be due to the fact that one trial per min in this paradigm is not sufficiently perturbing to induce significant changes above the normal metabolic levels as measured by scanning microdensitometry.

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244.5 DIRECT APPLICATION OF DOPAMINE TO RAT STRIATUM DECREASES GLUCOSE UTILIZATION IN THE STRIATUM AND RELATED STRUCTURES. Lucy L. Brown and Leslie I. Wolfson*. Dept. of Neurology, Albert Einstein College of Medicine, Bronx, New York 10461.

Tracer amounts of ^{14}C deoxyglucose (DG) were used to quantify glucose utilization (GU) in rats by the autoradiographic method of Sokoloff et al. (1977). Ten days after placement of intrastriatal cannulae, 21 rats were tested for behavioral effects of intrastriatal dopamine (DA) (50 ug DA HCl in 0.5 ul of a 0.1% solution of ascorbic acid in Ringer's; pretreatment: 50 mg/kg pargyline, 30 min before DA). All rats showed a contralateral body twist or active contralateral turning after DA injection. At least two days later, the rats were partially restrained and given an IV bolus (0.5 cc) of DG (50 uCi) at variable intervals (10 to 80 min) after an intrastriatal injection of DA (N = 16) or of the vehicle solution alone (N = 5). Forty-five min after DG injection, rats were decapitated and brains were prepared for autoradiographic study. The results showed that, with only one exception, DA caused a decrease in striatal GU which varied in area and in magnitude among animals, probably because (a) the amount of DA effectively delivered varied, and (b) some cannulae were placed at the very edge of the striatum. Four of five animals injected with DG 45 to 80 min after intrastriatal DA showed less dramatic decreases than did eleven others injected within 10 to 25 min. This effect of DA over time on GU may be significant because we also observed that the latency to turn or orient contralaterally was twenty minutes or more. A consistent decrease in GU was also seen in the ventrobasal thalamic area. A more variable change in GU was found in substantia nigra: animals with large striatal GU decreases also had a decrease in substantia nigra; animals with small striatal GU decreases had an increase in substantia nigra. None of the patterns of GU described above differentiated the active turners (N = 3) from those who maintained a contralateral body twist (N = 13). Thus, intrastriatal injections of DA which produce postural asymmetries also cause (a) a decrease in striatal GU, and (b) a consistently large GU decrease in ventrobasal thalamus. The variable effects seen in substantia nigra may not be important for the behavioral consequences of intrastriatal DA. Supported by NIH Grant NS09649.

244.6 THE METABOLIC RESPONSE TO INCREASING AMOUNTS OF EXPOSURE TO A VISUAL STIMULUS. A.W. Toga, and R.C. Collins, Dept. of Neurology, Wash. Univ. Sch. of Med., St. Louis, MO 63110

The functional organization of the rat visual system has been previously studied using ^{14}C-2-deoxyglucose autoradiography (2DG). Changes in glucose utilization reflect the intensity, the frequency of presentations and the pattern of the visual stimuli. However, little is known about the metabolic response to prolonged exposure to a stimulus.

One day prior to stimulus presentation hooded rats had one eye enucleated and femoral arterial and venous catheters inserted to allow free movement. Animals were fasted for 12 hours in the stimulus chamber. Full field photo flash was presented at 16.5/s. To study the effect of stimulus experience animals were assigned to one of 3 groups (N=3); novel, 5 hrs or 10 hrs of continuous flashing. In the novel group, animals received 60 uCi/kg of 14C-2DG at the same time the stimulus was initiated. The remaining animals received the 2DG following the assigned number of hours of experience with the stimulus continuing for the duration of the experiment. All subjects received homatropine prior to 2DG administration. Timed blood samples were drawn to determine plasma glucose and specific activity levels. Animals were sacrificed, perfuse-fixed, frozen, and brains cut for autoradiography.

Visual structures innervated by the stimulated eye compared to enucleated showed an increase glucose utilization regardless of the amount of experience. In all experimental groups, the greatest metabolic response was seen in the stimulated dorsal superior colliculus and dorsal lateral geniculate. Glucose utilization for these two structures in animals of the novel group was 1.56 ± .35 (mean ± S.D.) versus .53 ± .10 (enucleated) and 1.03 ± .38 versus .57 ± .20 μmoles/g/min. The metabolic response of the visual system was slightly depressed in animals exposed for 5 hrs compared to the novel group. Animals exposed for 10 hrs showed a bilateral increase in glucose utilization throughout brain. The ratio of stimulated/enucleated visual structures was also greatest in the 10 hr group. In addition, assymmetrical changes were noted in globus pallidus, entopeduncularis and lateral habenular nucleus.

This time-response curve indicates that glucose utilization in the visual system becomes depressed following experience with photic flash (5 hrs) but that with prolonged exposure (10 hrs) the metabolic requirements become greater.

244.7 EFFECTS OF LITHIUM TREATMENT ON MYO-INOSITOL-1-PHOSPHATE LEVELS IN SYNAPTOSOMES OF RAT CEREBRAL CORTEX. R.W. Wise*, A.L. Leavitt* and W.R. Sherman* (SPON: B.K. Hartman). Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

Lithium chloride, when administered to rats, has been shown to decrease the levels of myo-inositol (Allison, J.H. and Stewart, M.A., Nature, 233:267, 1971) and to increase the levels of myo-inositol-1-phosphate (MIP) (Allison, J.H. et al, Biochem. Biophys. Res. Commun. 71:664, 1976) in cerebral cortex of the rat. Both of these effects have been shown to be blocked by atropine. We have undertaken a study to determine whether the increased levels of MIP could be localized in subcellular fractions of the cerebral cortex.

In one experiment rats weighing about 350g were given 10 meq/kg LiCl intraperitoneally 18 hours before decapitation and removal of the cerebral cortex. Cortex was homogenized in 0.32 M sucrose and subcellular fractions prepared by Ficoll density gradient centrifugation. Results show an 8.3-fold increase in MIP levels in the synaptosome fraction from lithium treated animals with respect to controls.

The effect of lithium on synaptosome preparations in vitro will be discussed.

enriched fractions	mmoles MIP/kg protein	
	lithium	control
microsomes	1.56	<0.1
myelin	<0.1	<0.1
synaptosomes	4.00	0.48
mitochondria	1.37	1.58
low speed pellet	0.96	0.32

244.8 SPECTROPHOTOMETRIC MONITORING OF CEREBRAL CYTOCHROME c OXIDASE REDOX STATES AND HEMOGLOBIN SATURATION DURING HYPOTENSIVE SHOCK. A.L. Sylvia, H.J. Proctor* and F.F. Jöbsis*. Dept. of Physiology Duke University Medical Center, Durham, NC 27710 and Dept. of Surgery, U.N.C. School of Medicine, Chapel Hill, NC 27514

It has been recognized that severe blood loss results in hypotension and tissue hypoperfusion, and when not corrected early results in irreversible cellular brain damage. In many instances, despite correction of hypoxia and hypotension, fatal cerebral dysfunction still occurs.

In the present study, quadruple beam spectrophotometry was used to continuously and simultaneously monitor changes in the reduction/oxidation (redox) level of cytochrome c oxidase (Cyt. a,a_3) and hemoglobin saturation states (HbO_2) in rat brain during the course of hypotensive shock. By taking advantage of known absorption spectra for reduced Cyt. a,a_3 (605 nm) and oxygenated hemoglobin (577 nm), it is possible to noninvasively and nondestructively measure such parameters in the central nervous system.

Animals were anesthetized, tracheotomized, and femoral arteries and veins were cannulated. The rats were ventilated using 30% O_2/70% N_2. After baseline stabilization and established normal blood gases (PaO_2 100+ mm Hg; $PaCO_2$ 35-40 mm Hg) were obtained, the animals were subjected to a controlled step-wise hemorrhagic hypotension (MABP 30 mm Hg) plus hypoxia (FiO_2 8%) for a 30 minute period. The animals were subsequently resuscitated by reinfusion of the shed blood plus equal volumes of saline and by readministration of 30% O_2/70% N_2. Monitoring was performed continuously, i.e. during shock, end shock (ES) and both at 20 and 120 minutes after shock (S+20; S+120). The data are presented as absorbance differences (O.D. units) from the originally established baseline signals. We observed that Cyt. a,a_3 became increasingly reduced and failed to re-oxidize at a time when intracerebral hemoglobin became reoxygenated and the MABP and PaO_2 were restored to clinically acceptable values.

This work was partially supported by Grant NIH-AG00517.

244.9 OXIDATIVE METABOLIC ACTIVITY OF THE CEREBRAL CORTEX AFTER HEAD INJURY IN THE CAT RB Duckrow*, JC LaManna, M Rosenthal, JE Levasseur*, and JL Patterson, Jr* Department of Neurology, University of Miami School of Medicine, Miami, FL 33101 and Department of Medicine, Medical College of Virginia, Richmond, VA 23298

To assess the metabolic and vascular effects of head trauma, fluid-percussion pressure waves were transmitted to the brains of seven paralyzed and artificially ventilated cats using the technique of Sullivan (J Neurosurg 45:520,1976). Arterial blood gas tensions and pH were measured and maintained within physiological limits. Changes in the redox state of cytochrome a,a_3 and relative local blood volume were measured in situ by dual-wavelength reflection spectrophotometry of the cortical surface viewed through an acrylic cranial window implanted within the closed skull. Initial fluid-percussion impacts of 0.5 to 2.8 atmospheres peak pressure produced consistent transient oxidation of cytochrome a,a_3 and increases of cortical blood vloume. This oxidation occurred despite the presence of transient post-traumatic hypotension in some cases. Also, impact-induced alterations of vascular tone, indicated by the persistence of increased cortical blood volume, occurred independent of the presence or absence of transient hypertension in the post-traumatic period. Electroencephalographic (EEG) activity was suppressed immediately after impact and recovered in 24 to 72 seconds. Total suppression of EEG activity or persistent changes were not seen. In five animals, intracranial pressure (ICP) either decreased or stayed the same after impact. Two animals showed transient increases in ICP after trauma, but these transients resolved within 1.5 minutes. There were no persistent ICP increases over the two hour (maximum) observation period. These data demonstrate that hypoxia does not play a role in the immediate post-traumatic period in cerebral cortex and are consistent with the idea that following trauma there is increased cortical energy conservation. These data support the concept that head trauma alters the relationship of metabolism and cerebral circulation in the immediate post-trauma period. (Supported in part by Head Trauma Center Grant NS 12587, PHS grants NS 14319 and NS 14325, and a grant from the American Heart Association of Greater Miami)

244.10 COMPARATIVE EFFECTS OF ANOXIA ON EXTRACELLULAR POTASSIUM ION IN THE TURTLE AND RAT. T.J. Sick*, P. Lutz*, M. Rosenthal and J.C. LaManna. Depts. of Neurology and Marine and Atmospheric Science Univ. of Miami, Miami, FL. 33101.

To compare resistances to anoxia, extracellular potassium ion (K^+o) was measured with K^+ sensitive microelectrodes in cerebral cortex of turtles (Pseudemus Scripta) and rats. After establishing resting K^+o values, the rats and turltes were respired with 100% N_2. Brain hypoxia was determined polarographically with platinum microelectrodes and by monitoring the redox state of cytochrome a,a_3 by reflectance spectophotometry. The electrocorticogram (ECoG) was monitored continuously as an index of cerebral function. N_2 respiration in rats resulted in a fall in tissue oxygen tension and reduction of cytochrome a,a_3. These events were accompanied first by a slow elevation of K^+o from approximately 3 mM to 10-12 mM which occurred in 15-30 sec. This slow elevation was followed by a rapid rise in K^+o to 40-60 mM which lasted until N_2 was discontinued. The ECoG became isoelectric during the initial slow elevation of K^+o and remained isoelectric for 3-5 min after N_2 had been discontinued. In the turtle, respiration with 100% N_2 also resulted in a fall in tissue oxygen tension and reduction of cytochrome a,a_3 but the changes were considerably slower than those of rat. However, K^+o in turtle cortex remained at or near resting levels (4 mM) for over 3 hr. The amplitude of the ECoG was gradually reduced despite maintenance of K^+o at low levels, but the turtle brain remained excitable as demonstrated by a local negative DC shift and transient elevation of K^+o in response to direct cortical stimulation. To determine whether differences in body temperature are responsible for turtle's resistance to anoxia, rats were cooled from 37° C to 21-23° C. This resulted in moderate ECoG depression but did not prevent elevation of K^+o in response to N_2 and further depression of the ECoG.

These results suggest that there is no obligatory dependence of K^+ homeostasis upon oxidative metabolism in turtle brain in contrast to mammalian CNS. The results suggest, however, that energy demand in the turtle is reduced during anoxia but that depression of neuronal activity is not signalled by a rise in K^+o. In turtle, anoxia is signalled in another manner which results in energy conservation through depression of neuronal activity, without loss of Na-K transmembrane gradients. (Supported in part by PHS grants NS14319, NS14325 and NS06300)

244.11 TWO OPPOSITE EFFECTS ON SENSITIVITY TO INSULIN HYPOGLYCEMIC CONVULSIONS PRODUCED BY GOLD THIOGLUCOSE (GTG) TREATMENT. John Holliday*, Joan Luby*, Kathy Fish* and Mary Ann Marrazzi, Depts. Psychol. and Pharmacol. (Sch. Med.), Wayne State Univ, Detroit, Michigan 48201.

Systemically administered gold thioglucose (GTG) is well known to cause hyperphagia and a resulting obesity and to be associated with histological damage focused relatively selectively in the ventromedial (VMH)-arcuate hypothalamus. GTG lesions also alter the sensitivity to insulin hypoglycemic convulsions. Depending on the time after GTG treatment, this may be a decrease or an increase. Female CBA/J mice were treated intraperitoneally with GTG (0.8mg/g), gold thiomalate (GTM) (0.8mg/g) or saline. (GTM does not produce the selective GTG effects and is a control for non-specific gold thio-compound effects.) At 16 hours, insulin convulsions in the 3 groups were 3.8%, 65.6% and 68.8%, respectively. Similarly, at 24 hours, insulin convulsions were 0%, 25.0% and 31.3% respectively. In contrast to this decreased % convulsions, at 1 week the GTG group had increased % convulsions, 66% as compared to 0 and 6% in the GTM and saline groups. This effect can be seen for a couple of weeks after GTG before obesity becomes a significant complicating factor. Both the increased and decreased sensitivity are seen on the same population of mice tested at different times after the treatments. Blood glucose was the same in all 3 groups for both the increased and decreased % convulsions. In both cases, the difference must be in the brain's convulsive response to hypoglycemia, rather than in the degree of hypoglycemia resulting from insulin. Metrazol induced convulsions are not altered, so that the effects are not non-specific ones on stress or convulsion in general. Thus, GTG lesions seem to be affecting some control center(s) involved in the brain's functional adjustment to hypoglycemia. Moreover, the GTG lesioned glucostat seems to be the composite of 2 opposite regulatory components. Furthermore, the GTG lesion seems to be a relatively discrete brain lesion which alters a metabolic convulsion.

There is no change, however, in the convulsive response to hypoglycemia as early as 3 hours after GTG. Histological damage is visible with cresyl violet at 16 hours but not 3 hours after GTG, suggesting that the functional change does not occur prior to the visible histological damage. Concentration and hence localization of the cytotoxicity of systemically administered GTG, but not other gold thio-sugars, has been suggested to be due to binding of the glucose moiety of GTG to glucoreceptors, allowing the cytotoxicity of the gold thio-portion to be focused at that site. This hypothesis might predict an early functional deficit due to blocking by binding alone during the latency in establishing cytological damage, but this is not supported by these data. (Supported by NIH #5R01 AM 21476)

244.12 EVIDENCE THAT ATP DEPLETION MAY LEAD TO SYNAPTIC TRANSMISSION FAILURE DURING HYPOXIA IN THE IN VITRO HIPPOCAMPAL SLICE. Tim S. Whittingham* and Peter Lipton. Dept. of Physiology, Sch. Med., Univ. of Wisconsin, Madison, WI 53706.

The relatively high levels of phosphocreatine (PCr) and ATP in brain tissue suggest that these high energy phosphate compounds (~P) may be critical for normal neural activity. Recent experiments in our laboratory have indicated that the phosphocreatine system (PCr + ADP $\rightleftharpoons$ ATP + Cr) plays an important role in prolonging normal neuronal function in anoxic conditions, probably by maintaining neuronal ATP levels for a longer period of time.

In this laboratory, using transverse (.5 mm) hippocampal slices, synaptic transmission failure is observed to begin as early as 30-45 seconds after switching from bicarbonate buffer aerated with 95% O_2-5% CO_2 to buffer equilibrated with 95% N_2-5% CO_2. Field potentials are recorded from the dentate granule cell region following bipolar stimulation of the perforant path axons. If a decrease in [ATP] is leading to the hypoxic transmission failure, then there should be a decrease in ATP prior to any observed decrease in the evoked response. Last year we reported that when whole slice ~P levels were measured, there was no significant decrease in [ATP], indicating that a decrease in [ATP] was not leading to hypoxic transmission block. We now have evidence that there is a small, critical pool of ATP whose depletion is leading to hypoxic transmission failure.

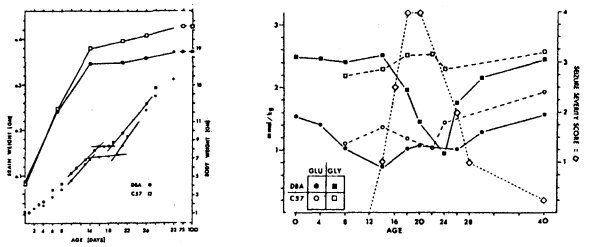
Hippocampal slices were frozen at the time of the first noticeable decrease in the evoked response (~35 sec). Earlier experiments indicated that the site of transmission failure was located in the molecular layer, a region predominantly composed of axon terminals and dendrites, which can be dissected away from the surrounding slice tissue. The ~P levels of molecular layer tissue were measured fluorimetrically as described by Lowry and Passonneau (1972). [PCr] has dropped ~32% (from the control value of 40.88±1.94 (SEM) to 27.97±1.56 at the time of first noticeable decrease) while [ATP] dropped ~20% (from 20.85±1.29 to 16.77±1.57). If the phosphocreatine system is of value in maintaining neuronal transmission due to its ATP buffering ability, then, increases in molecular layer [PCr] should result in prolonged neural function during anoxia. Slices were exposed to buffer containing 25 mM creatine for up to 3.5 hours. Over that time there was a gradual increase in [PCr] (from ~40 µMoles/mg protein to ~185 µMoles/mg protein) with no change in [ATP] (20.01±1.14 to 19.24±1.36). Over this same time period, there was a 3-4x increase in the time needed to observe the first noticeable decrease in the evoked response (from ~36 sec to ~2'0"). These experiments indicate that there may be a critical pool of ATP located in the synaptic region of the dentate gyrus, whose depletion results in synaptic transmission failure during anoxia. (USPHS grant# NS15878).

244.13 DEVELOPMENTAL CHANGES IN GLUCOSE AND GLYCOGEN CONCENTRATIONS IN BRAINS OF DBA/2J AND C57BL/6J MICE: POSSIBLE EVIDENCE FOR ENERGY RESERVES DEFICITS UNDERLYING SUSCEPTIBILITY TO AUDIOGENIC SEIZURES. Robert A. Schreiber, Dept. of Biochem., Univ. of TN. Center for the Health Sciences, Memphis, TN. 38163, USA.

During the developmental period characterized by suckling, the brain is primarily dependent of ketone-bodies derived from the dam's high-fat milk as a source of acetyl-CoA. The pups are weaned by the dam when the brain reaches near-adult size, and begins to shift to carbohydrate-derived sources of acetyl-CoA for brain functions.

The onset of susceptibility to audiogenic seizures (AGS) in genetically AGS-prone mice coincides developmentally with the above (see graph below). There may be a lag in the development of adequate immediately available energy reserves to last through a brief period of an external-stimulus-induced large energy expenditure (sec) until energy repletion processes can begin. On stimulation, CNS activity may then become disorganized, resulting in the onset of an AGS (Med. Hypot., 5, 487, 1979).

Since glucose and glycogen provide the bulk of the brain energy reserve, a developmental study was performed to measure the concentrations of these in brain tissue from DBA/2J mice (susceptible to AGS) and C57BL/6J mice (not susceptible). Mice were placed into a 6cm high by 8 cm diameter cage constructed of 1/4 in. hardware cloth for about two min. during which the cage was lifted by its wire handle a few times to the level of a Dewar flask containing liquid N_2. When the mouse showed no signs of agitation on lifting, the cage was plunged into the N_2. The mouse was then placed into a -20°C chamber, and 10 to 20 mg samples of frontal, temporal, cerebellar, and diencephalic regions were taken, weighed, extracted, and assayed for glucose, glycogen, ATP and phosphocreatine. Pooled preliminary data are shown below (N = 2 to 6 mice per age; no significant differences among areas). No difference with age have been found to date in the latter two metabolites.



Supported in part by MH 32583.

244.14 INCREASED ACTIVITY OF ADENOSINE - STIMULATED ADENYL CYCLASE IN CORTEX FOLLOWING ELECTROCONVULSIVE SEIZURES (ECT). Albert Sattin. VA Hospital and Institute of Psychiatric Research, Department of Psychiatry, I.U. Medical Center, Indianapolis, IN 46223

Many psychiatrists consider ECT to be the most efficacious treatment for endogenous depressions (Amer. Psychiat. Assn. Task Force Report 14, Electroconvulsive Therapy, Sept., 1978). Although the mechanism(s) of this "auto-pharmacological" treatment remain unknown, it is believed to work through the time-dependent cumulation of individual seizure effects to an end-point (clinical recovery) that persists following cessation of treatment. Since biogenic amine function may be altered in depression, ECT-induced changes in related systems are of interest. Vetulani et al have shown (N. Schmied. Arch. Pharm. 293:109, 1976) that 8 daily ECT's produce an apparent decrease in the NE-induced accumulation of cyclic AMP (CA) in chopped limbic forebrain that persists for at least 8 days after the last ECT. Chronic tricyclic antidepressant treatment produces similar effects which have also been directly correlated with a decreased number of β-receptors in cerebral cortex (Wolfe et al, J. Pharm. Exper. Therap. 207: 446, 1978).

Male Sprague-Dawley rats, age 2 mo., were given sham ECT or ECT, 60-80 millicoulombs, daily at 9:00 a.m. x 8 days through earclip electrodes. ECT induced seizures that almost always displayed hindlimb extensions. Rats were sacrificed 2 days after the 8th ECT. Cerebral cortex was dissected free of striatum, temporal lobes and anterior frontal cortex. The remaining cortex was chopped, (0.32 mm) randomized and (ca. 100 mg/20 ml) preincubated x 50 minutes in a K-R-B buffer, 37°, then transferred to buffer containing maximal doses of NE, 10μM, adenosine, 200μM or n-ethyl-carboxamide adenosine (NECA, an enzymatically stable analogue supplied by Dr. Jan Wolff), 1.0μM. After 10 minutes, the tissue was frozen in liq. N_2. cA was assayed by the Gilman method after Dow 50 purification. Mean control values in 2 experiments were 4.6 ± 1.1 (15) and 14.3 ± 2.0 (10) p moles/mg protein. Adenosine produced a 15.3-fold increase in sham ECT tissue and a 27-fold increase in ECT tissue (76% increase, $p<0.01$, 2-tailed t-test). In a third experiment, adenosine deaminase, 1U/ml was used to reduce the adenosine background. The NE-induced increase in cA was reduced by 41% in the ECT tissue while the NECA-induced increase was augmented by 38%. The two tissues did not differ in their cA response to NE + NECA which was potentiated 3-fold. These results suggest that ECT increases the responsiveness of α-NE receptors in forebrain by augmenting the adenosine component of the response. (Sattin et al, J. Pharm. Exper. Ther. 192:22, 1975). Other adenosine receptor effects might also be augmented.

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245.1 EFFECT OF TELENCEPHALIC CATECHOLAMINE DEPLETION ON FIXED INTERVAL PECKING IN PIGEONS: A MATTER OF INTERACTION. Irving Goodman, James Zacny*, Augustine Osman*, Albert Azzaro*, & Carol Donovan*. Depts. of Psychology & Neurology, West Virginia U. Morgantown, WV

Fixed interval (FI) key pecking rates are reduced and patterns are disturbed following the destruction of dopamine rich limbic or striatal structures in the pigeon forebrain (Wesp & Goodman, 1978). The present study initially attempted to determine the effects on FI responding of dopamine depletion achieved by destruction of dopamine bearing forebrain inputs, originating in nucleus tegmenti pedunculo pontinus (TPc), presumed homologue of the mammalian substantia nigra.

Intact pigeons, taught to key peck for grain reinforcement, responded on a schedule that had reinforcement delivered after the first response following a 2 min no reinforcement interval (FI-2). Following the establishment of stable response rates and patterns, birds were lesioned bilaterally in or near TPc, either by electrolysis (2mA, dc anodal current for 15 sec), 6-hydroxydopamine (6-OHDA)(8 ug/2uL saline) or pretreatment with desmethylimipramine (DMI)(15 mg/kg) followed by 6-OHDA. Control birds received 2 uL saline in TPc. Animals were run for 30 or more consecutive, daily, postoperative sessions, 30 min/session, on the FI-2 schedule. Initial analysis of postoperative performance was unimpressive; about half of the lesioned subjects showed increases and the other half decreases in response rates in comparison to preoperative performance. Subsequent assays of telencephalic levels of norepinephrine (NE) and dopamine (DA) in lesioned and control animals provided four outcome groups: (1) NE, normal/DA, normal; (2) NE,normal/DA, low; (3) NE, low/DA, normal; and (4) NE, low/DA, low. Low levels were defined as depletion greater than 25% of control level Catecholamine (CA) outcome groups were selectively associated with pecking rate changes. Individuals falling into groups (3) or (4) consistently showed increased key pecking rates from days 7 - 30. However, those in group (2) showed a marked decrease in key pecking over that same period. These data indicate that the reduction of forebrain DA provides insufficient information from which to predict decreased responding; likewise, increased responding is not always associated with a decrease in forebrain NE, since controls, which fell into group (1), were also seen to have an increase in FI responding. An interaction between NE and DA is clearly indicated. These findings in an avian species support the general view that behavioral task demands are not controlled by single neurotransmitter systems and that the analysis of multiple synaptic transmitters and modulators will be necessary to reach higher correlations between brain function and behavior.

245.2 INTRACRANIAL SELF-STIMULATION IN RELATION TO DOPAMINE TERMINALS IN THE SEPTAL AREA OF THE RAT. R. A. Prado-Alcala and R. A. Wise Physiol. Dept., Sch. Med., Natl. Univ. Mexico, Mexico 20, D. F. and Psychol. Dept., Concordia Univ., Montreal, P. Q., Canada H3G 1M8

Anatomical and pharmacological evidence implicate dopamine (DA) systems in the mechanisms of brain stimulation reward. Brain stimulation reward sites have an anatomical distribution in the ventral tegmental area which follows very closely the distribution of DA cells in that region.[1] Intracranial self-stimulation (ICSS) is obtained when electrodes are in the layer of DA cells, but not when they are in the interpeduncular nucleus or the zona reticulata, below, or the medial lemniscus, above, the nigral and ventral tegmental DA cells. The present study was designed to explore the reported correlation between ICSS sites and DA terminal fields particularly in relation to the distinct band of DA terminals on the ventro-medial aspect of the lateral septal nucleus (to which some of the tegmental DA cells project).

DA terminals in the septum are mostly restricted to a dense diagonal ribbon forming the medial border of the lateral septal nucleus throughout its anterior-posterior length. ICSS was tested above, within, and below this sheet of terminals. A moveable electrode was used to test 4-10 placements in a vertical penetration in each animal; rate of responding was assessed at currents ranging from 60 µa down in 2 µa steps to the lowest currents that would sustain responding (threshold).

ICSS did not vary in clear relation to dopaminergic terminal density as revealed by glyoxylic acid-induced fluorescence histochemistry. Self-stimulation was generally strongest in the dorsal region of the septum and was better in the area above the dopamine cells than within them. ICSS was sustained, though not as well, in the medial septal region also. Thus ICSS near the narrow band of DA terminals in the septum does not follow the close relation to DA anatomy that is seen near the cell bodies in the ventral tegmentum. It would thus appear that DA fibers to the septal area are an unlikely candidate for a brain stimulation reward substrate, although non-dopaminergic fibers from the septal area to the ventral tegmentum (conforming to the anatomy of the tegmental DA cell layer perhaps because they terminate there) remains an interesting possibility.

Supported by the National Institute on Drug Abuse (U.S.) to R.W. (DA 01720) and by a fellowship from CONACYT-Mexico to R. P.-A.

[1]Corbett, D. and Wise, R. A., Brain Research, 185:1, 1980.

245.3 HYPERACTIVITY AND IMPAIRED T-MAZE PERFORMANCE AFTER LESION OF MESOLIMBIC DOPAMINE TERMINAL REGIONS. P.H. Kelly and J.J. Shih*. Department of Physiology and Biophysics, University of Southern California, School of Medicine, Los Angeles, CA. 90033.

Rats with electrolytic lesions of the nucleus accumbens and olfactory tubercle were hyperactive compared to controls when tested in photocell cages 18 days after the lesion. The hyperactivity was most marked during the nocturnal phase of the light-dark cycle. Twenty-three days postoperatively, animals were placed on a one hour per day feeding schedule. Acquisition of a position discrimination in a T-maze (five trials per day, four 45mg Noyes pellets per trial) for food reward was begun after familiarization with the maze and a spontaneous alternation test with food in both arms of the maze. During a 10-minute familiarization session with food in both arms, control and lesion groups did not differ in the number of transitions made from one portion of the maze to another. The spontaneous alternation test showed that lesion and control groups did not differ in position preference nor in their tendency to alternate entries into the two arms of the maze. During five days of acquisition training the lesion group was slower to learn the postion preference and made fewer total correct responses. After five days of no training they retained the preference as well as controls. When the rewarded arm of the T-maze was reversed, the lesion group was significantly impaired in reversing the previously learned habit. Since hyperactive children with the "minimal brain dysfunction" syndrome demonstrate learning impairments, there appear to be similiarities between this syndrome and the effects of lesions in regions of mesolimbic dopamine terminals.

245.4 ALTERED BEHAVIOR OF MACAQUES FOLLOWING DOPAMINE INJECTION INTO CAUDATE N., N. ACCUMBENS, AND SUBSTANTIA NIGRA. M.F. Dubach* and D.M. Bowden. Depts of Physical Anthropology and Psychiatry & Behavioral Sciences and Regional Primate Research Center, Univ of Washington, Seattle, WA 98195.

To investigate the neuroanatical substrate of amphetamine psychosis, a technique was devised for making repeated intracerebral (IC) injections of dopamine (DA) into discrete sites in the monkey (Macaca fascicularis). Ninety µg of DA in 0.1 µl injections were made over a period of two minutes via 33 ga needles.

Behavior of the monkey, freely moving in a 4'x4'x5' cage, was recorded on video tape for one hour following an injection of DA or vehicle. Using a digitizer as an ethological recording device, a detailed numerical account of the animal's social behavior and activity pattern was generated. The data represented a continuous tabulation of location, posture, direction of orientation, and proximity to a companion monkey, as well as behavioral activity, including handedness and object involved. The first thirty seconds of each minute were scored, and data were stored on microcomputer floppy disk. Later, computer analysis provided a breakdown of duration and frequency data for each behavior code and each period in the hour, as well as an account of locomotor rate and repetitiveness of path, turning behavior, and stereotypy of activity and posture.

Analysis of early runs in three monkeys has indicated that significant behavioral changes occur within minutes of injection; different behavioral patterns are elicited by DA administered to different sites and the pattern obtained from a given site in a given animal is consistent from test to test. Injections have been made in substantia nigra, nucleus accumbens, and two sites in the head of the caudate nucleus, one at the AP level of the anterior commissure and the other 6 mm more rostral. At least three weeks intervened between injections at a given site.

Effects included changes in oral, locomotor, exploratory, and social behavior, reminiscent of changes observed in monkeys treated peripherally with CNS stimulants. Particularly interesting were increases in social distance, decrease in grooming and being groomed, and increase in grimacing when the companion animal was nearby. Responses to IC-DA in a given site, like behavior of untreated monkeys, varied considerably from one animal to another, but using each animal as its own control, consistent repeatable drug effects have been observed for each site. The results suggest that the neostriatum, as well as mesolimbic sites, may be involved in emotional and social responses to increased stimulation of DA receptors.

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245.5 SPIROPERIDOL-INDUCED ANTAGONISM OF (+)-AMPHETAMINE LETHALITY IN ADULT RATS. P.A. Burger*, J. Malles*, and L.A. Baez. Psychology Dept., Sch. of Medicine, Southern Illinois University at Carbondale, Carbondale, IL 62901.

Previous investigations in this laboratory (Baez, Kerns, & Smith, 1977) have indicated that both the behavioral and lethal actions of (+)-amphetamine sulfate can be successfully blocked by pretreatment with low doses (.25 mg/Kg) of the neuroleptic drug, spiroperidol. Those investigations raised the possibility that spiroperidol might act in a noncompetitive manner in its ability to block the behavioral effects of (+)-amphetamine , since the spiroperidol blockade was not overcome by doses of amphetamine as high as 60.0 mg/Kg. The present study further investigated this problem by exploring the nature of the spiroperidol antagonism of amphetamine lethality.

Phase I of the present study established the LD100 value for (+)-amphetamine sulfate in this laboratory. Adult male Long-Evans rats (225-300 g) housed in isolation, were injected intraperitoneally with (+)-amphetamine sulfate. Mortality counts were taken at four hours post-injection. 80 mg/Kg (+) amphetamine sulfate was found to represent an LD100 value.

Phase II explored the spiroperidol-induced antagonism of (+) amphetamine mortality. Adult male Long-Evans rats were injected subcutaneously with spiroperidol thirty minutes prior to an intraperitoneal injection of 80 mg/Kg (+)-amphetamine sulfate. Spiroperidol (.00078, .0015, .003, .006, .0125, .025, .050, .10 mg/Kg) was found to significantly reduce (+)-amphetamine mortality (χ^2= 22.1;p<.001) at four hours post-injection. A spiroperidol dose of .025 mg/Kg represented an ED100 value for the protective effect.

Phase III investigated the nature of the spiroperidol-induced protective effect. Adult male Long-Evans rats were injected subcutaneously with .025 mg/Kg spiroperidol thirty minutes prior to an intraperitoneal injection of (+)-amphetamine sulfate (40, 80, 90, 100, 120, 140, 160 mg/Kg). Because doses of (+) amphetamine greater than 100 mg/Kg were able to overcome the spiroperidol-induced protection against the lethal action of the agonist, the hypothesis of noncompetitive antagonism was not supported.

245.6 EFFECTS OF CHRONIC ANTIDEPRESSANT TREATMENT ON PASSIVE AVOIDANCE IN OLFACTORY BULBECTOMIZED RATS: IMPORTANCE OF THE TREATMENT-TEST INTERVAL. L. Noreika*, G. Pastor* and J. M. Liebman. Res. Dept., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901

Bilateral olfactory bulbectomy in rats markedly disrupts acquisition of a passive avoidance task. Using a step-down procedure, Van Riezen et al. (Br. J. Pharmacol. 60:521, 1978) have demonstrated that this effect can be reversed by repeated antidepressant (AD) treatment. In the present experiments, which examined this phenomenon in further detail, the interval separating the end of treatment from the passive avoidance test has been found to be crucial for the emergence of this AD-induced effect. These findings suggest that this proposed model of AD activity may reflect induced receptor sensitivity changes.

Two to three weeks after bilateral ablation (by suction, under surgical anesthesia) of the olfactory bulbs, chronic (once daily) i.p. drug treatment was initiated. At various intervals (4, 24, 48 and 72 hr) following the end of treatment, the animals were tested in a shuttlebox passive avoidance task. After completion of this test, brains were removed for visual confirmation of the ablation.

Daily AD treatment for 7 days (imipramine, 5 mg/kg; amitriptyline, 10 mg/kg; doxepin, 30 mg/kg; viloxazine, 5 mg/kg; mianserin, 20 mg/kg; bupropion, 30 mg/kg) attenuated the passive avoidance deficit in olfactory bulbectomized rats. This AD effect was most apparent in animals tested 48 and 72 hr after termination of treatment, and was absent or less consistent when testing took place 4 or 24 hr after completion of these treatments. When imipramine (5 mg/kg) was administered for 2 or 4 days, antagonism of the passive avoidance deficit was less consistent, and no effect was apparent when imipramine was given for one day. Neither haloperidol (0.1 mg/kg x 7 days) nor tranylcypromine (10 mg/kg x 7 days) had any effect at any post-treatment interval. At a high dose (5 mg/kg x 7 days), d-amphetamine reversed the deficit 48 hr after the end of treatment, but not at the 4-hr interval. A lower dose of d-amphetamine (0.5 mg/kg x 7 days) was ineffective.

The fact that the passive avoidance deficit was not reversed until 48-72 hr after repeated AD treatment suggests that an indirect process may be involved rather than a direct action of the AD. Repeated AD treatment is reported to reduce sensitivity of beta-adrenergic receptors in the brain (Nature 268:455, 1977) and to increase that of forebrain serotonin receptors (Science 202: 1303, 1978). The effects of ADs on the olfactory bulbectomy syndrome may reflect such an induced change in receptor sensitivity.

245.7 ANALYSIS OF THE MOTOR STIMULANT EFFECTS INDUCED BY INJECTION OF THE DOPAMINE AGONIST SK&F 38393 INTO THE NUCLEUS ACCUMBENS. Maryann Malesky* and Paulette E. Setler (SPON: R. Krell) Smith Kline & French Laboratories, Philadelphia, Pa. 19101.

SK&F 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine) is a dopamine (DA) agonist having unique properties which include production of contralateral rotation in rats with unilateral lesions of the substantia nigra (SNX), activation of dopamine-sensitive adenylate cyclase, and contralateral rotation after infusion into the denervated caudate nucleus. SK&F 38393 does not stimulate prolactin secretion or produce stereotyped behavior and does not cause emesis in dogs(Setler et al., Eur. J. Pharm. 50 (4), 419-430, 1978). Freedman et al.(Br. J. Pharm. 67(3), 430-431P, 1979) have reported that bilateral injections of SK&F 38393 into the nucleus accumbens (NA) of conscious rats produce a long-lasting increase in locomotor activity. The present study was undertaken to analyze this phenomenon.

Drugs (0.5 µl) were injected bilaterally in the NA of rats through permanently implanted cannulae (A 9.5, V-0.6, L 1.5, König and Klippel, 1963) and motor activity was measured in doughnut-shaped photo-cell activity chambers.

SK&F 38393 produced a dose-related (5.0 - 25.0 µg) increase in forward directed locomotion. Stereotyped behavior was not observed. Locomotor activity reached a maximum between 2 and 3 hours and lasted longer than 5 hours. This effect was antagonized by chlorpromazine or pimozide. These data suggest that the stimulating actions of SK&F 38393 in the NA are mediated by DA receptors as originally postulated for other agents by Woodruff et al. (Brain Res. 115, 233-242, 1976).

SK&F 38393 causes contralateral rotation when injected unilaterally at low doses (0.04-5.0 µg) into the DA depleted caudate nucleus, an effect blocked by chlorpromazine but not by pimozide. Unilateral injection of SK&F 38393 into the intact caudate nucleus is without effect even at high doses (30.0 µg) (Setler et al.; Abstract 1940, SN Meeting, 1979). The data suggest that the locomotor stimulant effects of SK&F 38393 given in NA may be mediated by a dopaminergic mechanism different from that mediating the caudate effects of this DA agonist.

245.8 PRODUCTION OF CONTRALATERAL ROTATION BY INJECTION OF DOPAMINE INTO THE PARS RETICULATA OF THE SUBSTANTIA NIGRA. Joseph T. McDevitt* and Paulette E. Setler. Smith Kline & French Laboratories, Philadelphia, Pennsylvania 19101.

Rotation behavior, produced by electrical stimulation of the striatum, or by dopamine agonists in unilaterally dopamine depleted animals, was originally believed to be due to striatal dopamine receptor activation. More recently, it has been observed that numerous agents when injected into substantia nigra also cause rotation, which could in some cases be due to modulation of the nigro-striatal dopamine pathway.

We have investigated the effects of dopamine stereotaxically injected into the rostral, middle, and caudal segments of the pars reticulata of the substantia nigra (SnR) of male Charles River rats, which were pretreated for 3 hrs with tranylcypromine (1.0 mg/kg, i.p.) and anesthetized with sodium brevital (10.0 mg/kg, i.v.). Dopamine, in concentrations of 5.0, 10.0, or 20.0 µg/0.5 µl, injected into the rostral segment of SnR caused significant dose dependent contralateral rotation, lasting >2 hrs. As the SnR injection sites were moved caudally, contralateral rotation decreased, and at extremely caudal sites dopamine caused slight to moderate ipsilateral rotation.

Another complexity was introduced by the observation that only some, but not all dopamine agonists cause contralateral rotation after intranigral injection. The novel benzazepine dopamine agonist SK&F 38393-A,tested at concentrations of 2.5, 5.0, or 10.0 µg/0.5 µl, also produced contralateral rotation after injections into SnR. Apomorphine, Piribedil, and the ergot derivative lergotrile, were ineffective.

Inhibition of dopamine induced rotation by systemically administered dopamine antagonists suggested that the effect of dopamine in SnR was mediated by dopamine receptors. Dopamine induced contralateral rotation does not appear, however, to be dependent on the nigro-striatal dopamine system. Enhancement rather than antagonism of the intranigral effects of dopamine agonists was observed in animals with prior unilateral 6-hydroxydopamine lesions of the ipsilaterally treated nigra. Similar results in 6-hydroxydopamine lesioned rats have been obtained with the GABA agonist muscimol (Arnt et al., Psychopharm 62, 1979).

The results of our studies suggest that rotational behavior produced by systemic administration of some dopamine agonists may not be entirely due to activation of striatal dopamine receptors, or reflective of nigro-striatal dopamine activity, but may also reflect activation of dopamine receptors in SnR. Which system(s) is modulated by these SnR dopamine receptors remains to be determined.

245.9 EFFECTS OF AMINERGIC STIMULATION OF THE AMYGDALA ON RAT MURICIDAL BEHAVIOR. John F. Miller* and Carlton E. Lints. Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

Although numerous reports have implicated brain serotonin (5HT) in the inhibition of rat muricidal behavior, one frequently quoted study (Leaf et al., 1969) suggested that norepinephrine (NE) may play a similar role within the amygdala. The results of the present experiments provide further support for this suggestion.

In experiment 1, separate groups of established mouse killers were given bilateral intracranial injections of different doses (45, 90 and 180 nmole) of either saline, 5HT, NE or the beta-adrenergic agonist isoproterenol (ISOP) aimed at the central nucleus of the amygdala. Only the 45 and 180 nmole doses of ISOP significantly elevated the kill latencies of the killer rats without significantly affecting their attack latencies. In experiment 2 the bilateral intracranial injection of 300 nmole of either ISOP or NE produced a complete and reversible block of muricide in 60-70% of the rats tested without affecting righting or placing reflexes and without producing any overt signs of motor ataxia.

The results of these experiments support previous reports of noradrenergic inhibition of rat muricidal behavior and suggest that the effect is mediated by beta-adrenergic receptors within the amygdala.

245.10 Dual role of substantia nigra dopamine neurons in mnemonic and motor functions. Ellen H.-J. Kim and Aryeh Routtenberg. Dept. of Psychiatry, MRRC, UCLA, Los Angeles, CA 90024 and Cresap Neurosci. Lab., Northwestern Univ., Evanston, Illinois 60201.

Retention of learned passive avoidance behavior can be disrupted by 5-min post-trial microinjection of either picrotoxin into substantia nigra (SN) or dopamine (DA) into neostriatum in rats (Brain Res., 113, 620, 1976; Neurosci. Abst., 2, 445, 1976). In this study, 6-OH DA, DA, or GHBA (each dissolved in 0.5 µl of physiological saline) was injected unilaterally into SN either 5-min or 22-hr post-learning. Only 6-OH DA (2 µg) injected 5-min post-learning produced a retention deficit. Delayed (22 hr) injection of 6-OH DA was ineffective. The performances on retention testing of DA (50 µg) or GHBA (75 or 100 µg) injected subjects did not differ from those of saline injected controls. Fluorescence microscopic examination of soma-dendritic DA fluorescence of ventral tegmental DA cells in a rat that received SN 6-OH DA 30 min prior to sacrifice indicated that this drug may initially have stimulated the A9 DA neurons. The A10 DA cells appeared to be minimally affected. Thus, hyperactivity of SN DA neurons (i.e., supra-normal release of DA in neostriatum and other areas of DAgic nigral projection), when induced shortly following learning, appears to interfere with the consolidation processes of memory for passive avoidance.

The present 6-OH DA injection caused no overt motoric abnormality. Nor did any of saline vehicle injections. In contrast, both DA and GHBA produced circling away from the side of injection. When DA or GHBA was injected into SN of experimentally naive rats, similar contraversive circling was observed.

In sum, 6-OH DA-induced post-learning alteration suggestive of an increase in the activity of nigroneostriatal DA neurons disrupted retention of learned passive avoidance without eliciting any motor asymmetry. On the other hand, intranigral injections of DA or GHBA that resulted in motor asymmetry did not interfere with passive avoidance retention. This indicates that the neural mechanisms underlying the two phenomena are dissociable. Finally, the fact that both DA and GHBA resulted in similar circling behavior suggests that the actions of the two drugs in SN may share a common route to produce such an asymmetric motor response. One possible route is via the down-stream action of non-DA neurons in SN that receive GABA terminals of extra-nigral origin (Neurosci.Abst. 5, 71, 1979). It is possible that DA produced a result similar to that of GHBA by mimicking dendritic DA action on GABA terminals (Brain Res., 136, 45, 1977), hence causing release of endogenous GABA in SN (Nature, 268, 652, 1977). -Supported by NIMH 25281 & NSF 20630 to A. R.-

245.11 CONTRASTING ROLES OF STRIATAL AND ACCUMBENS DOPAMINE IN MOTOR ACTIVITY IN SQUIRREL MONKEYS. Daniel L. Jones, R. E. Dill, Roy L. Dorris* and Sherry L. Berg*. Depts. Microscopic Anatomy and Pharmacology, Baylor College of Dentistry, Dallas, Tx.

Male squirrel monkeys (10) were chronically cannulated bilaterally in the nucleus accumbens septi and head of the caudate nucleus for subsequent drug injection. In all cases drugs were injected in a volume of 2 µl, followed by 2 µl of saline 2 min later to flush the cannula. Locomotor activity was assessed by means of a photocell activity cage coupled to a Heathkit LSI-11 computer. Catalepsy was judged present if the animal remained suspended (involving the hind limbs) from a 0.25 inch dia. rod for at least 10 min.

Intra-accumbens (i.a.) injection of dopamine (DA) produced a dose-dependent locomotor stimulation following a period of depressed activity the duration of which was dose-related in a positive manner. The DA antagonist, haloperidol (0.05 mg/kg s.c.), given 30 min prior to intracranial injection, completely blocked the locomotor stimulating effects of 25 µg DA given i.a. bilaterally, but did not alter the locomotor effects of 100 µg DA i.a. The depressed phase was not affected by the haloperidol in either case. This level of haloperidol (0.05 mg/kg s.c.) produced catalepsy in 30-40 min in 5 young monkeys (600-800 g) but not in mature animals (900-1100 g). The catalepsy induced by systemic haloperidol was not altered by the i.a. DA (25-100 µg). Haloperidol (0.4 to 10 µg) injected bilaterally i.a. or intra-caudate (i.c.) was not effective in producing catalepsy. However, i.a. haloperidol (10 µg) rapidly blocked ongoing locomotor activity induced by 100 µg i.a. DA. Intra-accumben DA was more effective than i.c. DA (50 µg both sites) in producing locomotor stimulation ($P < 0.01$).

These data confirm in a primate that the i.a. injection of DA is effective in producing locomotor stimulation, while i.a. haloperidol is not effective in producing catalepsy. The caudate does not appear to be critically involved in either motor effect. Supported by NIH Grant NS-15020.

245.12 SULCAL PREFRONTAL CORTICAL SELF-STIMULATION: EFFECTS OF 6-HYDROXYDOPAMINE OR KAINIC ACID LESIONS AT THE SITE OF STIMULATION. C.R. Gerfen, R.M. Clavier, and D.H. Henkelman*. Dept. of Anatomy University of British Columbia, Vancouver, B.C. V6T 1W5

We have previously reported that sulcal cortical intracranial self-stimulation (ICSS) in rats is abolished by 6-hydroxydopamine (6-OHDA) lesions of the A-10 and A-9 dopaminergic (DA) cell systems ipsilateral to the electrode. Contralateral lesions had only a temporary attenuating effect. However, destruction of the A-10 projection to the sulcal cortex was not necessarily implicated in the attenuation of sulcal ICSS since all ascending ipsilateral DA systems were destroyed. To determine whether DA innervation of the sulcal cortex is necessary for sulcal ICSS we have injected 6-OHDA (5 ug in 2.5 ul 0.9% saline with 0.3 mg/ml ascorbic acid) into the area around sulcal stimulation sites using a combined electrode-cannula system. Using this procedure sulcal cortical ICSS was maintained at prelesion rates after the removal of the DA innervation of that cortex. Verification of the lesions was made using the glyoxylic acid technique for catecholamine (CA) histofluorescence of Lindvall and Bjorklund (1974). The procedure revealed normal CA fiber and terminal patterns in the medial prefrontal cortex, nucleus accumbens, and olfactory tubercle ipsilateral to the electrode-lesion. The sulcal cortex, which normally contains a large number of DA fibers and terminals, had only trace numbers of DA fibers around the electrode in 8 of 10 animals tested. These data suggest that DA innervation of the sulcal cortex is not essential for ICSS elicited from that cortex.

In a second study, kainic acid (4 nmoles/1 ul) was injected into the sulcal cortex in sulcal ICSS animals. 7 of 10 animals receiving this treatment stopped bar pressing for sulcal ICSS for the duration of a 21 day post lesion trial period. All 10 animals had extensive neuronal cell loss in the sulcal cortex as determined by cresyl violet staining. However, only those 3 animals that recovered to prelesion ICSS rates had surviving neural perikarya around the electrode tip. In the 7 animals that showed attenuation of sulcal ICSS after the lesions, anterograde degeneration of fibers could be traced from the sulcal cortical ICSS area to, among other areas, the ventral tegmental area (site of the A-10 DA cells) and to the substantia nigra (site of the A-9 DA cell group) with Fink-Heimer staining.

The data presented here combined with our previous report suggest that sulcal ICSS may result from the stimulation of sulcal efferent systems with the possible subsequent activation of DA neural substrates.
Supported by MH 33987 to RMC.

245.13 RELATIONSHIP BETWEEN ENDOGENOUS BRAIN TYROSINE HYDROXYLASE AND SOCIAL BEHAVIOR OF RATS. S.L. Salman, J.M. Weiss*, W.H. Bailey and T.H. Joh. Rockefeller University, and Lab. of Neurobiology, Cornell Univ. Medical College, New York, NY 10021.

In constructing animal models of psychopathology, it would seem highly desirable to develop models in which abnormal behavior could be identified and studied in a natural social context, since abnormal behavior in humans is normally identified within such a context. The well known catecholamine hypothesis identifies disturbances in noradrenergic function as a basis of behavioral depression. Therefore, we asked the question: are there differences in endogenous catecholamine activity among animals in a population which are related to social 'dominance' behavior? Using the basic colony conditions described by Ellison (Brain Res.103:81,1976), we investigated the relationship between the activity of endogenous brain tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, and the behavior of animals in a colony hierarchy.

Male hooded rats were left undisturbed for ten weeks in a large colony enclosure while they established a dominance hierarchy. Behavioral observations were then scored on various measures: boxing, wrestling, fighting,'broadsiding',etc. Animals were then sacrificed, brains dissected into hypothalamus (Hypo), substantia nigra, locus coeruleus (LC), olfactory tubercles, amygdalas, caudate n., and frontal cortex. These regions were sampled for TH activity according to the method of Joh (Reis et. al. JPET 193:775,1975). Significant correlations between TH activity and dominance rank are presented below:

Spearman Rank-Order Correlations Between TH and Dominance Rank.

	Exp. 1 (N=9)	Exp. 2 (N=8)	Exp. 3 (N=9)
LC	.84*	.88*	--+
Hypo	.84*	.45	.63*

* p<.05
\+ samples lost

There was no significant correlation between behavior and TH activity in other brain regions. These data suggest that brain noradrenergic function is related to social dominance in rats. It remains to be determined whether social dominance results in elevated TH activity, or whether high TH activity in LC produces a dominant animal. However, the latter is suggested by recent investigations (Eison et.al. Pharm.Biochem.&Beh. 7:87,1977) in which LC-lesioned animals were found to be dominated in a social environment.

245.14 NORADRENALINE AND DISCRIMINATION LEARNING: FAILURE TO SUPPORT THE ATTENTIONAL HYPOTHESIS. M. Pisa and H.C. Fibiger, Div. Neurol. Sci., Dept. Psychiat., Univ. British Columbia, Vancouver, B.C.

The hypothesis of a role of forebrain noradrenaline (NA) systems in selective attention (Mason, S.T. and Iversen, S.D., Brain Res. Rev.1:107,1979) predicts that lesions of the dorsal noradrenergic bundle should impair both learning and reversal of discrimination tasks with only one relevant dimension available, and should facilitate nonreversal shift. Results of recent studies appear to support these predictions (Mason, S.T. et al., J. Comp.Physiol.Psychol., 1980, in press).

Using either a T maze or a cross maze, vehicle injected rats and rats with bilateral injections of 6-OHDA (4 µg in 2 µl) in the dorsal bundle (DB rats)were trained in either a position, a visual or a response discrimination task. Both the visual (white vs. black) and the kinesthetic (left vs. right turn) dimensions were relevant in the position discrimination. In contrast, the visual dimension was relevant, and the kinesthetic irrelevant, in the visual discrimination, and vice-versa in the response discrimination. On reaching learning criterion, rats of the position discrimination group were trained on multiple reversals, those of the visual and the response discrimination groups were either trained on multiple reversals or shifted to response and to visual discrimination respectively. Surprisingly, no significant differences among control and DB rats were found in either acquisition,reversal, or nonreversal shift, in any of the discrimination tasks.

It has also been reported that DB rats are impaired in left-right alternation learning (Mason, S.T. et al., J. Comp. Physiol. Psychol., 1980, in press) a finding thought to support further the attentional hypothesis. To evaluate whether increasing the distinctiveness of the choice arms had a differential beneficial effect on the alternation performance of DB rats, different groups were trained to alternate in a T maze with either similar grey arms or with distinctive, white vs. black, arms. Both control and DB rats performed significantly better in the T maze with distinctive arms than in that with similar arms. Surprisingly, however, no significant differences between control and DB rats were found either in learning or in retention (two weeks after discontinuation of acquisition training) with progressively increasing delays.

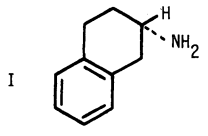
Biochemistry showed that the NA concentration in the cortex of DB rats was reduced to approximately 5-10% of control values.

Thus, previous reports that dorsal bundle lesions impair visual discrimination, facilitate nonreversal shift, and impair spatial alternation could not be confirmed, and no evidence was found to support the attentional hypothesis. Procedural differences possibly responsible for the discrepancy between present and previous results remain to be specified.

M. Pisa is a MRC fellow.

245.15 STIMULANT EFFECTS OF A NEW AMPHETAMINE ANALOGUE: 2-AMINO-1,2-DI HYDRONAPHTHALENE (2-ADN). M.B. Nichols*, G.K.W. Yim, B.A. Hathaway*, and D.E. Nichols. Depts. of Pharmacology and Toxicology and Medicinal Chemistry and Pharmacognosy, Purdue Univ., West Lafayette, IN 47907.

We have previously reported (Nichols et al., Brain Res. Bull., 2:169, 1977) that (-)-2-amino-1,2,3,4-tetrahydronaphthalene (2-AT, I) depresses spontaneous motor activity (SMA) in mice and resembles hallucinogens such as mescaline in its effects. In further extensions of this work we have now synthesized and screened 2-amino-1,2-dihydronaphthalene (2-ADN, II). Unlike 2-AT, which depressed spontaneous motor activity, 2-ADN increased



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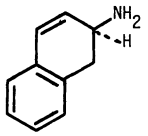


II

SMA. Although it was found that the (-) isomer of 2-ADN is most active, it's stereochemical configuration is opposite to that of (-)-2-AT. A parallel line bioassay indicated that racemic 2-ADN was one-fourth as potent as (+)-amphetamine in increasing SMA. 2-ADN also produces stereotypy similar to that elicited by (+)-amphetamine, indicating that it has an action on dopaminergic nigrostriatal systems. The central effects of 2-ADN were blocked by both reserpine and alpha methyl para tyrosine. Thus, the effects of 2-ADN, like that of ephedrine, may involve the release of catecholamine both from newly synthesized reserpine resistant, as well as from reserpine sensitive pools. It is intriguing that the introduction of a double bond into 2-AT should lead to a reversal in the pharmacological action. This feature may relate to a subtle structural requirement of the catecholamine uptake site. (This work was supported, in part, by Pharmacol. Toxicol. Training Grant GM-709504 (M.B.N.) and USPHS grant DA-01916).

245.16 LOWER ENDOGENOUS LEVELS OF NOREPINEPHRINE AND SEROTONIN IN THE BRAINS OF RATS FROM A GENETICALLY SEIZURE SUSCEPTIBLE STRAIN. H. E. Laird II, L. Chin*and A. L. Picchioni*. Dept. of Pharmacol. and Toxicol., Coll. of Pharmacy, Univ. of AZ., Tucson, AZ. 85721.

It has been previously demonstrated in our laboratory that genetically seizure susceptible (GS) rats have lower thresholds for convulsions due to sound, pentylenetetrazol, minimal, maximal and intracerebral electroshock than genetically seizure resistant (GR) rats (Laird and Huxtable, In: Taurine and Neurological Disorders, 1979; Duplisse et al. J. Int. Res. Comm. 1:(9),1973; Jobe, Picchioni and Chin, J. Pharmacol. Expt. Ther. 184:1-10,1973) A comparison of the endogenous levels of the inhibitory neurotransmitters, norepinephrine (NE) and serotonin (5HT) in the brains of these two strains of rats with differing seizure susceptibilities has revealed that NE and 5HT levels are lower in several areas of the brains of GS rats. Biogenic amines were estimated spectrophotoflurometrically. These results are presented below:

	NE CONTENT ng/g (+SEM)					5HT CONTENT ng/g (+SEM)				
	CH	MB	HT	PM	CB	CH	MB	HT	PM	CB
GR	430 (±30)	560 (±20)	2930 (±160)	710 (±40)	210 (±20)	730 (±40)	1070 (±50)	1950 (±130)	730 (±30)	220 (±10)
GS	270* (±10)	300* (±10)	1740* (±120)	420* (±40)	180 (±10)	600* (±20)	750* (±10)	1670 (±40)	690 (±20)	230 (±10)

* P 0.02 CH=Cerebral Hemispheres; MB=Midbrain; HT=Hypothalamus; PM=Pons-Medulla; CB=Cerebellum

Although this study does not distinguish between the relative roles of NE and 5HT in the modulation of seizure activity, it does provide data that agree with correlative studies involving drug-induced reduction of brain biogenic amine levels and changes in seizure thresholds. Furthermore, these data support the postulation that animals with lower endogenous brain biogenic amine levels have a reduced inhibitory function in the central nervous system (Scudder et al., Neuropharmacol.5:343-51,1966). In view of the differential distribution of the biogenic amines it is tempting to speculate that the low levels of NE in the CH, MB, HT,and the low levels of 5HT in the CH and MB of the GS rats are responsible for the greater seizure sensitivity of these animals.

Supported in part by Epilepsy Foundation of America and NIH grant NS14405.

245.17 THE EFFECT OF PERIPHERALLY ADMINISTERED SEROTONIN ON FOOD INTAKE IN THE RAT. J.D. Pollock* (SPON: D.S. Levine). Psychobiology Program, University of Pittsburgh, Pittsburgh, PA 15260.

The present experiments demonstrate a dose dependent suppression of food intake by serotonin (0.5 to 5.0 mg/kg free base) with or without the MAO inhibitor, clorgyline, 5.0 mg/kg ($p < .05$) in rats. This effect cannot be attributed to the creatinine in the serotonin creatinine sulfate complex since creatinine equal to the amount in the highest dose of serotonin creatinine had no effect. The high dose of serotonin, 5.0 mg/kg serotonin free base, particularly with clorgyline showed motor impairment as revealed by front end catalepsy. At this dose no significant impairment was observed on open field tests and back end catalepsy. Lower doses of serotonin creatinine sulfate (free base) manifest no apparent motor impairment. These results suggest that the dose dependent suppression of food intake at doses less than 5.0 mg/kg serotonin free base is not related to motor impairment. The relationship to a peripheral satiety mechanism and symptoms of the carcinoid syndrome will be discussed.

245.18 SELF-STIMULATION OF THE MEDIAL PREFRONTAL CORTEX DOES NOT INVOLVE THE MEDIAL FOREBRAIN BUNDLE. D. R. Corbett, A. Laferriere and P. Milner. (SPON: N. White). Dept. of Psych., McGill Univ., Montreal, Quebec, H3A1B1.

Intracranial self-stimulation (ICSS) has been reported from two areas of the neocortex, the medial prefrontal cortex and the sulcal prefrontal cortex (Routtenberg & Sloan, 1972). Opinion has been divided as to the neural substrates of ICSS in these two areas. Some have suggested that the ascending dopaminergic systems underlie the observed ICSS, while others have argued that descending systems from the cortex are responsible. Since both of these neural networks traverse the medial forebrain bundle (MFB) and peri-capsular regions we sought to assess their involvement in ICSS from the medial prefrontal cortex.

Each animal was implanted with a bipolar stimulating electrode in the infralimbic area (area 32) of the medial prefrontal cortex and a monopolar lesioning electrode on the far-lateral border of the MFB, ipsilateral to the stimulating electrode. The rats were then trained to lever press for brain stimulation in daily 15 min. test sessions. Once responding had stabilized, anodal electrolytic lesions (2.0 mA/10 sec.) were made via the MFB electrode. Behavioral testing resumed 24 hrs. after the MFB lesions and continued at intervals of 4, 7, 14 and 21 days post lesion.

Medial prefrontal cortex ICSS was not affected by these lesions. This finding was especially surprising because a number of the lesioned animals were hypophagic, they ceased grooming and they displayed reduced sensory-motor abilities. Histological examination of the lesions showed them to be large, extending from the fornix to the medial portions of the internal capsule and from the zona incerta to the base of the brain.

These data suggest that the ascending dopaminergic systems do not have an important modulatory role in medial prefrontal cortex ICSS, as they do at many other ICSS sites in the forebrain and midbrain. Moreover, lesions of the MFB-lateral hypothalamic area much smaller than the lesions reported here are known to eliminate or attenuate ICSS from a number of other limbic and mid- and lower brainstem sites. This observation suggests that whatever neural systems support ICSS in the medial prefrontal cortex they do not traverse the ventral diencephalon.

Thus it appears that prefrontal cortex ICSS involves very different neural systems and may therefore exhibit different characteristics than the commonly studied MFB ICSS system.

245.19 MULTIPLE SITE MONITORING OF DOPAMINE RELEASE IN FREELY MOVING RATS: BEHAVIORAL CORRELATES. John D. Salamone*, Darryl B. Neill*, Wayne S. Lindsay*, Brad Kizzort*, and J. B. Justice* (SPON: David Freides) Depts. of Psychology and Chemistry, Emory University., Atlanta, Georgia 30322

Recently several groups have utilized electrochemical techniques for in vivo monitoring of electroactive compounds in the brains of experimental animals.

We have designed and constructed a microcomputer-controlled electrochemical system for multiple-site in vivo monitoring of monoamine release in freely moving animals. The system consists of a microcomputer, A/D and D/A converter board, and a multiplexed potentiostat-amplifier. Sixteen working electrodes can be monitored, allowing near-simultaneous evaluation of monoamine release at different brain sites.

The circuitry can be used for all controlled potential electrochemical techniques. We have written software (Intel 8080 microprocessor) to perform chronoamperometry. The computer has control over the length of time the potential is applied, the time between measurements, and the number of points collected during each pulse.

We have used this sytem to monitor dopamine release in forebrain terminal areas of the nigrostriatal and mesolimbic dopaminergic systems of rats. Experimental results will be presented showing:

(1) Correlations of the chronoamperomentric signal with more conventional measures of dopamine release.

(2) The effects on the signal of systemically administered drugs which alter dopaminergic transmission with concurrent behavioral evaluation of locomotion activity and stereotypy.

(3) Correlations of the signal with both spontaneous and conditioned behaviors in non-drugged rats.

This project has been supported by grant BNS-79-06815 from the National Science Foundation.

245.20 RISE IN URINARY 5-HYDROXYINDOLEACETIC ACID (5-HIAA) ASSOCIATED WITH PRACTICE OF THE TRANSCENDENTAL MEDITATION PROGRAM. R.K. Wallace*, B. Simon*, S. Guich*, P.F. Tomlinson*, L. Petrick*, S. Beth* and K.G. Walton. Biochem. Lab., Institute of Psychophysiology, Maharishi International University, Fairfield, IA 52556.

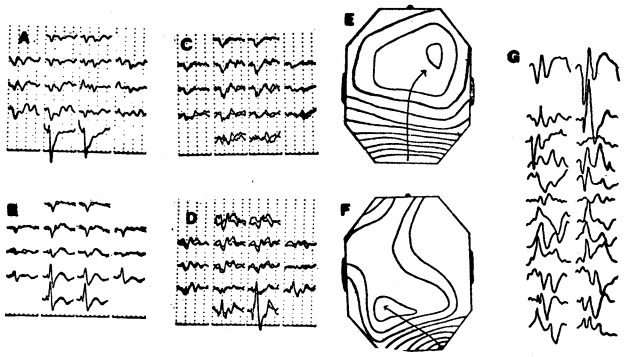
Transcendental Meditation (TM) is the name applied to an effortless mental technique for expanding the conscious mind introduced over 20 years ago by Maharishi Mahesh Yogi, a world-renowned teacher and scholar of the ancient Vedic tradition of knowledge. Maharishi has been instrumental in reviving the Vedic science of consciousness, of which the TM program, including the recently rediscovered TM-Sidhi techniques, may be considered the practical or laboratory aspect.

In the past 10 years a number of psychological, physiological and biochemical correlates of the TM practice have been identified (Scientific Research on the Transcendental Meditation Program: Collected Papers, Vols. I and II, MERU Press, 1977 and 1980). The nature of these correlates suggests that a unique behavioral state, referred to as transcendental consciousness (TC), is produced periodically during each practice session. Such periods are characterized by a large drop in respiratory rate, decreased heart rate, increased EEG coherence at all frequencies, and subjective alertness with little or no mental activity. The observed long-term effects of the TM practice, including a broad spectrum of improvements in mental and physical health, increased perceptual-motor coordination, increased creativity and enhanced enjoyment of life, appear to be related to the repeated experience of this TC state.

An increase of urinary 5-HIAA, the main metabolite of serotonin, was reported previously to be associated with the TM practice in 11 meditators (Bujatti, M. and Reiderer, P., J. Neural Transmission 39:257, 1976). We have confirmed and extended this observation using three experimental paradigms which appear to exclude the possibility that the observed rise in urinary 5-HIAA could be accounted for by normal circadian rhythms of 5-HIAA. Other researchers have reported changes in lactate, phenylalanine, prolactin, TSH, FSH, ACTH, T_4, cholesterol, cortisol and catecholamines in blood or urine, or both, which appear to be associated with practice of the TM program. Most of these substances are either produced or indirectly regulated by the pituitary. Brain monoamines, including serotonin, are known to affect pituitary secretion. Thus, while the increased 5-HIAA excretion correlated with the TM practice might reflect altered metabolism of peripheral serotonin, the hypothesis we are pursuing, namely, that the TC state involves central serotonergic and possibly other monoamine systems, seems more tenable. (Supported in part by a grant from the Rogers Foundation.)

246.1 TOPOGRAPHY OF 16 CHANNEL PATTERN SHIFT POTENTIALS (P-VERS) IN NORMAL SUBJECTS AND LEARNING DEFECTIVES. R. G. Bickford, D. G. Brunet* and M. L. Scholl*. BIK Foundation, La Jolla, CA 92037.

Studies of 70 normal subjects and 30 patients with learning defects were made from 10-20 system electrodes positioned by "electrocap". Results were computed by ACE (Automated Cerebral Electrogram) software and Mini-CEARS (BIK Systems) and DEC PDP/1140 computers. In addition these systems generated spectral bin averages, area displays, direct and integrated 16 channel averages (VER, AEP, BAER, and SER) for overall comparison. Results, normals: great variety in response in both contour and distribution was seen (A, B, C) indicating involvement of frontal lobes in processing. Patients: approximately 20% show O1-O2 voltage asymmetry greater than normals. O1-O2 integrated P-VERS allow estimation of ratios and setting of normative cut-off. Conclusions: 1) P-VERS are widely processed; 2) alpha and P-VERS generators are independent;3) a group of learning disordered patients with R/L processing problems has been delineated. Legend - 16 channel averages, linked ear reference, 350 msec. sweep, 100 stimuli binocular pattern viewing 34 min. arc checks. A, B, C, - varieties of response in normal subjects; D - response in learning defective patient; E - computer generated area display at 84 msec.; F - same display for learning defective patient 75 msec. Note line indicating direction of voltage gradient is different from E. G - display of 12 P-VERS in 12 learning defective patients O1 and O2 responses show frequent asymmetry, 350 msec. sweep.



Supported by BIK Foundation.

246.2 SIMULTANEOUS RECORDINGS OF THE AUDITORY BRAINSTEM (ABR) AND MIDDLE LATENCY RESPONSES (MLR) IN NORMAL SUBJECTS AND NEUROLOGICAL PATIENTS. N. Kraus, O. Özdamar*, L. Stein*, and D. Hier*. Siegel Institute, Michael Reese Hospital, Chicago, Illinois 60616.

Although auditory middle latency responses in man (8-50 msec, post-stimulus onset) have been shown to be generated intracranially, their source of origin is still uncertain. Recent studies suggest that these responses are cortically mediated (Picton et al, 1974). MLRs have been extensively investigated as a test of hearing (Mendel et al, 1975). As a neurological test, MLR has received little attention. This study was undertaken a) to examine the feasibility of recording these responses in a clinical setting, b) to ascertain the validity of audiological applications of MLRs using ABRs as a reference and c) to examine MLRs in patients with neurological impairments involving cortical dysfunction.

ABRs were recorded simultaneously with MLRs to assess the integrity of the auditory periphery and brainstem. Click stimuli were presented at several intensities and repetition rates. Filters were set at 3 and 2000 Hz and a 20 μsec sampling time was used. To be of clinical value, MLR recording should be applicable to uncooperative patients and individuals with whom communication is difficult. As sedation is frequently required, the effect of chloral hydrate on MLRs was examined. ABR and MLRs were obtained in normal adults with and without chloral hydrate sedation (1000 mg, p.o.). All patients received chloral hydrate. Adult patients were stroke victims with cortical lesions defined by CAT scans and behavioral evaluations. Children were language delayed, encephalitis and meningitis cases.

In normal subjects the major MLR component (P_a) was easily identified with a mean peak latency of 30 msec. The latency and amplitude of both P_a and ABR components were not significantly affected by sedation or the repetition rates employed. While the amplitude of ABR waves continued to increase with click intensity, P_a leveled off at 50-60 dB HL. ABR wave V and P_a were identifiable down to 10 dB HL. A number of patients showed normal ABRs in the absence of MLRs. This suggests that ABR wave V is a more reliable index of auditory sensitivity than MLR. Middle latency responses in pathological cases were evaluated with respect to normative data.

246.3 STUDIES ON PERIODIC AUDITORY EVOKED POTENTIALS AND 40 HZ RHYTHM INTERACTION IN THE AMYGDALA AND LIMBIC STRIATUM. C. C. Turbes, G. T. Schneider*, J. M. Simard* and R. J. Morgan. Dept. of Anatomy, Creighton University Sch. of Medicine, Omaha, NE 68178.

The interaction between the 40 Hz rhythm and auditory evoked potential (AEP) are considered in these studies. The 40 Hz rhythm is of special interest in this relationship since it is associated physiologically to respiration, alert and oriented states.

Recordings are made on free-moving cats using telemetry. Chronic electrodes are implanted in the sigmoid gyrus, amygdala and limbic striatum. The interaction of slow wave activity of these brain areas are analyzed using spectral, coherence spectral and crosscorrelation methods.

Autospectral analyses show the auditory evoked potentials and the 40 Hz activity are present in these areas of the brain. Coherence spectral estimates show the 40 Hz activity and the AEP between these brain regions are phase locked. This is shown by the per cent coherence and is a possible indication of the degree of physiological interaction between these regions of the brain.

The correlation levels of AEP between the nucleus accumbens and the amygdala ranges between 59% and 90% at 8 to 10 Hz. The associated 40 Hz activity ranges from 56% to 80%. The shortest time delays (tau), an indication of long pathway synaptic transmission, is from the nucleus accumbens to the amygdala. These are within the limits of synaptic delay and fit neuroanatomical findings.

In the case of the sigmoid gyrus and amygdala, the correlation levels range from 35% to 91% at 5 to 7 Hz. The 40 Hz activity showed correlation levels at 60% to 79%. In each case the shortest synaptic transmission was from amygdala to sigmoid gyrus.

The interactions between sigmoid gyrus and nucleus accumbens of AEP showed correlations at 76% to 90% at 12 to 14 Hz.

The 40 Hz activity showed correlation levels at 54% to 77%. The direction of shortest synaptic transmission was nucleus accumbens to sigmoid gyrus.

Where no periodic auditory stimuli are used, the average correlation levels for these frequencies are lower and show great fluctuation. This is possibly due to the changing behavior of drowziness, sleep and arousal during the absence of the stimulus.

246.4 THE EFFECTS OF SPINAL CORD INJURY ON SEPs PRODUCED BY NEURONAL INTERACTION, Richard K. Simpson, Jr., John G. Blackburn, Henry F. Martin and Sidney Katz, Dept. of Physiology, Medical University of South Carolina, Charleston, S.C. 29403.

The purpose of the present study was to determine the utility of somatic evoked potentials (SEPs) produced by neuronal interaction for spinal cord injury evaluation. Interaction between spatially separate afferent inputs was measured as alterations in SEP configuration compared to controls. In contrast to cats under choralose (Katz, S. et.al. EEG clin. Neurophysiol. 45:45-52 1978), monkeys anesthetized with N_2O were used. Stimulus intensities were sufficient to excite all nerve fibers. The conditioning stimulus (CS) was applied to the left peroneal nerve and test stimulus (TS) to the left radial nerve. CS-TS intervals were 100 msec in duration. SEPs were recorded from the primary cortical receiving area for the forelimb. Different surgical lesions were made at spinal cord level T_3-T_4.

Amplitudes of TS produced SEPs remained unchanged post lesion. CS produced SEPs were found to be dependent upon the integrity of anterolateral column pathways. Accentuated interaction was observed following dorsal column ablation. Little interaction was observed following anterolateral column ablation. Interaction was enhanced after left hemisection but diminished after right hemisection or central cord lesion. Interaction between spatially separate afferent inputs as measured by SEP alteration is a sensitive indicator of spinal cord injury. (Supported by NINDS grant P-5P81-NS-11066).

246.5 DETECTION OF SENSORY SPINAL TRACT DYSFUNCTION WITH SIGNAL DETECTION THEORY. Richard J. Schneider and Ronald Burke*. Maryland Institute for Emergency Medical Service Systems, Baltimore, Md. 21201.

A somatosensory discrimination task was established using either hair follicle or electrocutaneous stimuli. The hair follicle task required subjects to discriminate either two different frequencies or amplitudes of hair displacement on the anterior aspect of the calf. The electrocutaneous task required discrimination of two amplitudes of current pulses delivered over the sural or peroneal nerve. The paradigm was arranged to allow the relative ambiguity of the various stimuli to be manipulated. The task required the subject to push a button in response to one stimulus and to refrain from pushing in response to the complementary stimulus. In theory of signal detectability (TSD) terminology, hits and false alarms were recorded and used to construct a receiver operating curve (ROC). Normal human subjects and subjects diagnosed to have MS with spinal symptoms were tested for discriminative capacity in this paradigm. In an analogous fashion, the capacity of M. mulatta to discriminate electrocutaneous and hair follicle stimuli was compared.

The data obtained with this paradigm were described adequately by TSD. Thus, a reliable measure of discriminative capacity was obtained which was independent of response biases. Results suggest that normal subjects possess superior discriminative capacity compared to MS subjects. This result was evident irrespective of individual differences in surface hair density. Normal humans and M. mulatta demonstrated comparable discriminative capacity across several levels of task difficulty. The evidence suggested that when hair follicles were not stimulated uniquely, i.e. other receptor populations like low-threshold skin were activated, performance improved dramatically. When peripheral nerves were blocked with local anesthetic in M. mulatta discriminative acuity decreased to hair follicle and electrocutaneous stimuli. Following dorsal funiculus section discriminative capacity to hair follicle stimuli was diminished in these subjects but electrocutaneous capacity did not show the same effect.

These data support the conclusion that this paradigm together with TSD may be used to differentiate normal and MS subjects. Further, hair follicle stimulation preferentially assesses dorsal funiculus function while electrocutaneous stimulation is less specific. While these results were not dependent on receptor density, it was necessary to stimulate hair follicle receptors uniquely to obtain high resolution results.

Supported by Research Grant RG-1207-A-1 from the National Multiple Sclerosis Society.

246.6 THE EFFECTS OF SPINAL CORD LESIONS ON THE SOMATOSENSORY EVOKED POTENTIALS IN MACACA FASCICULARIS. S. Katz, J.G. Blackburn*, H.F. Martin, and R.K. Simpson. Dept. of Physiology, Medical University of South Carolina, Charleston, S.C. 29403.

The effects of selected spinal lesions on the somatosensory evoked potential (SEP) were studied in chronic animal preparations (Macaca Fascicularis). The left and/or right posterior tibial nerves were stimulated percutaneously at .3-.2 Hz, 0.3 msec, and 3-4 mA. Recordings were taken from chronic, epidural electrodes over the leg (Cz) area in response to both unilateral and bilateral nerve stimulation. Following well-defined control recordings, the spinal cord was surgically transected. Transections included the dorsal columns, right and left hemisections, and anterolateral columns. Central cord lesions were made using an RF lesion generator. All lesions were placed at T_3-T_4. Recordings were taken at intervals ranging from one day to 10 weeks. All recordings were made under N_2O-O_2 anesthesia.

A bilateral dorsal column transection (which also included the spinocervical tracts) was followed by an attenuation of the early, primary components of the SEP with minimal alteration of the late, secondary responses. Similar responses were seen with both unilateral and bilateral nerve stimulation. Significant alterations in the late components were seen following bilateral transection of the anterolateral columns in response to both unilateral and bilateral stimulation. Left hemisections were followed by loss of the primary components with little attenuation of the late components in response to left tibial nerve stimulation. Similar results were observed with right hemisections in response to ipsilateral nerve stimulation. Bilateral nerve stimulation revealed SEPs with both early and late components. Variable alterations in all components of the SEP were seen in response to both unilateral and bilateral stimulation following central cord lesions. Some deterioration of the evoked potentials with time was observed following several of the spinal lesions. All lesions were confirmed histologically.

The results obtained support previous observations in acute preparations indicating that both the dorsal and anterolateral columns contribute to the SEP. (Supported by NINCDS Grant P-5P81-NS-11066).

246.7 ACRYLAMIDE TOXICITY: EFFECTS ON CORTICAL EVOKED POTENTIALS AND LOCOMOTOR ACTIVITY IN RATS. William K. Boyes*, R. Dana Laurie*, G.P. Cooper (SPON: R. S. Manalis). Dept. of Environmental Health, Univ. of Cincinnati, Cincinnati, OH 45267 and Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH 45268.

Most electrophysiological investigations of chronic acrylamide neurotoxicity have been devoted to studies of peripheral nerve despite ultrastructural evidence of significant CNS damage. In this experiment we investigated ascending sensory systems by recording somatosensory and visual evoked potentials (SEPs and VEPs) in acrylamide-poisoned rats. In addition, electrophysiological measures were correlated with a behavioral indication of intoxication by housing acrylamide treated rats in standard running wheel cages. Acrylamide monomer was given to adult male Sprague Dawley rats in drinking water at concentrations of 0, 50 or 100 ppm. Cortical evoked potentials were recorded under nembutal anesthesia from chronically implanted electrodes before, and after 1, 3, 5, 7 and 24 weeks of exposure. Running wheel activity was monitored for 2 weeks before and 12 weeks following onset of exposure. The mean weekly number of wheel revolutions turned by the 100 ppm group was significantly less than the other two groups for each week beyond the fourth week of exposure. After 5 to 6 weeks of exposure, some of the 100 ppm animals demonstrated signs of ataxia including outward splaying of the hind limbs. After 7 weeks of exposure the latency of the N1 peak of the SEP was significantly increased in the 100 ppm group. The latency of both P1 and N1 was prolonged in the 100 ppm group after 24 weeks. No effect was found on the latency of later components of the SEP. Alterations in VEP waveforms were less severe. Measurements from the 50 ppm group were not significantly different from control in either behavioral or electrophysiological experiments. The finding that the early peaks of the SEP, which are thought to arise from specific somatosensory projection fibers, were altered by acrylamide and the later peaks, thought to arise from slower nonspecific systems, were not altered, suggests that acrylamide effects ascending somatosensory fibers in a manner similar to that reported in peripheral nerves. Large, fast fibers are more sensitive to acrylamide than small, slow ones. The fact that behavioral deficits were evident well before SEP alterations indicates that either (a) the SEP, as measured, is insensitive to small amounts of nerve damage or (b) that the initial acrylamide lesions occur in systems other the ascending somatosensory systems. (Supported by the EPA, and NIEHS Grant ES00159 and the Alice B. Ryan Foundation).

246.8 ACUTE TRIETHYLTIN ALTERS VISUAL EVOKED POTENTIALS AND HIPPOCAMPAL AFTERDISCHARGES W. E. Howell and R. S. Dyer. Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Acute exposure to triethyltin (TET) is known to produce intramyelinic vacuolization and edema (Magee et al., J. Path. Bact., 1957, 73:107), and to inhibit mitochondrial oxidative phosphorylation and coupled respiration (Stockdale et al, Eur. J. Biochem., 1970, 15:342). The visual evoked potential (VEP) and hippocampal afterdischarge (AD) were used to study the effects of TET on visual and limbic system function, respectively. Fifty male Long-Evans hooded rats were surgically implanted with a bipolar electrode in the dentate gyrus of the dorsal hippocampus and an epidural screw electrode overlying visual cortex; two frontal cortex screws were used as reference and ground electrodes. Animals were tested for 10 consecutive days using both methods (VEP and AD). On days 2-6 animals received an i.p. injection of either 0.000, 0.188, 0.375, 0.750, or 1.500 mg/kg TET bromide in saline. Pupils were dilated prior to VEP testing using a 1% atropine sulfate solution. Daily average VEPs were derived from 256 individual responses to a 10 μsec strobe flash. Immediately following the VEP test session, AD threshold stimulus intensity level was determined by stimulating the dentate gyrus with a series of 50 HZ 2 sec trains of 0.2 msec biphasic pulses at ascending current steps once per minute (AD1). Suprathreshold AD properties were determined by retesting 15 min later at a 4x threshold current (AD2). Beginning 10 min after AD2, a measure of posticta1 excitability was obtained by stimulating at 2x threshold current every 2 min until a third AD (AD3) was elicited. Animals that received 1.500 mg/kg TET exhibited significantly increased VEP peak latencies (P1, N1, P2, and N3) compared to controls; no TET-induced amplitude differences were observed. ADs recorded from animals treated with 1.500 mg/kg TET exhibited a significant increase in spike frequency during the first segment of the AD. In contrast to this apparent excitatory effect, there was an increase in severity of the post-ictal EEG depression (PID) and an increase in time required to elicit AD3. These findings indicate that excitability in the hippocampus and visual system is differentially affected by TET. The visual system findings imply altered conduction velocities but no effect upon cellular responsiveness (no amplitude changes). The AD findings imply a profound depression, measured by both the increased PID severity and longer post-AD refractory period. Failure of TET to increase AD thresholds dissociates TET-induced depression from barbiturate-induced and ethanol-induced depression, both of which increase AD thresholds.

246.9 DELTA-9 TETRAHYDROCANNABINOL SLOWS PRIMARY CORTICAL EVOKED RESPONSE TO SOMATOSENSORY STIMULATION IN CATS. D.M. Wilkison* and M.J. Hosko. Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

In an attempt to establish the relationship between the altered sensory preception induced by drugs of abuse and the neurophysiological mechanisms that are responsible for those alterations we have undertaken a study in which a series of agents commonly involved in abuse will be evaluated using modality-specific and non-specific event-related potentials. The effects of delta-9 tetrahydrocannabinol (THC) on modality-specific (primary) somatosensory stimulation in acutely prepared alpha-chloralose-anesthetized cats will be reported here. Nucleus ventralis posterolateralis of the thalamus (VPL) and the sigmoid gyri of the cortex were mapped bilaterally with recording electrodes for the location of the maximal positive-negative potential in response to contralateral radial nerve electrical stimulation. After mapping, one thalamic electrode was used to evoke a thalamocortical response, the other served to record thalamic responses to radial nerve stimulation.

THC (0.5-4.0 mg/kg) produced a dose-dependent slowing of cortical responses to radial nerve stimulation characterized by a decrease in the slope and an increase in the latency of the primary positive potential. Cortical responses evoked from the thalamus were delayed similarly. The effects of THC on the cortical evoked response to either VPL or radial nerve stimulation were dependent on the intensity of the stimulus. The 2 mg/kg dose of THC typically delayed the primary cortical response to a near maximal stimulus by 1-3 milliseconds while the response to a near threshold stimulus was markedly attenuated. In contrast to the effects on cortical responses, THC did not alter the latency of potentials evoked from VPL by radial nerve stimulation. A cannabinoid analog, dimethylheptalpuran, which is alleged to have little or no psychotropic activity while retaining the cardiovascular activity of THC delayed the cortical response approximately 1 millisecond but did not exhibit the stimulus intensity-dependent characteristic seen with THC. These data support that THC alters thalamocortical processing of primary somatosensory activity without significantly altering the thalamic response to radial nerve input. (Supported by PHS Grant #DA-01754).

246.10 CAPSAICIN MODIFIES SENSORY EVOKED POTENTIALS FROM HABENULA, ANTERIOR HYPOTHALAMUS, DORSAL RAPHE, AND SUBSTANTIA NIGRA FROM FREELY BEHAVING RATS. L. S. Rabe*, L. M. Moreno*, and N. Dafny. Depts. of Anesthesiol. and Neurobiol. and Anat., Univ. of Texas Med. Sch. at Houston, Houston, TEX., 77025

Peripheral injections of a homovanillic acid derivative, capsaicin, have been shown to cause changes in primary sensory neurons and decreases in the amount of substance P in the dorsal horn of the spinal cord of rats. The present study was designed to assess the effects of peripheral administration of capsaicin on sensory evoked responses recorded from several brain sites. Male Sprague Dawley rats were stereotaxically implanted with permanent stainless steel teflon-coated electrodes (120μm in diameter) in areas of the brain which had been shown to have high concentrations of substance P: substantia nigra (SN), habenula (HB), dorsal raphe (DR), and anterior hypothalamus (AH). Three to five days after recovery from surgery, the unrestrained, unanesthetized rats were placed in a shielded electrophysiological test chamber for recording averaged sensory evoked responses, (AER's). A set of four AER's to both acoustic and visual stimuli (32 repetitions) were taken as controls. Another set of AER's to both acoustic and visual stimuli was recorded following each injection of capsaicin (in successive doses of 5, 10, and 20 mg/kg, sc). Between stimulations spontaneous EEG-like activity was recorded from the same electrodes as the AAER's.

A dose of 5 mg of capsaicin elicited some spiking activity from HB. At the dose of 10 mg, more frequent bursting and spiking activity appeared in HB and AH. After 20 mg all four structures exhibited spike activities. The capsaicin injections in general modified sensory evoked responses in all four brain areas. The averaged acoustic evoked responses exhibited the most consistent and apparent effects of capsaicin. The recordings from the HB and the AH had significant increases in the amplitudes of N_2 and P_3 components. This increase was dose-related, in that the highest dose of capsaicin produced the greatest increase in amplitudes. However, the averaged visual evoked responses from HB and AH showed a biphasic dose-response effect of capsaicin consisting of an increase in the amplitude of the N_2 component at the lowest dose and a decrease at the two higher doses.

The results of this study show that peripherally administered capsaicin modifies sensory information transmission in the CNS and that this effect is not identical in all brain areas. The HB exhibited the greatest effects, both to acoustic and visual stimuli : the AH was somewhat less sensitive, and the DR and SN had minimal sensitivity to capsaicin.

247.1 PREPARATION OF SYNAPTOSOMES FROM THE CENTRAL NERVOUS SYSTEM OF THE HORSESHOE CRAB Limulus polyphemus. R. Sukumar*, R.F. Newkirk*, W.E. Thomas*, and J.G. Townsel. Dept. of Physiol. and Biophys., University of Illinois Medical Center, Chicago, Il. 60612.

Subcellular fractions from the central nervous system (CNS) of Limulus were separated in 0.8 M sucrose using a floatation procedure previously applied in the preparation of synaptosomes from invertebrate nervous tissue [Newkirk, et al., Brain Res. 101:103-111, 1976]. A low speed pellet (P_1) a high speed supernatant (S_2), a floating pellicle (P_2L) and a high speed pellet (P_2^2H) were isolated from a 10 per cent homogenate of Limulus CNS. These fractions were biochemically characterized. The P_2H fraction was determined to be enriched in fumarase activity, a mitochondrial marker enzyme, while the S_2 fraction was enriched with lactic dehydrogenase activity, a cytoplasmic marker. The P_2L fraction was enriched with choline acetyltransferase activity, a presumed marker of synaptosomes. Further, a marked enrichment of acetylcholine (ACh) was seen in the P_2L fraction. This fraction also took up ^{3}H-choline in the high affinity range [Maleque, et al., Biochem. Pharmacol. 28:985-990, 1979]. Greater than 80 per cent of the ^{3}H-choline taken up into this fraction was released by osmotic shock. These results are consistent with the presence of a sequestered compartment within the P_2L (i.e. synaptosomes). Ultrastructural studies of the P_2^2L fraction revealed a diverse population of membrane bound structures. Some appeared to be large featureless membrane bound sacs while others presented well organized profiles containing mitochondria and vesicles, typical of synaptosomes. These results provide evidence that the CNS of Limulus offers a reasonable source of cholinergic synaptosomes. (Supported by NIH grant HL 24140)

247.2 **ISOLATION AND CHARACTERIZATION OF GOLGI ENRICHED FRACTIONS FROM ISOLATED NEURONS OF RAT BRAIN.** **D.S. Deshmukh*, W.D. Bear* and S. Kuizon* (Spon: G.Y. Wen). Dept. Neurochem., Institute for Basic Research in Mental Retardation, Staten Island, NY 10314.**

In order to investigate the role of Golgi apparatus (GA) in the synthesis and assembly of the plasma membranes during active period of neuronal proliferation and synaptogenesis, attempts were made to isolate GA membranes from the neuronal cell enriched fraction of brains of 8 day old rats. The procedure of Farooq et al. (Brain Res. 124, 69, 1977) was adopted for the bulk preparation of neurons, with the use of trypsin and a defined orifice nozzle for dissociation of the tissue. Neurons were obtained in a fraction at the bottom of 48% sucrose. The cells suspended in 10% sucrose medium were colected by low speed centrifugation. Yield of neuronal cells was 32 x 10^6 cells/g or 5.8 mg protein/g brain. Values of DNA and RNA/DNA ratio were 6.8 pg DNA/cell and 1.6 respectively.

The cells were homogenized with the help of a Tekmar tissuemizer in a medium containing 0.5 M sucrose and the optimal concentrations of $MgCl_2$, KCl, EGTA and dextran (BBA 542, 248, 1978), and centrifuged at 5,000 x rpm for 5 min. Density-gradient centrifugation of the supernatant yielded distinct bands of the fractions at the top of 0.72M, 0.88M, 1.13M, and 1.17M sucrose. The specific activities of the Golgi-marker enzymes, N-acetyllactosaminyl synthetase (NAL-synthetase) and thiamine pyrophosphatase (TPPase) of the 0.72M and 1.13M fractions, were about 4 to 8 times enriched over those of the whole neurons. The specific activities of these enzymes (3.8 for NAL synthetase; 2.5 for TPPase) were also enriched over those of the original brain homogenates. The recoveries of the enzyme activities were 1.2% for NAL-synthetase, 26% for TPPase in the 0.72M fraction, 5.5 and 30% in the 1.13M fraction. Recovery and relative specific activities of marker enzymes for other subcellular organelles were low in two fractions. Thus, GA was found in two fractions, at the 0.5-0.72M interface, possibly enriched in GA vesicles, the second at the 0.88-1.13M interface, probably representing the GA cisternae. **This work was supported by U.S.P.H.S. grants NS14073 and NS14480 from NIH, Bethesda, MD.**

247.3 NORMAL ONTOGENESIS OF NEURO-MUSCULAR TISSUES *in vivo* and *in vitro*. CONSTITUTIVITY OF CREATINE KINASE DURING PHYSIOLOGICAL DIFFERENTIATION OF BRAIN, HEART AND SKELETAL MUSCLE AND THE EXISTENCE OF THE TERMINAL, FUSION-CAPABLE MYOBLAST. [1,3]Oscar Ramírez, [2]Efraín Mercado*, [2]Omar Hernández*, [3]John Takahashi* and [1]Margarita Hernández*. [1]Centro de Investigación del Inst.Politécnico Nal. [2]Div. Bioquímica Inv. Científica, Centro Médico Nal. del I.M.S.S. México 14, and México 7, D.F., and [3]C.V.R.I. University of California Med. Center, San Francisco, 94143. Adult tissues of higher vertebrate with high energy requirements, such as muscle and brain, contain high levels of creatine kinase (CK) *In vivo*, it has been proposed that the first abrupt increase in cytosolic brain CK is associated with neuronal multiplication at early stages and the second increase with neuronal maturation before hatching, in chick development (Ramírez et al., (1977) J. Neurochem. 28, 411).

The aims of the present study were: 1) to determine the early ontogenesis of cytosolic chick brain and limb-bud (muscle) CK activity *in ovo*, to see if such CK activity was measurable before the advent of creatine during chick embryo development; 2) to learn whether insulin might be physiologically involved in the increase of CK activity in neuro-muscular tissues *in ovo*; 3) to decide whether thigh myogenic cells of 11-12 day-old chick embryos can fuse typically in the absence of chick embryo extract (CEE) in primary culture; 4) to investigate the role that calcium and insulin play in syncytium formation and muscle CK activation in a CEE-free medium; and 5) to elucidate if chick skeletal fibro myogenic CK was also constitutive in cultures fed continuously in a creatine-free medium. The brain enzyme appeared at stage 11.5 (≃ 42 h) and increased sharply, before creatine and insulin advent *in ovo*. First detection of limb-bud CK and creatine in the whole embryo occurred at stage 24 (4.5 days) before myogenic cells can be distinguished histologically. Phosphocreatine has not been formed in the embryo before stage 28 (5 1/2 - 6 days).

In primary culture, competent (terminal) myoblasts from 11-12 day-old chick embryos could form typically aligned myotubes with 20 or more nuclei in a CEE-free medium. Typical myotube formation and CK activation depend on the conditioning of the micro-environment in creatine-free media containing from 0.3 to 1.5 mM calcium.

In early development of the chick heart, skeletal muscle and brain, CK is a constitutive enzyme regarding creatine and independent of insulin, *in vivo* and *in vitro*.

247.4 *LECTINS AS CYTOCHEMICAL PROBES FOR VISUALIZATION OF MEMBRANE* OLIGOSACCHARIDES IN HUMAN MUSCLE. S. D. J. Pena*, B. B. Gordon*, G. Karpati and S. Carpenter*. Department of Neurology and Neurosurgery, Montreal Neurological Institute, Montreal, P.Q., Canada H3A 2B4.

Biotynyl derivatives of seven plant lectins — concanavalin A (Con A), Ricin I (RCA I), wheat germ agglutinin (WGA), peanut agglutinin (PNA), soybean agglutinin (SBA), Ulex europeus agglutinin (UEA I) and Dolichos biflorus agglutinin (DBA) — were allowed to bind to cryostat sections of biopsied normal human muscle. The sections were then reacted with avidin-horseradish peroxidase conjugates and stained with 3,3'-diaminobenzidine. The most general staining was observed with Con A, RCA I and WGA which permitted strong visualization of the plasmalemma-basement membrane unit, tubular profiles in the interior of muscle fibers, blood vessels and connective tissue. PNA gave virtually no intracellular or blood vessel staining while SBA and UEA I selectively stained blood vessels. DBA was unique in providing good visualization of myonuclei. In each case, lectin staining could be blocked by appropriate sugar inhibitors. Neuraminidase treatment of the cryostat sections altered the pattern of staining of all lectins except UEA I and Con A. Staining with RCA I became stronger while that with WGA became less intense. Staining with PNA, SBA and DBA became stronger and more generalized, resembling that of RCA I. These effects of neuraminidase treatment are in conformity with the known structure of the oligosaccharide chains of membrane glycoproteins and the specificities of the lectins involved. Lectin histochemistry will certainly provide a useful new tool for the investigation of muscle disorders.
(Supported by grants from the Medical Research Council and the Muscular Dystrophy Association of Canada).

247.5 THE IMMUNOCYTOCHEMICAL LOCALIZATION OF ALCOHOL DEHYDROGENASE IN ADULT RAT CEREBRAL CORTEX. Barry Goldstein* and David S. Maxwell. Department of Anatomy, U.C.L.A., Los Angeles, California 90024

Alcohol dehydrogenase (AdH) activity in normal rat brain has been demonstrated biochemically in many laboratories. The presence of the enzyme suggests that the central nervous system can metabolize ethanol, although this capacity appears to be quite limited. This study utilizes the immunocytochemical method to determine the cellular localization of rat brain alcohol dehydrogenase in the cerebral cortex.

Antisera against alcohol dehydrogenase was obtained from rabbits that were injected with horse liver alcohol dehydrogenase. Appropriate biochemical and immunochemical studies were completed to establish the titer of the antisera and determine the cross-reactivity and specificity of the rabbit anti-alcohol dehydrogenase antibodies. These methods included spectrophotometric assays for AdH, the Ouchterlony double diffusion method, and immunotitration (enzyme-inactivation) studies.

Three immunocytochemical methods were compared, the peroxidase antiperoxidase (PAP) method, the indirect horseradish peroxidase-labeled antibody method, and the indirect fluorescein-labeled antibody method. A modified PAP method yielded the best results for both light and electron microscopy.

Neurons in the deeper layers of the cerebral cortex were the only cells that contained reaction product after staining. Cell bodies, processes and specific synapses were intensely stained. Astrocytes and oligodendrocytes did not contain any reaction product. Parallel control sections were all negative.

The light microscopic appearance of the cortical neurons displayed cell bodies with unstained nuclei and long apical processes continuing towards the surface of the brain. All areas of the cortex contained labeled neurons but the greatest density of cells was observed in the motor cortex. Electron microscopy demonstrated the reaction product in the cytoplasm of neurons and in many profiles of sectioned processes.

This study has established a means for the morphological localization of alcohol dehydrogenase in the rat cerebral cortex. This method offers an approach to study the distribution of this enzyme in different brain regions and after various experimental conditions. In addition, alcohol dehydrogenase might prove to be a useful neuronal-specific enzyme. (Supported by Grant AA03513-03 from the National Institute on Alcohol Abuse and Alcoholism.)

247.6 ULTRASTRUCTURAL LOCALIZATION OF NON-SPECIFIC ESTERASE IN NORMAL AND PATHOLOGIC HUMAN MUSCLE. V. Askanas, E.K. Worthington*, W.K. Engel and G.G. Cunningham*. Neuromuscular Diseases Section, NINCDS, NIH, Bethesda, MD 20205

Light microscopic evaluation of non-specific esterase (NSE) staining on fresh-frozen sections of abnormal human muscle is of diagnostic value. Highlighted by the NSE reaction are positively stained small, angular fibers (diagnostic of denervation), tubular aggregates, regenerating-degenerating fibers and macrophages (in inflammatory-cell reactions). In normal muscle the NSE reaction stains end-plates and distinguishes fiber types and subtypes of type-I fibers. We have developed an ultrastructural technique for NSE and studied 39 normal and pathological muscle biopsies from patients with different neuromuscular diseases. The technique of Nachlas and Seligman (J. Biol. Chemistry 151: 343, 1949) was modified for EM. Elimination of any component of the histochemical reaction prevented staining. The NSE-EM reaction was fully inhibited by phenylmethyl sulfonyl fluoride 10^{-2}M. Eserine 10^{-4}M, iso-OMPA 10^{-3}M, or BW284C51 dibromide 10^{-4}M minimally decreased (but did not abolish) the NSE-EM reaction. In both normal and abnormal muscle the reaction product of NSE-EM was localized to plasmalemma, t-tubules (but not sarcoplasmic reticulum), and mitochondrial outer > inner membrane (but not cristae, suggesting different enzymatic composition of cristae cf. inner mitochondrial membrane). In abnormal muscle, bizarrely shaped mitochondria in carnitine deficiency were highlighted by NSE including their proliferated cristae. Tubular aggregates (considered to be proliferations of sarcoplasmic reticulum, Engel, Bishop, Cunningham, J. Ultrastruct. Res. 31: 507-525, 1970) of type-II fibers were also positively stained with NSE. The dark staining of angular fibers could be only partly attributed to the denser accumulation of mitochondria per cross-sectional unit: the individual mitochondria had darker outer and inner membranes, and, especially, the myofibrils of small, angular fibers stained darker than normal, suggesting that loss of neural trophic influence changes enzymatic properties of both mitochondria and myofibrils.

Thus, under different circumstances pathologic mitochondria (outer and inner membranes and/or cristae), sarcoplasmic reticulum, and myofibrils can be stained excessively by the NSE-EM. Accordingly, ultrastructural localization of NSE may become an important tool for demonstrating enzymatic properties of different organelles in pathological muscle.

247.7 TISSUE CULTURES OF GOLDFISH BRAIN EPENDYMAL ZONE CELLS: IMMUNOHISTOCHEMICAL IDENTIFICATION OF β AND γ EPENDYMIN, NGF AND GFA CONTAINING CELLS. R. E. Majocha* and V. E. Shashoua (SPON: G. Hauser). Mailman Research Center, McLean Hospital, Harvard Medical School, Belmont, MA 02178.

In previous investigations, two proteins (β and γ Ependymins) localized in the ependymal zone of goldfish brain were found to be associated with the process of acquisition of new patterns of behavior (Shashoua, V.E., Science, 193:1264, 1976). The ependymal zone also has NGF containing cells (Benowitz, L. and Shashoua, V.E., Brain Res., 172:561, 1979). These findings have stimulated us to develop a tissue culture system for the growth and characterization of ependymal zone cells.

Excised ependymal tissue from the ventricular surface of optic tecta was used as a source for several types of cells by mild trypsinization. These were grown on polylysine-coated glass slides in Medium 199 with 10% fetal calf plus 5% calf serum in an atmosphere of 1% CO_2 in air at 22° C.

At 3-5 days after plating, the initially uniformly dispersed round undifferentiated cells begin to sprout processes and to form micro-aggregates 50-200 μm in diameter. At about 10 days multiple fiber connections develop between islets of cells. Such culture could be maintained at least 5 weeks in vitro. Immunohistochemical studies with antisera to the β and γ Ependymins, to NGF and to GFA (antiserum obtained from Drs. Bignami and Dahl) showed that each antiserum stained a different but distinct cell type within the cultures.

Cells containing β and γ sent out multiple processes which could be seen as early as 24 hours after plating. Brilliant staining varicosities were visible on the membrane surfaces and along their processes. Morphologically the cells resembled glia.

The NGF-containing cells had a fibrous appearance with maximum staining localized in their processes; cell soma were only faintly stained. GFA containing cells were similar to NGF cells morphologically. They differed, however, in their staining patterns; anti-GFA could intensely stain processes as well as cell bodies. Light microscopy studies of cresyl violet stained cultures indicate that these ependymal zone cultures also contain "neuron-like" as well as a background of fibroblast cells. These results demonstrate that the ependymal zone of goldfish contains a multiple population of cells which can be maintained in culture and that at least three types retain their in vivo capacity to produce specific proteins which can be visualized by immunohistochemical methods.

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

247.8 NEURON SPECIFIC ENOLASE (NSE) AND NON-NEURONAL ENOLASE (NNE) IMMUNOREACTIVITY IN PRIMARY CULTURES FROM FETAL RAT BRAIN. J.A. Weyhenmeyer*, M.K. Raizada*, P.J. Marangos, and R.E. Fellows. School of Basic Medical Science, University of Illinois, Urbana, IL 61801, Department of Physiology and Biophysics, The University of Iowa, Iowa City, IA 52242, and National Institute of Mental Health, Bethesda, MD 20205.

Recently the existence of two physiologically distinct glycolytic isoenzymes, neuron specific enolase (NSE) and non-neuronal enolase (NNE) has been reported in mammalian brain. The purpose of this investigation was to determine whether antisera to these enzymes can be used as specific neuronal and glial markers in primary cell cultures from fetal rat brain. Cells were dissociated from 20-day fetal brain with trypsin and were grown in Dulbecco's modified Eagle's medium (DME) with 10% fetal bovine serum in 35mm tissue culture dishes containing 11x22mm glass coverslips. Brain cells were used for experiments after 4, 6, 8, 10, 12 and 14 days in culture. Cells were stained by the peroxidase-anti-peroxidase method using highly specific antisera to NSE or NNE and buffered diaminobenzidine-H_2O_2. Typically, these cultures consist of phase-dark cells over a background monolayer of large flat cells of glial origin. The phase-dark cells have been characterized on the basis of morphological and physiological criteria as neurons. NSE immunoreactivity was demonstrated as dense staining limited to the neuronal cell body and processes, without staining of glial elements. NSE was also localized in varicosity-like structures along the length of neurites. Immunoreactive NNE was observed only in large, flat cells of glial origin, with no staining of neurons. A progressive increase in percentage of NSE-positive neurons was observed with increasing time in culture between 4 and 14 days. A decrease in NNE immunoreactivity of glial elements also occurred over the same time period. The observation that the number of positively stained neurons increases with time suggests that the neuronal population is capable of undergoing differentiation under the conditions of cell culture. This research is supported by NIH grants HD11184 and HL24402 to REF.

247.9 CNA-1: A NEURAL AND AVIAN SPECIFIC ANTIGEN. J.D. Redshaw* and D.J. McCallion*, (Spon.: M. Bisby). Department of Anatomy, McMaster University, Hamilton, Ontario L8S 4J9, Canada

Chicken neural antigen-1 (CNA-1), a saline soluble antigen has been isolated and purified from adult chicken brain tissue extract. The purification of CNA-1 was performed by using salt fractionation, chromatographic methods (ion exchange and gel filtration) and polyacrylamide gel electrophoresis. Each step of the purification procedure was monitored using quantitative immunoelectrophoretic techniques. A 76.9-fold purification was achieved with a 0.9% yield. The purified antigen demonstrated alpha-1 globulin mobility during immunoelectrophoresis, a molecular weight of 65,000 daltons by molecular exclusion chromatography, and two identical subunits (30,000 MW) during SDS-polyacrylamide gel electrophoresis. CNA-1 accounted for about 1.3% of the total soluble protein within the adult brain extract. By immunochemical criteria, CNA-1 was demonstrated to be both avian restricted (not present in brain extracts of mammalian species) as well as neural specific (not present in non-neural tissue extracts). The CNA-1 antigenic determinant was found to be protein in nature, since treatment with either chymotrypsin or trypsin resulted in a loss of antigenicity. Molecular weight heterogeneity of CNA-1 on molecular exclusion chromatography (> 1,500,000 and 65,000 MW) was also revealed with the use of a monospecific antiserum directed against the antigen. CNA-1 was detected as early as the seventh day of incubation in embryonic brain extracts and found to accumulate within the developing embryonic brain from Day 7 to hatching. Both neurogenesis and synaptogenesis are known to occur during this period within the chicken CNS. Prehatching (Day 20) levels were observed to be only 54.0% of adult CNA-1 levels, therefore accumulation of the antigen continues until adult levels are achieved. Immunohistochemical localization studies demonstrated CNA-1 specifically localized on neuronal plasma membranes and processes. However not all neuronal types expressed the antigenic label. Further studies are in progress to elucidate the role of CNA-1 in embryonic development and adult function.

247.10 MONOCLONAL ANTIBODIES TO THE SYNAPSE. E. Niday and R. Hawks*. Freidrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.

We wish to identify markers specific to cell types and regions of the brain. To this end we are producing a series of monoclonal antibodies to the rat brain synaptic plasma membrane (SPM). These membranes, composed of pre- and postsynaptic components, were isolated by differential centrifugations of brain homogenates. Spleen cells from mice immunized against SPM were fused to a myeloma cell line and the hybridomas were selected according to Köhler and Milstein (Eur. J. Immunol., 6:511, 1976).

Antibody production was detected in the culture media with a peroxidase labelled sandwiching technique. Millipore filters were spotted with 1.0 µl of a 100 µg/ml solution of antigen, incubated with conditioned media containing concentrations between 3-15 µg of putative antibody, then allowed to react with commercially available peroxidase labelled rabbit anti-mouse immunoglobulin.

We can use these monoclonal antibodies to determine the distribution of specific proteins in the brain. Firstly, we can identify the specific protein antigen by separating the SPM proteins on SDS-PAGE, electrophoretically transferring the proteins onto nitrocellulose filters following the method of Towbin et al. (Towbin, H., Staehelin, T., and Gordon, J., Proc. Natl. Acad. Sci. USA, 76:4350, 1979), and reacting the "Towbin Blot" with monoclonal antibodies. Secondly, we determine the distribution of that protein in the brain by immunohistochemistry.

247.11 IMMUNOHISTOCHEMISTRY OF MONOCLONAL ANTIBODIES TO SYNAPTIC ANTIGENIC DETERMINANTS. R. S. Lasher and P. F. Erickson*. University Colorado Medical School, Denver, Co. 80262

In an attempt to develop immunologic probes for use in the analysis of the membrane components of synapses and their role in synapse formation, synaptosomes or fractions of synaptosomal membranes were used to produce antibodies in BALB/c mice. Spleen cells from these mice were fused either with P3X63-Ag8 or with P3-NSI/1-Ag4-1 myeloma cells using PEG 1000. The cells were cultured in 24-well dishes containing DME+20%FBS+HAT+50µM 2-ME. Wells containing the desired hybridomas were identified by utilizing a binding assay involving incubation of the culture medium with synaptosomes and I-125 protein A. Cells in positive wells were cloned, all of the clones were assayed, and positive clones were grown up and used to produce ascites in Pristane-primed mice.

Eight different monoclonal antibodies have been produced thus far, and light and electron microscopic immunohistochemical studies have been initiated on 5 of them (A45,A78,A105,A110,A112). Anesthetized rats were perfused through the ventricle with cold 5% paraformaldehyde in 0.15M phosphate buffer. After fixation for 3 hrs., 50 µm sections of the desired areas were cut using a Vibratome, and then were processed using a number of indirect procedures employing protein A as a linker and either anti-HRP + HRP or PAP. In one case (A78), 0.2% Triton X-100 was required in the initial incubation step. Either normal mouse serum or ascites produced by P3X63-Ag8 cells was used as a control. Sections were postfixed in 1% OsO4, stained briefly in 1% aqueous uranyl acetate, dehydrated, embedded in Epon and sectioned.

The antibodies were found to react with the following antgenic determinants (only the areas listed were examined). A45: (a) in the nuclei of neurons in the cerebellum, choroid epithelial cells and smooth muscle cells; and (b) within dendrites and to a small extent on plasma membranes of Purkinje cells. Biochemical evidence suggests that the antigenic determinant is in a carbohydrate present on a large number of glycoproteins. A78: in the presynaptic dense projections and postsynaptic density of many (all?) synapses in the cerebellum and substantia nigra. A105: mainly in postsynaptic dendritic profiles (on the plasma membrane, around microtubules and mitochondria; cerebral cortex, cerebellum, spinal cord); also in some presynaptic terminals, esp. cerebellar glomeruli, on the plasma membrane, around synaptic vesicles and mitochondria; and in neuronal soma (spinal cord). A110: in the plasma membrane and cytoplasm of climbing fibers and terminals, and associated regions of dendrites of Purkinje cells (cerebellum). A112: mainly in Purkinje cell dendrites and processes of Bergmann glial cells. Characterization of the antigenic determinants involved is underway. Supported by NINCDS grants NS-09199 and NS-13133.

247.12 SPATIAL AND TEMPORAL DISTRIBUTION OF SYNAPTIC VESICLES IN NERVE TERMINALS VIEWED WITH HIGH-VOLTAGE ELECTRON MICROSCOPY. W.D. Niles and D.O. Smith. Department of Physiology, University of Wisconsin, Madison, WI 53706.

The spatial and temporal distribution of synaptic vesicles in the terminals of the excitor axons of the crayfish walking leg were viewed with high-voltage electron microscopy. The tissue was fixed in 4% glutaraldehyde in either phosphate or cacodylate buffer. After postfixation in 1% OsO4, dehydration and embedding in Spurr, sections ranging in thickness from 0.1 to 0.5 µm were cut and stained with uranyl-magnesium acetate and Sato's lead. Synaptic terminals of the excitor axon were readily identified by their round vesicles, which fell into size groups with diameters of 40 to 70 nm and larger than 100 nm.

In nonstimulated tissue, the smaller vesicles clustered around the active zone and the larger vesicles were situated around the peripheral regions of this cluster. The entire population of vesicles was surrounded in most terminals by large, irregularly shaped mitochondria. Microtubules were seen to project from the more proximal region of the axon, to pass in close apposition to these mitochondria, and then to continue through the cluster of vesicles towards the active zone. In some preparations, vesicles were seen in contact with these structures.

Electrical stimulation of 30 impulses/s of the axon before and during fixation altered the ultrastructure of the terminals. In some cases, a filamentous network was seen to radiate from the microtubules. The axolemma overlying junctional folds was smooth in nonstimulated tissue but exhibited extensive involutions into the interior regions of the terminal following 15 min of stimulation or of incubation in physiologic saline with 20 mM K^+. After 90 min of stimulation, elongated cisternae measuring about 50 nm x 200 nm were intermingled with the synaptic vesicles.

When horseradish peroxidase (HRP) was added to the extracellular environment, uptake into the terminals was not observed in the absence of stimulation. After 15 min of stimulation (30 impulses/s), HRP appeared in the involutions and in a few synaptic vesicles located at the periphery of the vesicle cluster in the center of the terminal. After 90 min of stimulation with HRP added during the final 30 min, labeled vesicles were present throughout the cluster and apparently in contact with the mitochondria.

It is hypothesized that vesicles are recovered via the involutions and cisternae in the central region of the terminal. Then they are transported along microtubules past mitochondria, with which they may come into contact, into the cluster overlying the active zone. Supported by NIH grants NS13600 and NS00380 and the Alfred P. Sloan Foundation; W.D.N. was supported by an NIH training grant to the Neurosciences Training Program.

247.13 GROWTH CONE MOTILITY: HIGH-VOLTAGE ULTRASTRUCTURAL OBSERVATIONS OF INDIVIDUAL NEURONS WITH DOCUMENTED MOTILE HISTORIES. K. W. Tosney* and N. K. Wessells* (SPON: L. Landmesser). Biol. Dept., Stanford U., Stanford, CA 94305 and MCDB, U. Colorado, Boulder, CO 80309.

The motile activity of neurons is characterized by the extension, movement, and retraction of lamellepodia ("veils") and rodlike filopodia ("microspikes") along the leading edge of "growth cones", the tips of elongating neurites. We have filmed the motile activity of individual chick ciliary ganglion neurons and examined the same cells with high-voltage electron microscopy. Since this combination of techniques allows, for the first time, a direct correlation between the ultrastructure and the local, short-term motile history of a cell, we hoped to gain insights into the mechanisms of cell surface extension, adhesion, and turnover during cellular motility.

We find that recently extended veils contain only cortical lattice elements; microtubules and large membrane-bound organelles are absent. In contrast, sites of recent veil retraction contain strings of vesicles between the cortical lattice and the naked, complexly folded, upper membrane. In general, other large vesicle groups occur only in bulbous, retracting growth cones or in glutaraldehyde-induced artifacts ("blisters"). Areas without veil activity contain a three-dimensional microtrabecular lattice without vesicle clusters. These observations suggest that the generation of new surface area during extension of processes does not involve local populations of large vesicular elements. However, the mechanism of cell surface retrieval may transiently produce vesicles during veil retraction, or may alter membrane-cytoskeletal relationships in a manner that renders the local area highly susceptible to blistering upon fixation.

We also find differences between fixed and moving processes. Attached microspikes contain closely packed and aligned lattice elements which often extend as a small stress fiber into the body of the growth cone. In contrast, unattached microspikes have a more open, criss-cross array of lattice elements. Similarly, in actively extending veils, the lattice elements, while planar, are also criss-cross and loosely arranged. Compact trabecular arrangements generally insert into areas of apparant adhesion located proximal to the area of newest extension. These results suggest that the mechanism of cell surface extension operates through loosely packed trabecular elements, rather than through more compact trabecular arrangements. The latter are probably generated at adhesion points subsequent to extension.

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247.14 A SINGLE NEURON EXPRESSES MULTIPLE FORMS OF TUBULIN. K.J. Sweadner and I. Gozes. Dept. of Neurobiology, Harvard Med. School, Boston, MA 02115 and Lab. of Neural Endocrine Regulation, MIT, Cambridge MA 02138.

Given the structural complexity of neurons in the mature brain it is of interest that a major cytoskeletal component, tubulin, has multiple molecular forms. In contrast to non-neuronal tissues, the brain has nine different molecular forms, which can be separated by isoelectric focusing[1]. This tubulin heterogeneity could result from different kinds of cells having different tubulin forms or from single cells having multiple tubulin forms, each serving different cellular functions.

We have analyzed the tubulin forms present in mature sympathetic neurons grown in dissociated cell culture. Non-neuronal cells are eliminated with cytosine arabinoside. Cultures of ~5000 cells were labeled with [^{35}S]methionine, and tubulin was isolated by vinblastine precipitation in the presence of unlabeled brain carrier. When analyzed by isoelectric focusing and autoradiography, tubulin from the cultures exhibited a similar pattern to brain tubulin. Solitary neurons growing in microwells of Terasaki tissue culture plates were labeled and analyzed in the same way; we are showing here for the first time the successful isolation and characterization of a protein from a single mammalian cell. Single neurons exhibited a tubulin isoelectric pattern identical to that of the mass cultures. Multiple tubulin forms thus can be found in a single cell.

Conditioned medium from heart cells induces the synthesis of acetylcholine in these otherwise adrenergic neurons[2] and alters the expression of several cell surface and extracellular proteins[3]. In dense cultures, conditioned medium also appears to cause the neuronal cell bodies to assume a more flattened morphology, as observed by phase-contrast microscopy. Close inspection of the tubulin forms present in cultures grown with and without conditioned medium reveals a small but reproducible quantitative difference which may correlate with the change in cell shape.

1 I. Gozes and U.Z. Littauer (1978) Nature 276: 411-413.
2 P.H. Patterson (1978) Ann. Rev. Neurosci. 1: 1-17.
3 K.J. Sweadner and S.J. Braun (1979) Soc. Neurosci. Abstr. 5:182.

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247.15 DEVELOPING NODES OF RANVIER IN FROG AND RAT PERIPHERAL AND CENTRAL AXONS. Jung-Hwa Tao-Cheng* and Jack Rosenbluth. (SPON: E.SIMON). Depts. of Physiology and Rehab. Medicine, New York University School of Medicine, New York, N.Y. 10016.

In an ultrastructural study of myelinogenesis in tadpole sciatic nerves it was shown that the length of the presumptive nodal membrane is generally much more extensive than that of the adult node and that the appearance of the nodal "undercoating" and E face particles are very variable during development. Paranodal regions develop asymmetrically, and typically the paranodal "loops" are widely separated from each other. Those closest to the node consistently exhibit the characteristic "transverse bands" and associated ER cisternae flattened against the junctional Schwann cell membrane while the loops further removed from the node usually do not.

Study of myelinogenesis in a mammalian peripheral nerve and in the mammalian and amphibian central nervous system shows that the sequence of events is comparable in these locations, but differs in some details. In the sciatic nerve of 2-3 day rats the developing nodes are also usually relatively long and the paranodal loops widely separated. Here too only the loops closest to the node exhibit distinct transverse bands in association with ER cisternae against the Schwann cell junctional membrane. However, unlike developing nodes in tadpole peripheral nerves, where well-differentiated paranodal junctions are frequently observed even when there are only two or three Schwann cell loops present, those in young rats usually do not exhibit distinct transverse bands until the sheath contains at least five layers. The staining of the transverse bands is also less intense in rats than in tadpoles. In the optic nerves of both young rats and tadpoles, developing nodes also tend to be very elongated compared with adult nodes. Paranodal loops are widely spaced, and again only those paranodal loops closest to the node have transverse bands. However, ER cisternae are not consistently observed in these loops, and when they are present they are not necessarily closely apposed to the junctional glial membrane.

Thus, in developing mammalian as in amphibian nerves, both centrally and peripherally, the paranodal transverse bands appear consistently in the glial or Schwann cell loops closest to the nodal region but not in those further removed; the node is initially elongated and gradually becomes restricted in length as the paranodal regions develop; the paranodal regions usually mature asymmetrically and the paranodal loops become more closely spaced as development pregresses. These observations indicate that the same mechanisms probably operate in myelin formation by oligodendrocytes in the central nervous system and by Schwann cells in the peripheral nervous system.

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247.16 MEMBRANE SPECIALIZATIONS IN FROG EPENDYMAL ASTROCYTES. Gary E. Korte* and Jack Rosenbluth. Depts. of Physiology & Rehabilitation Med., New York University School of Medicine, New York, N.Y.10016.

Ependymal cells in the amphibian central nervous system have characteristics of both ependymal cells and astrocytes, and hence have been called "ependymal astrocytes." Those in the cerebellum of the frog Rana pipiens have an orderly arrangement which makes them advantageous for study in thin sections and freeze-fracture replicas.

The cell bodies line the fourth ventricle and extend microvilli and cilia into the cerebrospinal fluid. The outer surface of the membrane facing the ventricle binds positively charged ferric oxide, signifying the presence of abundant anionic molecules at this surface. The binding is markedly reduced after neuraminidase treatment, indicating it is largely due to sialic acid. We did not observe binding on the membrane of the lateral and pial surfaces of the cells.

Basal processes arise from the cell bodies, traverse the granule cell layer and molecular layer, and terminate as subpial endfeet. At all levels they contain conspicuous glycogen particles and 9-10nm filaments. Along their course the processes give rise to lamellar appendages which, in the molecular layer, selectively enwrap synapsing parallel fiber boutons and Purkinje cell dendritic spines in one to several cytoplasmic sheets. Other types of synapses are not so ensheathed. Lamellar appendages also contact neuronal somata and their processes and form incomplete though extensive sheaths around capillaries.

In freeze-fracture replicas the intramembranous particles of the ependymal astrocyte plasma membrane have a higher concentration and a greater mean size than those of neuronal membranes in the same replicas. Numerous gap junctions occur, some with particle-poor "windows." Some windows appear to be forming by the addition of rows of particles at the periphery of the gap junction. Tight junctions were not observed; nor did we find orthogonal"assemblies"of particles such as those described in mammalian astrocyte and ependymal cell plasma membranes.

The observations support the notion that these cells combine the morphological characteristics of ependymal cells and astrocytes. Presumably, they also combine the functions that have been attributed to both cell types, such as isolation of synapses by ensheathment,and secretion, absorption or reception at the ventricular surface, requiring a biochemically specialized plasma membrane at this site. The freeze-fracture observations also emphasize the marked morphological differences between ependymal astrocyte and neuronal plasma membranes, which probably reflect significant biochemical differences between them.

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247.17 ELECTRON MICROSCOPIC QUANTIFICATION OF TRANSPORTED RADIOACTIVITY AND SYNAPTIC MORPHOLOGY IN AUTORADIOGRAPHS: A PRACTICAL APPROACH FOR ROUTINE USE. D.L. Oliver, L.T. Andrus*, and D.K. Morest. Dept. of Anatomy, Univ. Conn. Hlth. Ctr., Farmington, CT

EM autoradiography of synaptic endings after axonal transport of ^{3}H amino acids can be used to relate the fine structure of synapses to their connections. However, the method is limited by resolution and subjective observations. Quantitative methods accurately establish sources of radioactivity and discount silver grains due to beta scatter. Quantitative methods can demonstrate synaptic types that differ in subtle ways. In these methods, we use two batteries of FORTRAN programs with an LSI-11 microprocessor and a Graf Pen Digitizer.

We injected ^{3}H-leucine and/or proline in the caudal cochlear nucleus of cats and examined the resulting label in the inferior colliculus. Autoradigraphs were exposed for 8-34 weeks and quantified after the method of Blackett & Parry ('77). This method compares the actual distribution of grains to a hypothetical random distribution of silver grains and sources generated from the resolution formulae for the material. The silver grains were counted in the following compartments: postsynaptic structure, axon,glia, endings with round vesicles, with flattened vesicles, or combinations of these. A best fitting routine was used to minimize the differences between the real and hypothetical grain distributions and calculate the relative amounts of activity in each potential source. In our material, most silver grains were over axons, endings with round vesicles, or axon/glia. Our analysis showed that the labeling of endings was 3-30x greater than expected if random. The frequency of axonal and glial labeling was less than expected if random, yet it was not completely explained by scatter. Hence, the axons and glia also contain radioactivity.

In the digitizing process we measured area, perimeter, long axis, average diameter, and roundness of synaptic vesicles in normal sections and autoradiographs. We found that endings clustered into three groups containing synaptic vesicles of different average diameter and roundness. Each group may contain subgroups distinguished by terminal size and vesicle concentration. Most labeled terminals fell into the group with smaller-sized, round synaptic vesicles. Thus, the quantitative analyses systematically indicate each of the particular classes of axon terminals that are labeled following injections in the caudal cochlear nucleus.

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247.18 THE USE OF A WHEAT GERM AGGLUTININ - HORSERADISH PEROXIDASE CONJUGATE FOR ANTERO- AND RETROGRADE TRACING IN THE CNS. C.W. Scouten and C.W. Malsbury. Department of Psychology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X9.

Wheat germ agglutinin (WGA) binds to neuronal membranes and can be absorbed by phagocytosis, a more rapid and efficient uptake mechanism than that which allows non-binding molecules, such as horseradish peroxidase (HRP) to enter cells. Like HRP, WGA undergoes axonal transport and has been used for the retrograde tracing of neural connections. Pressure injections of radio-labeled WGA (^{125}I) have been used to demonstrate afferent connections of the hippocampus (Schwab et al. Brain Res., 1978) and hypothalamus (Wiegand & Price, Soc. Neurosci. abst., 1979). In these cases autoradiography was used to visualize WGA-labeled cells. One of the disadvantages of this method is the time required for exposure of the autoradiograms. Gonatas et al. (J. Histochem. Cytochem., 1979) have now reported that WGA coupled to horseradish peroxidase (HRP) can be used as a retrograde tracer. This has the advantage of enabling one to use an easy, rapid and sensitive histochemical procedure (Mesulam, J. Histochem. Cytochem., 1978) to visualize the location of the WGA-HRP conjugate. In addition, Gonatas et al. found that WGA-HRP was 40 times more sensitive than free HRP for the retrograde labeling of neurons innervating the submandibular gland of the rat.

The present results demonstrate that WGA-HRP is also an extremely useful tool for tracing connections when applied intracerebrally. We have used a commercially available WGA-HRP conjugate (Sigma #L9008, WGA to HRP Type VI) and have found: 1) as with free HRP, WGA-HRP can be applied using the iontophoretic method via micropipettes; 2) there appears to be less diffusion away from the injection site as compared to that seen with free HRP; 3) the vascular staining (uptake by pericytes) characteristic of injections of free HRP is nearly eliminated; and 4) the method is sensitive, as both anterograde and retrograde transport can be demonstrated even with small injection sites. The ability to produce well-localized injections with less diffusion than free HRP and no vascular staining gives WGA-HRP significant advantages for tracing CNS connections. These properties, and the sensitivity of the method, are probably due to the rapid binding of WGA to neuronal membranes near the injection site, followed by phagocytosis and transport.

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247.19 IDENTIFICATION AND ISOLATION IN-VITRO OF PRESUMPTIVE MOTONEURONS MARKED BY RETROGRADE TRANSPORT OF A NEW FLUORESCENT TRACER. Martha McPheeters and Lawrence M. Okun*, Department of Biology, University of Utah, Salt Lake City, Utah 84112.

As an approach to obtaining cell populations enriched for selected neuronal classes, we are exploring the automated sorting of neurons rendered fluorescent by retrograde transport.

We have prepared a tracer potentially suitable for this purpose from wheat germ agglutinin, a plant lectin that is efficiently accumulated in neuronal cell bodies when injected near their axon terminals (Schwab et al, Brian Res 152: 145-150, 1978), by conjugating it with Lucifer Yellow VS, a fluorochroming reagent that combines good quantum yield and resistance to fading under exciting illumination (Stewart, Cell 14: 741-759, 1978). This conjugate, WGLY, when injected into hindlimb muscles of chick embryos, yielded brightly fluorescent labeling, mostly in the form of small cytoplasmic granules, of neurons at expected positions in spinal cord (and at scattered positions in ipsilateral, lumbosacral DRGs) visible in formalin-fixed, frozen sections from embryos sacrificed 12-18 hours after the injections. The numbers and positions of spinal-cord neurons labeled by WGLY were comparable to those of cord neurons labeled by control injections of horseradish peroxidase (HRP), in 30-fold higher amounts, delivered to the same muscles.

Fluorescent cells were also found, at expected low frequencies, in dissociated-cell suspensions prepared from spinal cords marked by transport of WGLY from muscle, and the level of fluorescence in the dissociated cells was sufficient for detection by a commercially available, fluorescence-activated cell sorter (FACS III, Becton Dickinson) which could provide samples enriched, virtually to purity, for fluorescent cells.

From their appearance and location in situ as well as from their correspondence to populations marked by retrograde transport of HRP, the spinal-cord neurons labeled by WGLY injected into muscle may be regarded as motoneurons. It remains to be determined whether any cord cells unlabeled by WGLY in situ become fluorescent during dissociation or other preparative steps prior to the fluorescence-activated sorting. If they do not, then the sorted samples will constitute highly-enriched preparations of rigorously-identified spinal motoneurons. The same approach can, in principle, permit preparation of enriched samples of any neuronal class that can be selectively labeled by somatopetal transport of a fluorescent substance. (Supported by NIH Grant NS 10615, by a Graduate Fellowship from the Danforth Foundation to M.M. and by an Established Investigator Award from the American Heart Association to L.M.O. Lucifer Yellow VS was a gift from W. Stewart.)

247.20 IDENTIFICATION OF OSMIUM IMPREGNATED MYELINATED AXONS IN THE SCANNING ELECTRON MICROSCOPE UTILIZING THE BACKSCATTER ELECTRON MODE. Peter L. Friedman* and Mark H. Ellisman (Spon: S.D. Flanagan), Dept. of Neuroscience, U. Calif. at San Diego, La Jolla, CA 92093

No morphological criteria currently exists for identifying specific cellular or fibrillar components of the nervous system with the scanning electron microscope (SEM). This inability to differentiate neuritic from glial components or even axons from dendrites constitutes a primary deterrent to the reliable and routine use of the SEM in the further morphological characterization of the nervous system.

Three techniques have been combined that facilitate the identification of myelinated axons in the SEM: A) aqueous cryofracture; B) a modified osmium-thiocarbohydrazide-osmium (OTOTO) regime permitting unrestricted secondary electron (SE) mode examination of nervous tissue in the SEM at magnifications in excess of 80,000X, and C) backscatter electron (BSE) mode identification of myelinated axons.

Human spinal cord and spinal nerves, and rat cerebral and cerebellar cortex, retina and cochlea were fixed by immersion or perfusion in 3% glutaraldehyde and 1% paraformaldehyde in 0.135 M Na-cacodylate buffer, pH 7.35 containing 3.5% sucrose. Tissues were rinsed in buffer, post-fixed in 2% OsO_4 (buffered as above) for 1.5h., and again rinsed in buffer. Tissues were then frozen in slush point liquid nitrogen for 3-10 min. and cryo-fractured under nitrogen. Tissues were then placed immediately into 2% buffered OsO_4 for 30-60 min., rinsed, immersed in a fresh, saturated, filtered, aqueous solution of thiocarbohydrazide (TCH) for 15 min., rinsed, immersed in 1% aqueous OsO_4 for 30 min., rinsed, placed into TCH for 15 min., rinsed, immersed in 1% OsO_4 for 30 min., dehydrated with ethanol and critical point dried with CO_2. Tissues were examined in an ETEC Autoscan equipped with a solid state, independent BSE detector at 20kV.

The location and amount of osmium in spinal nerves and in bipolar neurons after OTOTO impregnation was determined by transmission electron microscopy, energy dispersive microanalysis of x-rays, and by the analog line scan of SEM specimens. These approaches yielded both qualitative and semi-quantitative evidence suggesting that the greatest concentration of osmium was confined to the myelin sheath. This enhanced impregnation of myelin by osmium appears to form the basis for the BSE identification of myelin sheaths.

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247.21 SILVER IMPREGNATION OF PRE-MOUNTED NEURAL TISSUE. A. Firl*, E. J. Mufson and D. G. Stein, Clark University and U. Mass. Medical Center, Worcester, MA 01610 and Beth Israel Hospital, Boston, MA 02215.

The Fink-Heimer technique for the demonstration of axonal degeneration involves the soaking of free floating sections in several solutions prior to mounting on glass slides. This procedure has several disadvantages including shrinkage of tissue, textural tissue changes, as well as tearing and wrinkling of tissue. These factors make tissue mounting and data analysis difficult. We present a modification of the Fink-Heimer method in which tissue is mounted on glass slides prior to staining.

Rats were perfused (.9% physiological saline followed by 10% formol-saline) 1-14 days after central nervous system (CNS) lesions and their brains were removed and placed in 10% formalin for several days and then soaked in 30% sucrose-formalin until the brains sank. The brains were then embedded in a 12% gelatin-egg yolk matrix, hardened in 10% formalin, placed in 30% sucrose-formalin for 5 days, cut at 25 µm on a freezing microtome and stored in 10% formalin. Sections were mounted from distilled water onto slides pretreated with 1% pig-gelatin solution and air dried. All solutions were prepared with distilled water. Following two 3 minute rinses, slides were placed in .05% potassium permanganate for a predetermined period, rinsed (twice at 3 min), bleached (equal parts of 1% oxalic acid and 1% hydroquinone), rinsed (twice at 3 min), placed in 2.5% uranyl nitrate (10 min), rinsed (1 min) and placed in 2.5% silver nitrate (60 min). Sections were then rinsed (3 min) and immersed for 2 minutes in a freshly prepared ammonical silver solution consisting of: Part A- concentrated ammonium hydroxide and 2.5% sodium hydroxide in a ratio of 3:1; part B- 2.5% silver nitrate and 100% ethanol in a ratio of 2:1. Solutions A and B were combined until the brown precipitate disappeared. The pre-mounted slides were transferred into a reducing solution (900 ml distilled water, 75 cc 95% alcohol, 17 cc 10% formalin, 20 cc 1% citric acid), rinsed, soaked in .5% sodium sulfate (1 min), dehydrated in 95 and 100% alcohol, cleared in xylene and coverslipped.

The results obtained with the pre-mounted method were consistent with results obtained with standard techniques for the demonstration of degenerating axons in the CNS.

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247.22 EARLY SPINAL CORD TRAUMA ASSESSED BY DIFFUSE AXONAL UPTAKE OF HRP. C.LaMotte, Sect. of Neurosurgery, Yale Univ., New Haven, CT,06510.

The most severe effects of spinal cord trauma result from interruption of fiber tracts. Damage from the primary traumatic lesion may to be further increased within a few hours by secondary lesions resulting from hemorrhages, changes in blood flow, edema, and necrosis. Determination of the contributions of both the primary and secondary effects to final neuronal destruction is of critical importance for early therapeutic intervention. However, traditional methods of staining for axonal injury, such as degeneration or Marchi methods, do not detect damage until 2 or more days after injury. This study was aimed at developing a method of direct assessment of damage to fiber tracts within the first 24 hours after impact, using diffusion uptake of horseradish peroxidase (HRP) as a marker for axonal injury.

To test uptake of HRP by normal and injured axons, lesions were studied in which the locations of cut axons and normal axons were known: in 15 cats, the dura was opened and the cord hemisected. Then a gelfoam 'garter' soaked with HRP was wrapped around the cord at the site of incision. After 1 to 24 hours, the animal was sacrificed by aortic perfusion. Sections were reacted with diaminobenzidine. Label was found in almost all the axons on the hemisected side; the axons on the intact side and in controls with no lesions were free of HRP.

The method was then applied to traumatized cords. Using a standardized weight-drop technique, a 20g weight was dropped from 10 or 25cm. onto exposed cat thoracic cord and the animal sacrificed after 3 hours. Results of 10 cats showed that in both 20g-10cm and 20g-25cm cats, most axons in the dorsal columns, lateral white and ventral white were stained. The 20g-25cm cords had more swollen axons and destruction of grey matter.

These results indicate HRP uptake occurs with partial or complete axon breakage or with increased membrane permeability sufficient for passage of the 40,000MW HRP molecules. The 20g-25cm impact produces severe paraplegia in cats and the widespread HRP uptake may mainly be by permanently broken axons. However, 20g-10 cm impacts initially produce paraplegia but cats usually recover function after a few days; in these, HRP uptake may reflect either membrane permeability changes or minimal breakage which is repairable but could account for temporary loss of function.

This study demonstrated that the diffuse uptake of HRP by damaged axons provides an immediate assessment of axonal distrubance following impact. We have used it to show that the large majority of spinal axons must suffer substantial and immediate damage even at mild impact (20g-10cm). This labelling method may be further applicable in assessing processes of natural and therapeutic recovery through measuring changes in membrane permeability associated with axonal repair. (Supported by NIH grant NS 10174).

247.23 LIGHT AND ELECTRON MICROSCOPIC STUDIES OF THE DORSAL VAGAL MOTOR NUCLEUS AFTER HRP INJECTIONS IN THE VAGUS NERVE AND BRAINSTEM IN THE CAT. J.H. McLean* and D.A. Hopkins. Dept. of Anatomy, Fac. of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

Two distinct neuronal types have been described in the dorsal motor nucleus of the vagus nerve (DMV) in the cat using light and electron microscopy (McLean and Hopkins, Can. J. Neurol. Sci., 1979). At all levels of the nucleus, medium-sized neurons (18 x 25 µm) containing a non-invaginated nucleus and abundant cytoplasm are at least three times more numerous than small neurons (9 x 15 µm) with an invaginated nucleus and scanty cytoplasm. In the present study, the efferent connectivity of these two neuronal types has been studied by examining the DMV using light and electron microscopy following horseradish peroxidase (HRP) injections in the cervical vagus nerve or brainstem.

After HRP injections into the cervical vagus, 40 µm frozen sections were processed according to the method of Mesulam (1978). A range of sizes and shapes of neurons in the DMV was retrogradely labeled but the large majority of labeled neurons were medium-sized, with average dimensions of 17 x 25 µm. Following vagus injections and incubation with diaminobenzidine (DAB) or Hanker-Yates reagents, blocks containing the DMV were processed for electron microscopy. The soma of almost all medium-sized neurons contained reaction product when observed in 1 µm sections with light microscopy. In thin sections stained with uranyl acetate but not lead citrate and viewed by electron microscopy, electron-dense reaction products in lysosomes were observed in the soma of the medium-sized neurons of the injected side. In contrast, lysosomes in the medium-sized neurons of the control side were unlabeled. No small neurons were labeled in either DMV. The results were similar using the DAB or Hanker-Yates reagent.

In order to determine if the small neurons project to higher levels of the neuraxis, injections of HRP (1-2 µl total) were made in one side of the mesencephalon or pons giving a hemi-injection of the brainstem at those levels. The medulla was then processed for light microscopy of retrogradely labeled neurons. In different cases, several small neurons were labeled in rostral to caudal levels of the DMV, although not all small neurons were labeled. No medium-sized neurons were labeled. Electron microscopic studies of the small neurons are now in progress.

The results indicate that in the DMV of the cat only medium-sized neurons project into the vagus nerve and at least some small neurons project to higher levels of the neuraxis.

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247.24 ULTRASTRUCTURAL CHANGES IN FACIAL AND VAGAL MOTOR NEURONS FOLLOWING AXOTOMY IN THE ADULT HAMSTER. S.K. Jacob. Dept. of Anat., Rush Coll. Hlth. Sci., Chicago, IL 60612.

Concomitant with a study of histological and population changes during the axon reaction in the facial and vagal nuclei of the adult hamster, an ultrastructural examination was performed on these nuclear groups after severance of the facial and vagus nerves. Our previous study has shown the facial motor neurons exhibited a transient chromatolysis and no neuronal loss following axotomy resulting in a relatively normal morphology by 30 days postoperative (dpo). On the other hand, the neurons in the dorsal motor nucleus of the vagus show dramatic morphological and population changes resulting in 60% loss by 30 dpo. The purpose of this project was to compare the cytological profiles in these two groups of reacting neurons.

Two groups of six adult hamsters underwent axotomy and removal of a distal segment of the right facial and vagus nerves. The left nerves remained intact to serve as a control. Brains of both groups were perfusion-fixed at 5 and 30 dpo. The facial and vagal nuclei were dissected out and further processed for routine ultrastructural examination.

Facial motor neurons showed few changes following axotomy. At 5 dpo, neurons contained few aggregates of RER and there were many scattered ribosomes throughout the cytoplasm. By 30 dpo, these cells showed an increase in parallel cisterns of RER. No autophagic vacuoles or accumulations of lipid inclusions were seen in either postoperative group.

Vagal neurons showed more dramatic changes following axotomy. At 5 dpo, neurons displayed a dilute cytoplasm with a reduction in the amount of organized RER, also observed were a few nuclear membrane infoldings. By 30 dpo, many degenerative changes were seen in the neurons. Most cells showed no aggregated RER and a highly folded nuclear configuration. The cytoplasm was vacuolated and contained many lipid accumulations.

This investigation has studied two extremes in the response to axotomy and has shown cytological changes unique to the degenerating neurons as compared to those which survive axotomy. While the reason for this range in response is unknown, it seems we have a model in the hamster that will allow us to readily compare different types of axon reactions within the same animal thus avoiding the problems of species variation in future studies.

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247.25 THE RAT CLAUSTRUM: A LIGHT- AND ELECTRON-MICROSCOPIC ANALYSIS. Lawrence R. Edelstein and Frank J. Denaro*. Dept. of Psychol., SUNY at Stony Brook, Stony Brook, NY 11794.

To date, there have been no contributions to the literature with respect to the rat claustrum from an electron-microscopic perspective. While the claustrum has been shown to be an area of high multisensory convergence (Spector et al., BR, 1974 - cat) and possessive of a high degree of reciprocity with respect to connections with vision-related cortical and subcortical regions (Kunzle, BR, 1976 - monkey; Buchholtz & Sanides, EBR, 1979 - cat; and Carey et al., JCN, 1979 - tree shrew) little has been done with respect to a comparative ultrastructural approach. Therefore, we offer the following preliminary data on our study of the claustrum of the albino rat.

Rats were perfused with 5% dextrose - 0.1M phosphate buffer (PB) with a pH of 7.2, followed by 2% EM grade glutaraldehyde - 1% paraformaldehyde - 0.1M PB. Postfixation was carried out in 2% OsO_4 - 0.1M PB at 4°C with tissue cores taken through a graded ethanol dehydration schedule. Cores were embedded in Araldite 502 resin. Semithin sections were stained with toluidine blue for light-microscopic observation. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEM 100B electron microscope.

Light-microscopic analysis reveals a fairly homogeneous organization in all planes with little variation in cell distribution. The predominant cell type is round/slightly elongated, medium in size (15-20um), with a large, relatively smooth nucleus containing slightly aggregated chromatin, and possessing a large prominent nucleolus. Dorsally, the claustrum is almost indistinguishable from cortex, while ventral claustrum is more readily demarcated. The area in and around the claustrum was found to be highly vascularized, with a large number of glia of several types generally staining more darkly than the claustral neurons.

Electron-microscopic examination reveals a predominance of axo-dendritic synapses of the asymmetric variety, possessing clear spherical vesicles. Axo-somatic were noted but with not nearly the frequency of the axo-dendritic synapses. A large number of multisynaptic areas were noted in most sections, in addition to "synaptic islands." Perikaryon was relatively narrow, with a fair number of round and elongated mitochondria and containing poorly organized ribosomes most often taking the form of rosettes.

247.26 A GOLGI STUDY OF THE PRIMATE MAMMILLARY NUCLEI. R.B. Veazey and D.G. Amaral. Dept. of Anatomy and Neurobiology, Wash. Univ. Sch. of Med., St. Louis, Mo. 63110

The mammillary and supramammillary regions of *Macaca fascicularis* have been examined in Golgi-Cox preparations. The majority of neurons in the medial mammillary nucleus (MMN) had smooth, ovoid or triangular perikarya, with diameters ranging from 10 to 50 µm. Generally, 1-4 large caliber dendrites radiated from the somata and these, in turn, give rise to a few, long, spiny branches which rarely passed beyond the capsule of fibers surrounding the nucleus, before terminating in delicate tufts. The axons of the MMN neurons were approximately 0.5 µm in diameter, and usually became myelinated a short distance from their origin. The somata in the medial portion of the MMN tended to be larger than those situated more laterally, and the cells in the ventrolateral part of the nucleus tended to have more highly branched dendrites and thinner axons.

Neurons in the lateral mammillary nucleus (LMN) had ovoid, or multipolar, cell bodies with diameters ranging from 24-36 µm and were generally larger than those of the MMN. Two to four main dendrites radiated from the somata with a dominant dorsomedial to ventrolateral orientation. These dendrites branched infrequently, and quite often left the confines of the nucleus. Widely-spaced, multiform, spines occurred along the dendritic shafts.

The midline region (nucleus medianus) between the medial mammillary nuclei contained mainly ovoid neuronal cell bodies from which 2-4 dendrites radiated dorsoventrally. These dendrites were branched more than those of the MMN or LMN and terminated by means of a thorny tufted appendage.

The supramammillary region presented a more complicated appearance being comprised of neurons with a variety of shapes and sizes. Interestingly, some large supramammillary neurons, near the midline, extended dendrites far into the contralateral side.

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247.27 FINE STRUCTURAL ANALYSIS OF THE INTRAVENTRICULAR NEURONAL COMPLEX OF THE GOLDEN HAMSTER BRAIN AND ITS RELATIONSHIP TO THE SUBJACENT MEDIAN EMINENCE. J.A. Mitchell and J.P. Card. Department of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48201.

The golden hamster is unique among mammalian species studied to date in that it possesses a highly organized intraventricular ganglion-like aggregation of neuronal cells and processes consistently located immediately rostral to the infundibular recess of the third ventricle. Previous studies from our laboratory (Card and Mitchell, 78, 79) have utilized scanning (SEM) and transmission (TEM) electron microscopy in descriptions of the fine structure of this cluster and its associated process network. The present report extends those observations with respect to the types of terminals found in the cluster and its relationship to the subjacent median eminence (ME). Adult golden hamsters of both sexes were perfused with buffered aldehyde solutions, the brain removed and the ME and surrounding tissue prepared for either SEM or TEM analysis. TEM analysis of the cluster confirmed previous observations of axon terminals containing spherical electron lucent vesicles (40 nm mean diameter) and dense-cored vesicles (60 nm mean diameter) in synaptic contact with neuronal perikarya and dendrites. However, of particular interest with respect to some of the specimens examined was the large number of terminals reminiscent of Herring bodies. Like Herring bodies in the posterior pituitary the majority of these endings contained large neurosecretory granules, small spherical electron lucent vesicles and mitochondria. These endings were generally ensheathed in fibrous astrocytic processes within the core of the cluster but were sometimes observed at the peripheral aspects of the cluster, free within the cerebrospinal fluid. Another interesting finding of the present study was the extensive interrelation that the cluster maintained with the ME via bundles of axons that connected the cluster with neuropil of the ME via discontinuities in the ependymal lining. At the SEM level faciculated bundles of axons coursing rostrally and caudally from the cluster became intimately enmeshed with processes of the exposed neuropil of the ME. Analysis of analogous regions with TEM confirmed this observation and also revealed numerous axon terminals forming synaptic contacts with dendrites at such sites. In conclusion, the present study has demonstrated two new features with respect to the intraventricular cluster of the golden hamster. First, some specimens contain large numbers of neurosecretory-like endings, some of which terminate freely within the ventricular lumen. Second, many specimens exhibit extensive interconnections between the cluster and the subjacent ME. The significance of the findings are presently under investigation. Supported by NIH RR-05387.

247.28 MORPHOLOGY OF HIPPOCAMPAL FORMATION NEURONS RESPONSIVE TO ELECTRICAL STIMULATION OF THE FIMBRIA-FORNIX. David M. Finch and Thomas L. Babb. Brain Research Institute and Reed Neurological Research Center, UCLA, Los Angeles, CA 90024.

Male Long-Evans strain rats were prepared for acute neurophysiological experiments under Equi-Thesin anesthesia (3 ml/kg, ip, supplemented as necessary). Twisted bipolar stimulating electrodes were implanted in the fimbria-fornix, and horseradish peroxidase-filled recording micropipets (4% HRP in 0.05 molar Tris and 0.2 molar KCl, buffered to pH 7.4 or 8.6 with HCl) were lowered to the hippocampal formation. The fornix was stimulated electrically with 0.2 msec pulses of 20-150 µA intensity, presented at 0.5/sec.

We have reported physiological data (Soc. Neurosci. Abstr., 5, 1979, 273; and Brain Res., 1980, in press) showing that the majority of subicular neurons respond to fornix stimulation with recurrent inhibition. HRP was injected into these responsive cells, and was visualized with the TMB technique of Mesulam (J. Histochem. Cytochem., 26, 1979, 106-117). The cells were identified in histological sections, and could be classified as pyramidal neurons. Therefore, both physiological and morphological evidence indicate that pyramidal cells of the subiculum have a recurrent inhibitory organization similar to that of pyramidal cells of the hippocampus proper. Responsive entorhinal neurons were also studied and reported on as well. The TMB technique used here - although sensitive - did not appear suitable for the study of fine structure such as dendritic spines, apparently because of the coarse size of reaction product granules.

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247.29 MORPHOLOGICAL CHANGES IN HIPPOCAMPAL CA1 PYRAMIDAL CELL DENDRITES AFTER CHRONIC ETHANOL CONSUMPTION IN THE RAT - A GOLGI STUDY. P.A. McMullen*, J.A. Saint-Cyr, T.L. Petit, P.L. Carlen, D. Durand. Playfair Neuroscience Unit, Toronto Western Hospital, Depts. of Anatomy, Medicine, Physiology, Biomedical Engineering, Psychology, University of Toronto, Addiction Research Foundation, Toronto, Ontario.

CNS damage associated with chronic alcoholism is particularly evident in limbic system structures. Depression of long term potentiation has been demonstrated in vitro in the Schaffer collateral-CA1 synapses (see Durand et al., Neuro. Sci. Abstr., Vol. 6, 1980) in slices taken from rats chronically fed ethanol compared to pair fed controls. Blocks of tissue from the contralateral hippocampi of 3 pairs of these animals plus 2 additional pairs were stained with the Golgi-Cox technique.

Male Sprague-Dawley rats had ad libitum access for 8-9 months, to measured amounts of liquid diet containing 35% of caloric content as ethanol (equivalent to an average of 10mg/kg/day.) The pair fed control group received isocaloric amounts of the same diet with maltose-dextrins replacing the ethanol.

Well stained CA1 pyramidal cells from both groups were drawn at 1500X with a camera lucida. Measurements of total, apical, and basilar dendritic lengths, basilar dendritic area as well as total, apical and basilar Sholl analyses were evaluated with the Mann-Whitney U statistic.

A Sholl analysis of the total neuronal tree revealed that the amount of dendritic branching in the ethanol group cells, 20-60 µm from the cell body, was significantly less than that of the controls at the $p \leq .05$ level. At distances of 60-380 µm, dendritic branching in the ethanol group cells was significantly less than that of the controls at the $p \leq .001$ level.

These studies quantitatively demonstrate that CA1 hippocampal pyramidal cell dendrites become attenuated in animals chronically fed ethanol.

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247.30 A BIMODAL POPULATION OF DENTATE GYRUS GRANULE CELLS DEMONSTRATING AGE-RELATED CHANGES. Steven F. Hoff*, Stephen W. Scheff and Carl W. Cotman (SPON: J. Angevine). Dept. Psychobiology, Univ. California, Irvine, CA and Dept. Anatomy, Med. Center, Univ. Kentucky, Lexington, KY 40536.

The hippocampal formation contains two primary cell types, pyramidal cells and granule cells. The densely packed granule cell layer of the dentate gyrus is commonly thought to consist of only one type of granule cell. We report here evidence for a bimodal population of granule cells. One population of cells stains more intensely with toluidine blue and appears different when examined with the electron microscope. These cells are more prevalent in younger animals.

Young (3 month) and aged (27-30 month) Sprague-Dawley rats were killed and the hippocampal formation processed for electron microscopy. Coronal sections through the dentate gyrus were stained to reveal cell bodies in semithin sections with toluidine blue. Camera lucida drawings of the cell layer from serial sections revealed a greater percentage of darkly staining cells in younger animals as compared to that observed in material from the aged rats. While the overall number of granule cells did not change the percentage of dark and lightly staining cells did.

Electron microscopic examination of the dentate gyrus also revealed two very distinct populations of granule cell bodies. The electron lucent variety conform to the classically described granule cell (J. Comp. Neurol., 128:359, 1966). The second and slightly smaller granule cell is quite different. The nucleus and cytoplasm of this cell type are very electron dense. The chromatin is evenly but more compactly dispersed than the electron lucent granule cell. The more commonly seen nucleoli in these cells is often centrally located as opposed to the eccentric location in the electron lucent variety. The electron dense cytoplasm of the dark cells displays an increase in the number of free ribosomes. While the dendrites of the electron lucent granule cells arborize throughout the dentate molecular layer, initial observations indicate that the dendrites of the dark cells are primarily restricted to the inner one-third of the molecular layer in the naive animal. Both the electron dense and lucent cell types receive axosomatic and axodendritic (including complex type) synapses.

The apparent decrease in percentage of darkly staining granule cells observed in the older animals may reflect a developmental change associated with the aging process. It might also indicate a change in the metabolic function of these cells. These data demonstrate a probable age-related cellular change in dentate granule cells. (Supported by research grant AG 00538)

247.31 THE MORPHOLOGY OF NONPYRAMIDAL NEURONS IN PHYSIOLOGICALLY IDENTIFIED RABBIT AUDITORY CORTEX. N. T. McMullen and E. M. Glaser, Dept. of Physiology, University of Maryland School of Medicine, Baltimore, Maryland, 21201.

Relatively little is known about the types and distribution of neurons in the auditory cortex and how they might correspond to those described for neurons of the visual and somatosensory cortices. Neuronal morphology of the latter areas has been rather extensively investigated in a variety of species. We have studied the morphology of nonpyramidal neurons in the physiologically identified primary auditory cortex of Dutch Belted and New Zealand rabbits. The primary area was identified by electrophysiological mapping experiments (see Glaser & McMullen, this volume). The brains were processed according to the Golgi Cox Nissl method and sectioned at 300 µm. Nonpyramidal neurons along the electrode tracks were surveyed and drawn using camera lucida techniques. Nonpyramidal neurons are found in all layers of the auditory cortex. They are located primarily in Lamina I-IV and are infrequently seen in the deeper cortical layers. At least three types of nonpyramidal neurons are seen in Lamina I: a) small, sparsely spined stellates, b) spine-free horizontal cells, and c) large, sparsely spined multipolar neurons. The stellates and horizontal neurons have tangentially running axons confined to Lamina I whereas the multipolar cells have descending axons that may reach Lamina IV. In Lamina II and upper Lamina III, nonpyramidal neurons are particularly heterogeneous with respect to dendritic and axonal projections. Both spiny and sparsely spined types are present as are types with ascending and descending axons. The density of nonpyramidal neurons is greatest in Lamina IV and lower Lamina III. The most common neuronal type in these layers is the large, multipolar, spine-free stellate with radiate and bitufted dendrites. The stout dendrites are frequently beaded and exhibit little taper. Spine-free, Lamina IV stellates with well stained axons were seen only rarely. Their axons are thick and ramify within Lamina IV and lower Lamina III. Less frequently seen in these layers were spiny stellates which have vertically aligned, spine-covered dendrites, and ascending axons with horizontal collaterals arborizing in Lamina II and III. Large multipolar sparsely spined nonpyramidal neurons were only occasionally seen in Lamina V and VI.
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247.32 THE SCANNING ELECTRON MICROSCOPE: VIEWS OF CEREBELLUM AND HIPPOCAMPUS. L.A. Paul*, I. Fried*, P.T. Duong*, and A.B. Scheibel. Depts. of Anatomy, Psychiatry, and Psychology, University of California, Los Angeles, CA 90024.

As demonstrated in our previous report (Scheibel et al., Soc. for Neursci. Abstr., 1979, #1464), scanning electron microscopy provides a bridge between Golgi-visualized microstructure and the ultrastructural detail of the transmission electron microscope (TEM). Using careful dissection techniques, "creative tearing," standard paraformaldehyde fixation in animal material, and standard critical point drying and sputter-coating, we have examined selected regions of hippocampus and cerebellum in rodent and in human. Comparative views of these structures following Nissl and Golgi staining both provide a familiar context and dramatize the tridimensionality of the tissue matrix. The accompanying photographs indicate the range of neuronal relationships and synaptic geometry found in these regions. Of special interest are the dense clusters of cerebellar granule cells and their fine axons contrasting with the large-caliber afferent mossy fibers and terminal tufts. Basket fibers often cover the Purkinje cell bodies and, where they have been stripped off by tissue preparation, residual finger-like indentations are visible on the somal surface. Climbing fibers, glial cells, and glomeruli can also be identified. Low magnification photographs of the hippocampal-dentate complex reveal the pyramidal cell layer, while closer views show densely packed pyramidal cells with their complement of afferent terminals, mossy tufts, and glial surround. Arrays of dentate granule cells embedded in a mesh of fine fibers are also visible. The synaptic scale covering somata in both regions is particularly prominent.

Scanning electron microscopy in the central nervous system offers, in addition to an unprecedented esthetic experience, a view of topographical reality heretofore approached only by serial reconstruction of TEM photomicrographs. It is apparently robust enough to provide data even in the case of suboptimally fixed human tissue.

(With grateful acknowledgment to the Brain Research Institute and Dr. J. Berliner, Dept. of Pathology, UCLA School of Medicine.)

247.33 $CANNING ELECTRON MICROSCOPY (SEM) OF THE RESPONSE TO CEREBRAL LACERATION OR CONTUSION. H. M. Murray, D. M. Feeney and W. G. DAIL Departments of Anatomy and Psychology, University of New Mexico, Albuquerque, NM 87131

Adult rats were used to study tissue response to cerebral laceration or contusion. Animals were perfused with 1% paraformaldehyde-2.5% glutaraldehyde in 0.2M phosphate buffer (pH 7.3) at 1, 4, 9, 15 and 30 days after injury. Brain specimens were prepared by routine SEM procedures. At one day after laceration (L + 1), the cavity formed by the lesion is filled with large numbers of erythrocytes and fine fibrils. When the lesion impinges on subcortical white matter, axons exhibit swelling. The lesioned area at L + 4 contains erythrocytes, macrophages and fibroblast-like cells; neutrophils or lymphocytes are not evident. Macrophages display many large surface blebs. Fibroblast-like elements are loosely arranged; a few of these cells have flattened morphology although the majority have a more rounded profile. By L + 9, fibroblast-like elements form a layer that is several cells in thickness. Deeper cells in the lesion wall are flattened and primarily smooth, although scattered, small microvilli are observed on their surface. More superficially-located cells are more rounded and display cytoplasmic projections extending across the surface of the more flattened cells. The projections give rise to randomly-oriented fine cytoplasmic filaments and elongated microvilli. Macrophages and crenated red blood cells are still evident within the lumen of the cavity. Fewer macrophages and erythrocytes are present within the cavity at L + 15 and L + 30 than at earlier time intervals. The formation of the wall is more advanced with the majority of fibroblast-like elements being flattened. Intercellular spaces between these cells are still evident at L + 15 but decrease in size and number by L + 30. No well-formed fenestrae are observed in the lining of the cavity. Extracellular material closely associated with fibroblast-like cells appears at L + 15 and is increased in amount at L + 30. The response to cerebral contusion differs little from that described above except that there is a delay of 7-15 days in the temporal sequence of events. The reason for this is not known at present but may be related to the amount of tissue damage produced by the contusion. The results of the study show that hematogenous elements are retained within a lesion cavity long after the insult and that fibroblast-like cells participate in the formation of neural-extraneural interface. Thus SEM provides a useful tool to study brain injury by allowing visualization of large areas (not possible with TEM) and at greater resolution than light microscopy. This provides a "3-dimensional" view of the complex morphological interactions that occur subsequent to the loss of structural integrity in the central nervous system.

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247.34 CLUSTERING OF SUPRAEPENDYMAL CELLS IN THE RAT THIRD VENTRICLE AFTER MULTIPLE INJECTIONS OF P-CHLOROAMPHETAMINE. L.C. Saland and A.T. Munger*. Dept. of Anatomy, Univ. of New Mexico School of Medicine, Albuquerque, NM 87131

P-chloroamphetamine (PCA) is an agent which causes long-lasting depletion of brain serotonin in rats, and appears to cause degenerative changes in "selected" central nervous system serotonergic fibers and cell bodies. The depression in brain serotonin levels after PCA appears at 3-5 days and lasts for weeks or months (Sanders-Bush and Steranka, '78). Three sequential injections of 10 mg/kg PCA reduce serotonin levels by 30% and cause degeneration of some neurites in the median eminence of the hypothalamus (Saland, Dail and Reyes, 1980, J. Neurobiol., in press). Several investigators have suggested that supraependymal neuronal elements within brain ventricles are serotonergic. This study examines effects of PCA on supraependymal cells and fibers in the rat third ventricle. Adult male Sprague-Dawley (150-200 gm) rats received three sequential injections (10 mg/kg each, separated by 24 h) of PCA, were anesthetized with pentobarbital and perfused intracardially with (0.15 M) cacodylate-buffered 1% paraformaldehyde/2.5% glutaraldehyde (pH 7.2, room temp.) at 8, 12 and 14 days after the initial PCA injection. SEM examination of medial basal hypothalamus (MBH) at 8 days post-injection revealed large clusters of cell bodies atop the ependyma in every experimental animal, with numerous fibers intermingling among the larger cells. Large numbers of fibers were also present on the ependymal surface, many with "varicosities". MBH regions from saline-injected controls contained variable numbers of supraependymal cells and fibers, usually isolated or in occasional small groups. Observations by TEM of sections of re-embedded SEM specimens illustrated parts of some cell clusters and processes which appeared to be neurons, while other cells were mononuclear with abundant free ribosomes. Macrophage-like elements were not usually seen after PCA. Cells and fibers did not appear to be degenerative at any time period. At 12 or 14 days, some brain regions appeared to revert to the appearance of areas in control rats, while others still contained some large cell clusters. Other studies (Card and Mitchell, 1978, J. Comp. Neurol., 180: 43; Coates, 1978, Anat. Rec., 190: 366) have shown clusters of supraependymal elements in normal vertebrates, and have suggested sensory and/or secretory roles for the cells within cerebro-spinal fluid. Consistent changes in the supraependymal region after PCA may indicate the ability of specific cells to respond to drastic alterations in serotonin levels.

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247.35 SCANNING ELECTRON MICROSCOPY OF MACROPHAGES IN THE BRAIN AND SPINAL CORD FOLLOWING EXPERIMENTAL INJURY. C. H. Phelps, J. C. Pearson*, J. C. Christian*, R. P. Nieser*. Department of Anatomy, Wright State University, Dayton, Ohio 45435.

Macrophages appear within central nervous system wounds within two or three days following experimental injury. The origin of these cells has been debated but current evidence favors their origin from blood monocytes (Fujita et. al. Prog. Neuropath. 3, 1976). In the present study macrophages were examined with scanning electron microscopy (SEM) following cortical or spinal wounding. Adult male Sprague-Dawley rats received either a small stab wound in the parietal cerebral cortex or a hemisection in the lower cervical spinal cord and the migration, transformation, and subsequent phagocytic activity of macrophages was characterized. After survival times of 2, 3, 5 or 7 days the animals were perfused with a mixture of glutaraldehyde and formaldehyde and tissue blocks containing the lesions were processed for SEM. Some tissues were embedded in Epon 812 and sectioned for light microscopy and transmission electron microscopy to confirm the identity of various cell types. On the second day following injury, macrophages were observed migrating into and around the blood clot within the wound. The macrophages are large cells with extremely irregular surface features including lamellopodia, filopodia and blebs. They were sometimes seen in the process of engulfing erythrocytes which had been trapped in the fibrin clot within the cavity of the wound. In the central canal of the spinal cord adjacent to the lesion, macrophages were seen associated with Reissner's Fiber. It appeared that the macrophages used the fiber as a substrate for migration either to or from the lesion and lamellopodia were often seen spreading out over the surface of Reissner's fiber. Sometimes erythrocytes were observed attached to the surface of these macrophages.

247.36

Withdrawn by Author

247.37 TOXICITY OF OXYGEN IN IRON-RICH AREAS OF RAT BRAIN. Robert C. Switzer, III. Laboratory of Brain Evolution and Behavior, National Institute of Mental Health, Bethesda, MD 20205.

As a part of a series of experiments demonstrating a component of globus pallidus in olfactory tubercle, oxygen at high pressure (OHP) was used to induce degeneration in pallidum. This was prompted by earlier reports of the effects of OHP by Ballentine who exposed female rats for 50-60 min daily to OHP at 57 psi until they were paralyzed. Histology revealed severe necrosis of several brain areas, including globus pallidus.

In the current study, the early effects of OHP on neuronal perikarya were sought using the cupric-silver method of deOlmos. Female rats were exposed to OHP at 57 psi for 50 min daily for 1-5 days and sacrificed 2 days after their last exposure. After only one exposure, degeneration was conspicuous in all of the auditory nuclei, facial motor nucleus, and a well defined patch in the extreme entorhinal cortex. With longer exposures, the degeneration increased in these areas and also occurred in other areas which include: ventrolateral thalamus, interpeduncular nucleus, medial dorsal thalamus, cerebellar nuclei, pallidum, and n. reticulata of substantia nigra. This list closely matches a list of iron-rich areas of the brain as revealed by the Perl reaction for ferric iron; however, the degree of degeneration does not appear to be directly related to the relative amount of iron.

There is convincing evidence that the damage of OHP is due to the production of lipid membrane-damaging free radicals. Since the free radicals superoxide, peroxide, and hydroxyl are readily generated by an iron-oxygen interaction, it is not surprising that the iron-rich areas would be susceptible to damage under these conditions. However, it is surprising that the areas with the highest amount of iron, globus pallidus and n. reticulata of substantia nigra, are not the first to be damaged. An explanation is provided by the metabolic activity maps using 2-deoxyglucose. Inferior colliculus and other auditory nuclei with a moderate amount of iron, have the highest metabolic activity while pallidum and nigra exhibit a lower metabolic rate. Other areas of high metabolism but without demonstrable iron did not show damage.

It is concluded that susceptibility of brain structures to damage by OHP is a function of the iron levels of a given region and its metabolic rate.

247.38 HISTOCHEMISTRY OF RAT SPINAL CORD VASCULATURE. [1]M.S. Cannon,* [1]J.B. Gelderd, [1]D.E. Bowers,* [2]W.P. Fife* and [2]D.W. Welch.* Dept. of Anatomy, College of Med.[1]; Dept. of Biology, College of Science,[2] Texas A&M Univ., College Station, TX 77843.

Sections of spinal cord from the mid-thoracic region of normal, adult Long-Evans female rats were surgically removed under sodium pentobarbital (200mg/kg body wt.) anesthesia for histochemical examination of arteries and arterioles. The histochemical reactions utilized represented a number of metabolic pathways, including; glycolysis, the hexose-monophosphate-shunt, Kreb's tricarboxylic cycle, β-oxidation of fats and the respiratory chain. Enzymes were generally evaluated from fast frozen material, while substrates such as glycogen and neutral fat were examined from fixed and/or frozen tissue.

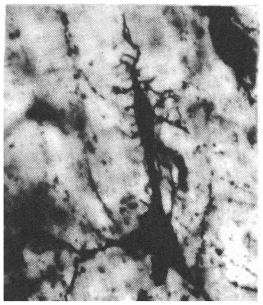
Some findings were as follows; Kreb's cycle: succinate, isocitrate and malate dehydrogenases showed more activity in larger than in smaller vessels. Glycolysis: utilizing the periodic acid-Schiff reaction, glycogen appeared to be present in nearly equal amounts in large and small vessels; lactate dehydrogenase showed a similar distribution. Hexose-monophosphate-shunt: DPN diaphorase demonstrated slight activity in both large and small vessels, while glucose-6-phosphate dehydrogenase showed moderate to strong activity in both types of vessels. The methyl green/pyronin and Azure B staining techniques showed DNA and RNA to be present in relatively small amounts in both large and small vessels. Respiratory chain: cytochrome oxidase demonstrated relatively little reactivity in both large and small vessels, while ubiquinone showed considerably more activity in large than small vessels. Lipid metabolism: lipase activity was minimal in both large and small vessels as was the presence of neutral fat; free fatty acids were present in considerably greater amounts in larger than in smaller vessels. β-hydroxybutyrate dehydrogenase showed more activity in larger than in smaller vessels. Energy consuming reactions: myosin- and Wachstein-Meisel ATPases demonstrated strong activity in both large and small vessels, while alkaline phosphatase showed virtually no activity.

The present preliminary histochemical data would tend to indicate that a strict metabolic heterogeneity (preference for aerobic or anaerobic metabolism) may not exist between large and small vessels in the rat spinal cord, although large vessels indicated some preference for aerobic metabolism. This would be in contrast to previous studies on coronary arteries and arterioles in the rat and dog which show that in general, arterioles have a higher capacity for aerobic metabolism than do arteries, which appear more adapted for anaerobic pathways. Supported by Grant No. 15800 from Texas A&M Univ. and Grant No. 55601 from Paraplegia Cure Research Foundation.

247.39 ECTOPIC DENDRITIC GROWTH AND MEGANEURITE FORMATION IN PORENCEPHALY WITH POLYMICROGYRIA: A GOLGI STUDY. R.F. Mervis and A.J. Yates. Division of Neuropathology, Ohio State University College of Medicine, Columbus, Ohio 43210

A six year old boy with a history of mental retardation and seizure disorder since 1 year of age died as a result of aspiration pneumonia. The brain was small (650 gm) and had bilaterally symmetrical clefts in the region of the Sylvian fissures allowing free communication between the subarachnoid space and the lateral ventricles (schizencephalic porencephaly). The cerebral cortex at the margins of the clefts grossly had the appearance of polymicrogyria. Histological preparations of this tissue and that from grossly normal areas were stained with H&E and Luxol blue-PAS stains and impregnated with the Rapid Golgi method. There was fusion of the molecular layer and along the lines of fusion were numerous astrocytes. Cortical layering and neuronal organization in these areas were abnormal, but in both the areas of polymicrogyri as well as the grossly normal cortex many stellate and pyramidal cells had somatic distortion and meganeurite-like structures with aberrant ectopic dendritic growth. In humans, meganeurites have been found previously only in ganglioside storage disorders. Most dendritic processes of abnormal as well as the normal-appearing neurons were otherwise heavily spined.

The findings in this case of schizencephalic porencephaly demonstrate that in the areas of polymicrogyria there are both architectonic and cytological abnormalities. While the specific pathogenetic mechanism leading to these structural abnormalities are unknown, it is tempting to speculate that both could be the consequence of a single, unknown defect resulting in abnormalities of neuronal shape and migration.



248.1 CORRELATION OF AGING CHANGES IN THE OLFACTORY EPITHELIUM AND OLFACTORY BULB OF THE RAT. J. W. Hinds and N. A. McNelly*. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston MA 02118.

In the present study two regions linked synaptically (olfactory epithelium and olfactory bulb) have been compared in an age-graded series of rats. Previous studies from this laboratory on the aging olfactory bulb in Sprague-Dawley Wisconsin (SD) rats have shown a striking increase in volume of the olfactory bulb, number of synapses, and size of mitral cell bodies and their dendritic trees from 3 to 24-27 months and then a decrease at 30 months. These findings have now been confirmed in Charles River rats (Crl:CD(SD)BR) and extended to include correlations with aging changes in the olfactory epithelium. In the olfactory bulbs of Charles River rats the volume of the layers, the number of olfactory axo-dendritic synapses in the glomeruli, the total volume of glomerular dendrites, and the size of mitral cell bodies all approximately double between 3 and 24-27 months, and then all have decreased by 36 months. Unlike SD rats, however, no loss in the number of mitral cells occurs in Charles River rats, and the increase in volume of the olfactory bulbs from 3 to 24 months is approximately double that of SD rats. In the olfactory epithelium the total number of septal olfactory receptors more than doubles between 3 and 18-24 months and then declines markedly, as does the volume of olfactory axons in olfactory bulb glomeruli. Comparing the regression lines for change in number of septal receptors with that of the size of mitral cell bodies discloses that the peak for number of receptors is reached several months earlier than for mitral cell size. This suggests that the atrophic changes in the olfactory bulb may in part be secondary to changes in the receptors in the olfactory epithelium. Synapses in the glomeruli appear to decline less markedly with age than the number of receptors, and a significant increase in number of synapses per receptor occurs in the oldest group studied (33 months), suggesting a compensatory increase in the relative number of synapses per receptor in the surviving receptors.

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248.2 DENDRITIC INCREASES IN THE AGED RAT SOMATOSENSORY CORTEX. J.R. Connor, S.E. Beban*, B. Hansen*, P. Hopper*, and M.C. Diamond. Dept. Physiology-Anatomy, Univ. of California, Berkeley 94720.

For the past two years, our laboratory has investigated basal dendrites from superficial pyramidal neurons in the occipital cortex of old rats. Specifically, we have observed increases in total dendritic branching, total spines per unit area and increased dendritic density from 444 to 630 days of age in the occipital cortex of socially housed animals. The present study supports our previous findings and extends them to the somatosensory cortex of the same animals.

Animals who had been housed 3 to a cage (30X20X28cm) were anesthesized with nembutal at 444 days of age (n=12). The brains were then removed and placed in a Golgi-Cox solution. Littermates of these animals remained in the social conditions until they were sacrificed at 630 days of age (n=7). Two or three coronal sections through the somatosensory cortex were taken and 7 neurons per section were drawn with the aid of a camera lucida. Data on dendritic branching and density were gathered from basal dendrites of superficial pyramidal cells.

The first order branching for both the 444 and 630 day old animals was identical. All other orders were greater in the older group than the younger group, but only the third ($p < .05$) and sixth orders ($p=.02$) were statistically different.

Dendritic density was analyzed according to the Sholl method and included an orientation factor introduced by Valverde (1968). Compared with the 444 day old rats, the dendrites from the 630 day old animals had increased density 0-50μm from the soma ($p < .05$), 100-150μm from the soma ($p < .01$) and 150-200μm from the soma ($p < .001$). The 630 day old animals had a greater density of branches in the upper angle in the 0-50μm range than the 444 day old animals ($p < .01$).

This study supports our previous findings in the occipital cortex and extends them to the somatosensory cortex. These data further suggest that brains from socially reared animals do not display the negative aging changes as reported by some other investigators. We thus raise the question of whether or not reported aging changes are due to age or are environmentally induced.

248.3 AXONAL MYELIN RELATIONSHIP FROM DEVELOPMENT INTO SENESCENCE IN THE LONG EVANS RAT CAUDATE NUCLEUS. Martin R. Krigman, Cynthia W. Brashear*, Jeffrey J. Gaynor*, and Robert C. Elston*. Depts. Pathology and Biostatistics, Univ. North Carolina at Chapel Hill, Chapel Hill, NC 27514

The axon diameter of myelinated axons and the number of their myelin lamellae were determined from electron photomicrographs in a preselected region from the head of the caudate nucleus. The studies are based upon three or more bundles of axons per caudate. These were made in 30, 60, 120, 365, and 730 day-old male rats, from two or more litters per age group.

Statistical analysis of the data reveals that both axon diameter and number of lamellae change significantly with age, and that within each age group the variability from rat to rat, as well as that from bundle to bundle, is very small compared to the variability within bundles. Axon-to-axon differences within bundles accounted for between 85% and 95% of the total variability within each age group. Average axon diameter decreases significantly with aging ($p < .05$), whereas the average number of myelin lamellae, either per axon or per unit of axon diameter, increases significantly with age ($p < .05$). This increase is of a cubic form: there is a sharp increase between 30 and 60 days, little change between 60 and 365 days, and a moderate increase between 365 and 730 days.

Myelination continues in the caudate nucleus of the Long Evans rat despite a significant drop in mean axon size. The myelinated axons examined represent a diverse population of corticopetal fibers and local connections twixt the diencephalic and subcortical ganglia. Examination of their distribution in the different age groups suggests that the changes in the number of myelin lamellae represent select population changes: in a majority of the axons the number of lamellae does not increase, while in a small group of axons the number of lamellae increases with age.

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248.4 SPECIFICITY OF AGE-RELATED CHANGES IN STRIATAL DA RECEPTORS: PLASTICITY IN THE FACE OF A DEFICIT. J. F. Cubells,* C. R. Filburn,* G. Roth,* B. T. Engel* and J. A. Joseph. (SPON: J. B. Wirth). Gerontology Res. Ctr. NIA, Baltimore, MD 21224.

Age-related differences in the development of striatal dopamine (DA) degeneration hypersensitivity were examined in forty (10/group) male and female mature (6-8 mo) and senescent (24 mo) Wistar rats. 3[H] spiroperidol specific binding and DA stimulation of adenyl cyclase activity were measured in striatal preparations from these rats one month after unilateral 6-hydroxydopamine (6-OHDA) lesions of the left substantia nigra. Striata from the lesioned (hypersensitive, left) hemisphere were compared to striata from the non lesioned (right) hemisphere in each group by computing a left/right ratio for each animal in each assay condition. Ratios of 3[H] spiroperidol specific binding in the left striata relative to the unlesioned striata were determined at the 3[H] spiroperidol concentration which gave the highest amount of specific binding in each experiment (5 to 10 nM). Ratios of DA stimulation of adenyl cyclase in lesioned and unlesioned hemispheres (left/ right) were determined from these same preparations. Results showed no age related decrements in L/R ratios of either 3[H] spiroperidol specific binding or 100 uM DA stimulation of adenyl cyclase (3[H] spiroperidol, young $\bar{X}$= 1.5±sem .18, old=1.5±.13; 100uM DA-AC, young 1.3±.06 old 1.2±.11).

Prior behavioral confirmation of these alterations in DA receptor density had been obtained in these animals by administering lergotril, a DA receptor agonist, which has its greatest action on the hypersensitive left striatum and produces contralateral rotation (in this case turning toward the right). Analysis of the right turns/left turns/30 min for each animal showed no age differences in rotational behavior (young 192.9 ± 16.0 old $\bar{X}$ 173.2 ± 35.0). Previous experiments indicate that specific binding to neuroleptics (i.e., 3H haloperidol), in the inact striatum declined with age. These findings, and those above, suggest that even though striatal DA receptor density is lower in the old animal, the ability to proliferate DA receptors following lesions of the substantia nigra remains intact. Further indirect evidence for this putative lowering of receptor density was provided by a second study in which rotational behavior was examined following intrastriatal injections of DA (50ug) through chronically implanted cannula in the right striata of young and old rats (10/group), previously lesioned in the left substantia nigra (6-OHDA). Results showed deficits in rotational behavior of the old animals (young $\bar{X}$=25.4 ± 4.9 old $\bar{X}$ 10.8 ± 1.64).

248.5 DIFFERENTIAL IMPAIRMENT OF DOPAMINERGIC RECEPTORS IN THE AGING BRAIN . M. Trabucchi, M. Memo, H. Kobayashi and P.F. Spano. Dept.s of Pharmacology, Universities of Milan and Brescia, Italy.

Several behavioural, neurochemical and pharmacological observations indicate that the activity of various neuronal populations is impaired during aging. In particular the dopaminergic system seems to be significantly affected. Changes in dopamine (DA) system, DA-sensitive adenylyl cyclase activity and DA receptor binding sites have been described in brain dopaminergic areas of aged rats. We and others have recently proposed the existence of distinct classes of DA receptors in the central nervous system, clas sified as D1 and D2 on the bases of their association to or independence from the enzyme adenylyl cyclase. In the present study we report radioreceptor binding experiments performed utilizing two different ligands for dopaminergic receptors, namely ^{3}H-spiroperidol, which seems to label both D1 and D2 receptors, and ^{3}H-(-)sulpiride, which seems to specifically label D2 receptors.

Mature (3-4 months) and aged (20-24 months) Sprague-Dowley male rats were used in our experiments. ^{3}H-spiroperidol and ^{3}H-(-)sulpiride bindings were performed as previously described. Kinetic studies of ^{3}H-spiroperidol specific binding in striatum of aged rats showed a decrease in the number of binding sites, with no si gnificant changes in the affinity constant (Bmax values are 188±9 and 121±8 fmol/mg prot. for mature and aged respectively). On the contrary, Scatchard analysis of ^{3}H-(-)sulpiride specific binding performed in striatal membranes obtained from mature and aged rats did not reveal differences in the number of binding sites or in the affinity constant. These results may be of significance in the light of the possible different functional roles of the D1 and D2 central dopaminergic receptors.

248.6 BEHAVIORAL AND BIOCHEMICAL EFFECTS IN OFFSPRING OF NURSING RATS EXPOSED TO DOPAMINE RECEPTOR ANTAGONISTS. J.A. Engel and P. Lundborg*. Department of Pharmacology, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden.

Previous studies in our laboratories have indicated that in rats and rabbits the dopamine containing neurons gradually develop their functions during the first week postnatally, suggesting that the first week of postnatal life may be considered as a vulnerable period for the functional maturation of central dopaminergic mechanisms. Accordingly the functional consequences of giving drugs that specifically affect central dopaminergicmechanisms during this vulnerable period was investigated.

Neonatal rats were given the dopamine receptor antagonists penfluridol or pimozide during the first postnatal week via their nursing mothers. This treatment resulted in deficits in the acquisition of an active avoidance response at the age of 4 weeks. Moreover the offspring of the rat mothers treated with penfluridol displayed an increased open field ambulation prepuberally followed by an abnormally decreased activity postpuberally. No deficits were found in the development of locomotion, air righting, startle response and eye opening.

Associated with the behavioral changes we have found a decreased synthesis(measured as the accumulation of dopa after inhibition of aromatic amino acid decarboxylase) and utilization (measured as the disappearance of catecholamines after inhibition of tyrosine hydroxylase) of dopamine in the mesolimbic, but not in the nigrostriatal, dopamine neurons at 4 weeks of age. Furthermore an increase in the specific bindning of ^{3}H-spiroperidol to membranes of the limbic forebrain, but not to those of the striatum, was found in the 4 weeks old offspring of penfluridol treated mothers. The finding that apomorphine caused a more marked decrease in the accumulatiom of dopa after inhibition of aromatic amino acid decarboxylase and pretreatment with gamma-butyrolactone in the 4 weeks old penfluridol treated rats suggest that also presynaptic receptors may be affected by the early postnatal penfluridol treatment.

In support of the assumption that the behavioral syndrome is related to a dysfunction of central, possibly mesolimbic, dopamine systems we have found that the learning deficits could be counteracted by amphetamine, which acts by increasing the functional activity of the catecholamine neurons.

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248.7 COMPARISON OF β-NERVE GROWTH FACTOR PRODUCED BY CULTURED FIBROBLASTS FROM CONTROLS AND PATIENTS WITH FAMILIAL DYSAUTONOMIA. M.H. Grossman[1], J.P. Schwartz[2], and X.O. Breakefield[1]. [1]Dept. of Human Genetics, Yale Univ. Sch. of Med., New Haven, CT 06510; [2]Lab. Preclinical Pharmacology, NIMH, Washington, D.C. 20032.

Nerve Growth Factor (NGF) is essential for the survival and differentiation of sympathetic and sensory neurons. Previous studies (Schwartz and Breakefield, P.N.A.S., 77:1154, 1980) have shown that cultured human skin fibroblasts produce relatively small amounts (10-20 ng/mg cell protein) of a protein which competes with mouse β-NGF (purified from submaxillary glands) for antibodies prepared against it. Further, extracts from these fibroblasts can elicit neurite outgrowth from 14-day chick embryo dorsal root ganglia in a dose-dependent manner and this response is blocked by antiserum to purified mouse β-NGF.

Familial dysautonomia is an inherited neurologic disease characterized by deficits in autonomic and sensory functions, and by reduced neuronal numbers in sympathetic and dorsal root ganglia (Pearson et al., J. Neurol. Sci., 35:77, 1978). A comparison of β-NGF in fibroblasts from patients with familial dysautonomia and controls indicated that immunoreactive β-NGF from patients had about one-tenth the biologic activity as that from controls. Current studies have focused on attempts to compare the structure of β-NGF produced by these two cell populations. After growing cells in ^{35}S-cysteine and a ^{14}C-amino acid mixture, immunoprecipitating labelled proteins with antiserum to mouse β-NGF and analyzing proteins by SDS-polyacrylamide gel electrophoresis, both control and dysautonomic cells were found to synthesize an immunoprecipitable protein which comigrates with authentic purified mouse β-NGF (MW = 13,250 daltons). There was no apparent molecular weight difference between the β-NGF from control and dysautonomic cells. We are currently employing isoelectric focusing, non-equilibrium pH gradient electrophoresis and two-dimensional peptide mapping to elucidate possible alterations in amino acid sequence of β-NGF from dysautonomic cells. (This work was supported by the Dysautonomia Foundation and by USPHS Grant GM20124.)

248.8 LEARNING/RETENTION DEFICITS IN THE RAT FOLLOWING MILD PRENATAL CARBON MONOXIDE EXPOSURE. C.F. Mactutus, Z. Annau & L.D. Fechter The Johns Hopkins University, School of Hygiene and Public Health Baltimore, MD 21205

Behavioral tasks which evaluate learning and memory abilities may be particularly sensitive indicies of neurotoxicity as these behaviors require the functional integrity of the central nervous system. It was recently reported that the offspring of rats exposed prenatally to a low concentration of carbon monoxide, which results in carboxyhemoglobin levels approximating those maintained by human cigarette smokers, may show reduced birth weight and decreased weight gain (Science, 1977, 197, 680), as well as retarded development of reflexive (negative geotaxis and auditory startle) and simple (homing) behaviors (Neurobeh. Tox., in press). We now report more serious functional consequences of low level prenatal CO with a behavioral index of learning and memory.

Pregnant female Long-Evans rats, bred in our laboratory, were housed throughout gestation in exposure chambers which received either compressed filtered air or air diluted with CO (150 ppm). Within 12 hr of birth the neonates were counted, weighed, and examined for gross teratogenesis. Litters were culled to 8 pups.

The ontogeny of learning and retention for two-way avoidance was assessed. Six pups from each litter were randomly selected; one male and one female were trained at 16, 23 and 30 days of age. All subjects received 100 acquisition trials followed by a 24-hr retention interval and 100 reacquisition trials.

Body weight data revealed that neither at birth nor throughout the developmental period sampled were the CO subjects significantly lighter than their AIR counterparts. No gross abnormalities were observed in either group.

Avoidance acquisition was minimal at 16 and 23 days of age. At 30 days of age a significant sex by exposure treatment interaction indicated the CO males, but not the CO females, made significantly fewer avoidance responses relative to their respective controls. Shock escape latencies on the initial five trials suggested the prenatal CO treatment had not altered pain sensitivity nor motivational factors. Significant savings were noted for the AIR but not CO males, and for both the CO and AIR females. Whether the lack of savings for the CO males reflects a retention impairment above and beyond the original acquisition deficit is under study.

The highlights of the present data are: 1) a significant deficit in learning/retention ability consequent to mild prenatal CO exposure which would have gone undetected by conventional measures of teratogenesis, enhanced mortality, or decreased birth weight, and 2) the greater resistance of female over male pups to the neurobiological consequences of prenatal CO exposure.

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248.9 Distribution of Acetylcholinesterase - positive fibers in the normal and reeler mutant mouse cochlear nucleus. Michael R. Martin, LNO, NINCDS, NIH, Bethesda, MD 20205

The reeler gene is an autosomal recessive mutation affecting cell migration, cell orientation and granule cell number in cerebral and cerebellar structures (c.f. Caviness and Rakic, Ann. Rev. Neurosci. 1: 297, 1978, for review). It is believed that these abnormalities are due to aberrant cell-to-cell recognition patterns rather than disorders of cell proliferation or synapse generating mechanisms. The dorsal cochlear nucleus (DCN) in the reeler shows a similar description of laminar organization and loss of granule cells. One of the few known inputs to the granule cells of the cochlear nucleus is the acetylcholinesterase (AChE)-containing fibers from the superior olivary complex (Rasmussen, In Sensorineural Hearing Processes and Disorders, A.B. Graham, ed., 1967). The current study was designed to ascertain the effects of the reeler gene on the AChE-positive fibers innervating the cochlear nucleus.

The direct-coloring method of Karnovsky and Roots (J. Histochem. 12: 219, 1964) was used to demonstrate cholinesterase activity. For controls, ethopropazine was used to inhibit pseudocholinesterases or BW 284C-51 to inhibit AChE. Reeler mutant mice and littermate controls were obtained from the B6C3 colony at Jackson Labs.

In the normal mouse AChE-positive fibers enter the medial anterior and posterior ventral cochlear nuclei (AVCN, PVCN) via the acoustic stria. The granule cell bridge between the DCN and PVCN receives a number of fibers. A few of these fibers enter the deep DCN and caudal PVCN. None continue into the molecular layer of the DCN. AChE-positive fibers reach the granule cell layer covering the lateral aspect of the PVCN by first traversing the PVCN. The PVCN and interstitial region of the caudal AVCN receive a large number of AChE-positive fibers. Very few fibers enter the other regions of the AVCN. In reeler AChE-positive fibers in the granule cell bridge between the DCN and PVCN are greatly reduced. No fibers innervate the DCN or the lateral granule cell layer. The number of fibers in the PVCN and interstitial region are also greatly reduced.

It is concluded that the AChE-positive afferent innervation of the cochlear nucleus is abnormal in reeler. Several possible factors may be involved including a lesion affecting the cells of origin for these fibers or an abnormality in cell-to-cell recognition patterns between AChE-positive fibers and the postsynaptic cells of the cochlear nucleus.

248.10 ELECTROPHYSIOLOGICAL CORRELATES OF ALUMINUM NEUROTOXICITY IN THE ABSENCE OF NEUROFIBRILLARY DEGENERATION. B.J. Farnell*, U. De Boni* and D.R. Crapper McLachlan. Dept. of Physiology, Univ. of Toronto, Toronto Canada M5S 1A8.

Elevated aluminum concentration occurs in several human brain diseases but the physiological significance of this observation is unknown. The in vitro hippocampal slice preparation was employed to investigate the electrophysiological consequences of an aluminum induced encephalopathy. Four week old rabbits received .05 ml of either 5 µmoles aluminum lactate (n=23) or 15 µmoles sodium lactate (n=12) into each dorsal lateral ventricle. Slices were prepared from the right dorsal hippocampus at 5,10,15 and 20 days post injection. Input-output curves relating stimulus strength to input fiber prepotential, population EPSP and population spike were analysed.

Thirty six slices from 5-day (n=5) and 10-day (n=5) post aluminum treated animals and twelve slices from six sodium lactate injected animals exhibited orthodromic and antidromic excitability characteristics indistinguishable from control slices. All 17 slices from 15-day aluminum treated animals (n=5) demonstrated a significant reduction in the magnitude of the CA1 pyramidal cell layer population spike evoked from both apical and basilar orthodromic inputs. Thirty one slices from either 20-day post aluminum treated animals demonstrated a further significant decline in the evoked population spike response. These changes occur without alterations in the input-output curves relating stimulus strength, input fiber prepotential, dendritic population EPSP and antidromic excitability.

All of the slices were tested for their ability to sustain Long Term Potentiation of the population spike. The conditioning stimulus was a 1 sec. 100 Hz train at a voltage selected from 1/2 to 2/3 along the input-output curve, and applied to the stratum radiatum input to CA1. The criteria for potentiation was a population spike showing an increase of at least 25% above the baseline response for thirty minutes after the tetanization. The percentage of slices meeting this criteria were; 68% untreated and sodium lactate treated controls, 50% for 5-day, 35% for 10-day, 30% for 15-day, and 20% for 20-day post aluminum treated animals.

These studies indicate that aluminum causes a delayed reduction in the ability of the CA1 pyramidal cell population to demonstrate long term potentiation as well as an even later developing decrement in its ability to generate the population spike in response to afferent input. The Bielchowsky stain revealed that these electrophysiological changes occur in the absence of neurofibrillary degeneration of the CA1 pyramidal cells. The development and time course of these electrophysiological changes is consistent with delayed effects of aluminum upon brain metabolism. Supported by the Canadian Geriatrics Research Society.

249.1 GENETIC VARIATION IN NUMBER OF DOPAMINE NEURONS IN HYPOTHALAMUS AND PREOPTIC REGION OF TWO INBRED MOUSE STRAINS: H. Baker, T. H. Joh, D. J. Reis, Laboratory of Neurobiology, Dept. of Neurology, Cornell Univ. Med. College, New York, NY 10021.

Strain dependent variations in the activity of tyrosine hydroxylase (TH) in the midbrain tegmentum of mice of the BALB/cJ and CBA/J strains is entirely consequent to variations in the number of dopaminergic (DA) neurons (Baker et al., PNAS, 1980). We sought to determine whether there are comparable strain-dependent variations in the numbers of neurons in the hypothalamic DA systems including those of the tuberohypophysial (A12) group, the incerto hypothalmic (A13) group and a rostrally extending periventricular (A14) group which in the mouse is quite large in the preoptic area. TH activity was determined in microdissected blocks of brain, one containing the A12 and A13 groups and the other the preoptic portion of the A14 group. In both regions TH (nmols/mg prot./hr activity was greater in BALB/cJ than CBA/J mice:

Area	BALB/cJ	CBA/J	CBA/BALB	P
A12-13	2.67 ± .07	1.58 ± .02	.59	<.01
A14	1.23 ± .03	0.92 ± .10	.75	<.05

The greater TH activity in the BALB/cJ than CBA/J mice reflects differences in TH activity in DA and not noradrenergic neuronal systems since dopamine-β-hydroxylase activity in the A12-13 region was greater in CBA/J than BALB/cJ mice. DA neurons were immunocytochemically stained with specific antibodies to TH and counted through the entire hypothalamic-preoptic region. The total number of stained neurons in the A12-13 region was significantly greater (P<.05) in BALB/cJ than CBA/J mice (7012 ± 537 and 4984 ± 359 respectively). Similarly, at the level of the medial preoptic nucleus there were more A14 neurons in the BALB/cJ than CBA/J mice (3876 ± 522 and 1623 ± 310 respectively). We conclude that: (a) there is a strain difference (BALB/cJ > CBA/J) in hypothalamic TH activity between two inbred mouse strains; (b) the differences in TH activity can be attributed to greater numbers of neurons in all DA cell groups in this region, including A12-A14. These data suggest that genetic control of the number of DA neurons in these mouse strains is not restricted to midbrain DA systems but may be generalized.

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249.2 LURCHER ↔ WILD-TYPE CHIMERIC MICE: CEREBELLAR PURKINJE CELLS ARE PRIMARY SITE OF GENE ACTION. Richard Wetts and Karl Herrup. Dept. of Human Genetics, Yale Med. Sch., New Haven, CT 06510.

Lurcher is an autosomal dominant mutation in the mouse which causes ataxia in the heterozygote (+/Lc). Morphological and developmental studies (Caddy and Biscoe, 1979; Swisher and Wilson, 1977) have shown a dramatic series of cellular degenerations in the +/Lc brain. The cerebellar cortex is most severely affected. Virtually all of the Purkinje cells and a large majority of the granule cells degenerate during the first postnatal month. In the brain stem, the number of cells in the inferior olive (source of cerebellar climbing fibers) is also greatly reduced. As with most other neurological mutants studied, one can ask what is the primary site of gene action and which of the described defects are secondary epigenetic phenomena.

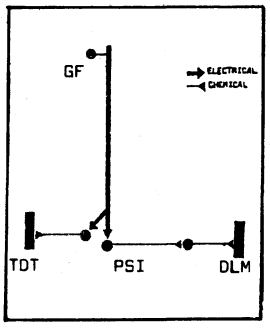
To answer this question we have made lurcher aggregation chimeras. Pairs of lurcher (+/Lc;a/a;Gus^{b}/Gus^{b}) and wild-type +/+;A/A;Gus^{h}/Gus^{h}) 8-cell embryos were aggregated, cultured overnight and transferred to a host female to finish development. All of the verified lurcher chimeras examined thus far have been behaviorly normal, including one animal (χ11) who was 90-95% lurcher in composition. Upon opening the skull, the chimeric cerebella were found to be intermediate in size between lurcher and wild-type. Cytological examination revealed a well ordered trilaminar arrangement in the cellular cortex. Both molecular and granule cell layers appeared roughly normal in width and cell density. The Purkinje cell layer, by contrast was sparsely populated. In sections from χ11, entire folia were commonly found with fewer than 5 Purkinje cells present. The inferior olivary nuclei appeared normal in size and cell density. β-glucuronidase histochemistry revealed that all Purkinje cells present in the chimeras were Gus^{h}/Gus^{h} hence +/+ in genotype. The cells of the inferior olive were a mixture of the two genotypes whose proportions reflected the overall ratio of genotypes in the chimera. Significant in this context was the finding that in χ11 most of the olivary neurons were +/Lc. β-glucuronidase is unfortunately not an appropriate marker for granule cells.

The findings strongly implicate the Purkinje cell as a primary site of gene action thus confirming the hypothesis of Caddy and Biscoe. Despite the mosaic of genotypes in which they develop, not a single lurcher Purkinje cell survived to 3 months of age. By contrast, the +/Lc olivary neurons are "rescued" by the presence of wild-type cells in the chimera suggesting their demise is a secondary event in the +/Lc mutant.

Supported by NIH (HD12213) and March of Dimes, Basil O'Connor starter grant.

249.3 MUTATIONS AFFECTING THE GIANT FIBER SYSTEM OF DROSOPHILA. John B. Thomas* (SPON: R.J. Wyman) Dept. of Biol., Yale Univ., Box 6666, New Haven, CT 06511.

Identified neurons of the giant fiber system in D. melanogaster, as diagrammed in the figure, have been studied in our lab using physiological and anatomical techniques. Each of the bilaterally paired giant fibers (GF) monosynaptically drives the ipsilateral TTM (jump muscle) and disynaptically, via the PSI interneuron, the contralateral DLM (flight muscle) (King and Wyman, J. Neurocytol., in press). The giant fibers can be reliably driven by a light-off stimulus in white-eyed flies of the brown; scarlet genotype.



Mutations on the X-chromosome affecting the structure and/or function of the giant fiber system were isolated using a behavioral screen to select flies failing to jump to the light-off stimulus. 40,000 flies carrying EMS-mutagenized chromosomes were screened. Non-jumpers were tested for physiological defects. Two mutants, nj75 and nj156, were found which have abnormal giant fiber output. In a third, nj42, there is apparently selective degeneration of the TTM.

In nj75 flies the TTM is driven normally by the giant fiber, but the DLM is not. The DLM can be driven only at abnormally low frequencies, and its latency is long and variable (2.5-3.0 msec vs. the normal 1.5 msec*). Since the neuromuscular junctions are normal, the defect is likely to lie in the PSI, or its synaptic input or output.

Neither muscle is driven normally by the giant fiber in nj156 flies, although the nmjs are normal. There is no response in the DLM; the TTM can be driven only at low frequencies and with a longer than normal latency (1.7 vs. 1.0 msec*).

In both mutants the morphology of the giant fiber appears normal using intracellular injection of Lucifer Yellow.

Supported by USPHS #NS-07314 to R.J. Wyman.

*For normal latencies cf. Tanouye & Wyman, J. Neurophys., in press.

249.4 ALTERED CONDUCTION OF IMPULSES AND REDUCED TRANSMITTER RELEASE INDUCED BY SINGLE GENE MUTATION IN MICE. A. Mallart, J.J. McArdle, D. Angaut-Petit and R. Bournaud. Lab. Neurobiol. Cellulaire, CNRS, 91190 Gif/Yvette, France.

Neuromuscular impairement in the single gene mutation of mice called motor end-plate disease (med) starts at 11 days of age and becomes increasingly severe until death occurs by the end of the 3rd week. We performed an in vitro electrophysiological study of impulse conduction by the motor nerves and of transmitter release at the motor endings both in med and in Swiss mice of the same age which served as a control. Altered conduction of nerve impulses was the more constant finding in med mice : all the examined motor axons innervating the deep thoracic muscle were affected in a more or less severe way irrespective of the age of the animal. The intercostal motor nerves were stimulated with suction electrodes and the nerve action potentials or end-plate potentials (e.p.p.s) recorded with microelectrodes. The conduction velocity was 2.68 ± 0.7 $m.s^{-1}$ (S.D., N = 35) for the whole med population as compared to 8.28 ± 3.3 (N = 59) for the controls. The absolute refractory period of conduction, measured by stimulating the nerve with paired shocks of variable interval was on the average 11.33 ± 3.6 msec, (N = 29) in med and 2.61 ± 0.6 msec (N = 28) in controls. By raising the bath temperature above 21°C, all or none conduction block of impulses occurred in med nerves at temperatures ranging between 23 and 36°C, while nerves from controls were blocked at 46-49°C. Spontaneous transmitter release in both young and old med mice was comparable to that observed in control at corresponding ages. On the contrary, evoked transmitter release in med aged 19-23 days was greatly reduced as compared to younger med and to controls. As a consequence 3-4 times less magnesium was required in the former to block neuromuscular transmission. We conclude that altered conduction of impulses is the major neurobiological defect in the med mice, yet we cannot ascertain whether defective transmitter release is a consequence of alterations of the motor axon or both correspond to independent expressions of the med gene. Supported by the D.G.R.S.T. grant n° 79 7 1066.

249.5 FIRST EYE-OPENING: BLACK VS. ALBINO C57BL/6J-c^{2J} LITTERMATE MICE. I. S. Westenberg. Ill. Inst. Devel. Disabil., Chicago, IL 60608.

Wang (1927) noted that pigmented rat pups' eyes open 1-2 days later than albino rat pups' eyes. This suggests that visual system anomalies recently associated with albinism in adult rodents may be related to abnormalities in the timing of the developmental sequence of the albinic visual system. Unfortunately, early comparisons were between pigmented rodents of one strain and albinos of some other strain. Thus, the variables of albinism and strain were confounded. This problem is solved here by comparison of black vs. albino littermates of the same inbred mouse strain, C57BL/6J-c^{2J}. The hypothesis is: For litters of blacks and albinos the first-detected eye-openings are more likely to be in albinos than in blacks.

Albino mice had 2 mutant c^{2J} genes at the albino, or c, locus (c/c); their black littermates had 1 mutant c^{2J} gene and 1 normal gene at the c locus (c/+). Each litter was checked daily; there were 3 possible outcomes: The first eye-opening could be in c/c (a), in c/c and c/+ (tie), or in c/+ (b).

Results in 32 litters were 9 a, 16 tie, and 7 b; these did not differ significantly from those expected by chance (χ^2 test). To detect sex effects the data for males and females were analyzed separately; neither males' nor females' frequencies of a and b differed significantly from chance. To detect maternal effects the data for litters by c/c mothers and litters by c/+ mothers were analyzed separately; again, a, tie, and b frequencies were close to chance. To detect c/c - c/+ differences in litters with early first eye-opening the data for litters with first openings on or before day 12 were analyzed separately; a and b frequencies were not significantly different from chance. In summary, no c/c - c/+ differences were found. Thus, at least for this strain of this species of rodent, precocious eye-opening does not appear to be specifically associated with albinism.

The difference between these results and Wang's may be related to: Experimental design (within-strain vs. between-strains comparison); species (mouse vs. rat); genotype of pigmented rodents (c/+ vs. +/+). The within-strain design was chosen as more efficient for isolating the effects of single mutant genes. Mice were used for genetic controls not yet available in rats. It is unlikely that c/+ black mice's results would have differed from +/+ black mice's, but this remains to be tested. It is possible that c/c - c/+ comparisons in other mouse strains will produce different results. Of course, the present results do not rule out the possibility of abnormalities in the timing of the developmental sequence of the visual system in albinos; the entire sequence must be examined with proper genetic controls.

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249.7 Neurobiological defects of muscle and nerve in murine muscular dysgenesis (mdg/mdg).

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Muscular dysgenesis (mdg) in the mouse is a single recessive autosomal lethal mutation. Affected embryos never show any sign of contractile activity during the whole gestation period and are dead at birth. Mdg/mdg muscles present immature cytological aspects, although less accentuated in diaphragm. Cytochemically demonstrable acetylcholinesterase is localized all over mdg/mdg muscles, in discrete foci of high acetylcholinesterase activity. Isolated single myofibers possess several areas of acetylcholinesterase accumulations and acetylcholine receptors clusters, suggesting multiple innervation. Silver nitrate staining of the motor innervation of skeletal muscles in mdg/mdg embryos at the time of birth evidences a spectacular overgrowth of mdg/mdg nerves and a generalized collateral and ultraterminal sprouting of axons. Neuromuscular transmission exists in mdg/mdg embryo's diaphragm, but the spontaneous myofiber membrane activities are higher in mdg/mdg then in +/mdg? muscle. The spontaneous activities are generated by the release of acetylcholine : they can be blocked by d-Tubocurarine in both +/mdg? and mdg/mdg phrenic nerve - diaphragm preparations. Direct evidence for multiaxonal innervation is found in both +/mdg? and mdg/mdg muscle, since the end-plate potentials varie in a step-wise manner with increasing stimulation. Moreover, an mdg/mdg myofiber can be innervated in several locations, as it was often found that different intensities of stimulation evoke end-plate potentials with different latencies and time course. Thus, mdg/mdg myofibers and axons are in functional contact, in several regions of each myofiber and in morphologically variably mature appositions, and multiple innervation develops more in mdg/mdg embryos than in normal ones.

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249.6 WEAVER MUTANT GRANULE CELL DEFECT EXPRESSED IN CHIMERIC MICE. D. Goldowitz and R.J. Mullen*. Dept. of Anatomy, Univ. of Utah College of Medicine, Salt Lake City, Utah 84132.

The cerebella of both heterozygous and homozygous weaver (wv) mutant mice have abnormalities in granule cells, Bergmann glia (Golgi epithelial cells), and Purkinje cells. To determine in which cell type(s) the mutant gene is acting we produced experimental chimeras containing mixtures of genetically mutant and genetically normal cells. For Purkinje cells, allelic differences in β-glucuronidase activity (Gus) was used as an independent cell marker to determine the genotype of a cell irrespective of its phenotype. In wv/+ $Gus^b/Gus^b \leftrightarrow$ +/+ Gus^h/Gus^h chimeras we found ectopic Purkinje cells included some that were Gus^b (high glucuronidase) and hence wv/+ but also some that were Gus^h (low glucuronidase) and hence +/+ in genotype. The latter we interpret to mean that the Purkinje cell ectopia is not caused by the wv locus acting within the Purkinje cell.

In normal mice a fraction of one percent of the granule cells are found ectopically within the molecular layer whereas in wv/+ mice many granule cells are stranded there during their migration from the external granule cell layer to the internal granule cell layer (IGL). For granule cells, the ichthyosis (ic) locus was used as an independent cell marker. In ic/ic cells there is a greater tendency for the heterochromatin to be clumped in the center of the nucleus rather than being distributed along the nuclear membrane. In wv/+ +/+ ↔ +/+ ic/ic chimeras the internal granular layers were a mixture of +/+ and wv/+ cells. The ectopic granule cells, however, were almost exclusively wv/+ in genotype regardless of the proportion of wv/+ cells in the IGL. These results with wv/+ chimeras suggest that the wv locus does act intrinsically within the granule cell.

At present, we do not know whether the Bergmann glia are directly affected by the wv locus or what interaction might be occurring between the neuron and glia. Additional studies and studies of homozygous weaver chimeras might shed light on these questions. (Supported by NIH Grant NS 16156 and the March of Dimes Birth Defects Foundation.).

250.1 EARLY ONTOGENETIC RESPONSES TO THE SEROTONERGIC AGONIST, QUIPAZINE. Linda A. Ristine* and Linda Patia Spear, Department of Psychology, S.U.N.Y., Binghamton, N.Y. 13901.

Three day old Sprague-Dawley rat pups were given saline, 1, 2.5, 5, or 10 mg/kg quipazine and placed in an incubator at room temperature (22±1°C.) or nest temperature (35±1.5°C.). Beginning 5 min. post-injection, behavioral-time sampling data were collected every 20 sec. for a test duration of 20 min. Quipazine induced a marked dose-dependent increase in activity which included increases in forward locomotion, wall climbing, rolling onto back, forelimb paddling, and hindlimb treading movements. Quipazine also induced a characteristic posture and tremor of the fore- and hindlimbs frequently evident when the animal was stationary -- the paws were held up in the air with the elbows on the floor of the apparatus. Straub tail was induced by the highest doses of quipazine only in neonates tested at room temperature. Quipazine induced an increase in mouthing which was much more prevalent at nest temperature than at room temperature. Not only was this mouthing temperature dependent, it also was positively correlated with length of deprivation of the pup away from the mother.

These results suggest that administration of a serotonergic agonist can have marked behavioral effects in the neonatal rat pup. Some aspects of these behavioral responses are similar to those seen after serotonergic agonist administration to adult animals (e.g., forelimb paddling, Straub tail, unusual limb posture) (Jacobs, Life Sci., 1976, 19, 777), while other behavioral responses are unique to developing animals (e.g., increases in motor movements and mouthing). That quipazine induces temperature- and deprivation-dependent mouthing early in life is interesting as quipazine inhibits rather than accentuates suckling behavior in deprived weanling rats (Nock, et al., Pharmacol., Biochem. Behav., 1978, 8, 277). Further implications of these results will be discussed.

250.2 ENKEPHALIN-LIKE IMMUNOREACTIVITY IN DEVELOPING AVIAN BASAL GANGLIA AND NUCLEUS SPIRIFORMIS LATERALIS. B.M. Davis*, N. Brecha and H.J. Karten. Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, N.Y. 11794.

Immunocytochemistry was used to follow the expression of enkephalin-like immunoreactivity (ELI) in two areas of the embryonic chick brain, the basal ganglia, and a pretectal nucleus, nucleus spiriformis lateralis (SpL).

Staged embryos (Lillie 1936) were dissected free of egg membranes and perfused with periodate-lysine-paraformaldehyde fixative. Whole embryos or brains were post-fixed for 2 hours and stored overnight in 30% sucrose phosphate buffer at 4°C. The embryos or brains were sectioned at 10μm on a cryostat. Sections were picked up on to warm gelatin coated slides and subsequently processed for ELI as described elsewhere (Brecha, et al 1979).

SpL is a nucleus located at the meso-diencephalic junction, whose cells receive a massive input from a basal ganglionic nucleus, paleostriatum primitivum (the avian equivalent of mammalian globus pallidus). SpL gives rise to a large projection upon the ipsilateral optic tectum. In the adult, all cells in SpL have ELI. SpL neurons arise between the 5th and 6th day of embryogenesis (Kuhlenbeck, 1936). Enkephalin positive cells are first seen in the pretectal area and probably presumtive SpL on the 6th day of embryogenesis. These cells are small neuroblast-like cells. In the adult, the cells are multipolar with diameters measuring approximately 25μm.

The avian basal ganglia is composed of four nuclei, distinguishable on the basis of their cytoarchitecture, connectivity and histochemistry. These nuclei are: paleostriatum augmentatum (PA), paleostriatum primitivum (PP), lobus parolfactorius (LPO), and intrapeduncularis telencephali (INP). Preliminary autoradiographic data indicate that PA cells begin to develope during the 4th day of embryogenesis. ELI was first observed in small, round neuroblast-like cells of five day old embryos (Stage 26). Enkephalin positive cells were first found near the external border of the telencephalic vesicles, at the level of the ventricular ridges, the proliferative zones which give rise to the basal ganglia. At later stages, enkephalin positive cells are located more internally. The pattern of expression of ELI results in an "external-internal" spatial temporal gradient. Enkephalin positive cells form a dense accumulation of somata and processes. The density of immunoreactive somata and processes increases up to stage 36, at which time the enkephalin positive cells begin to disperse. In the adult chicken, the enkephalin positive somata are widely separated and confined exclusively to PA and LPO. Fibers with ELI originate in PA and LPO and terminate in PP and possibly in INP. In the adult the enkephalin positive cells are 13-15μm in diameter.
This research supported by EY-02146 and NS-12078 (H.J.K.)

250.3 ACETYLCHOLINESTERASE ACTIVITY IN NEURAL CREST CELLS OF THE AVIAN EMBRYO. P. Cochard* P. Coltey* and N.M. Le Douarin* (SPON: I.B. Black). Institut D'Embryologie Du CNRS 94130 Nogent Sur Marne - France.

Recent work by our group has demonstrated that mesencephalic neural crest cells in the process of migration possess the ability to synthesize significant amounts of acetylcholine (ACH) and, in fact, exhibit detectable activity of the ACH synthesizing enzyme choline acetyltransferase, a fundamental marker of the cholinergic system. The significance of this observation still remains to be established. The present study was undertaken to determine whether another trait of the cholinergic metabolism, the ACH catabolic enzyme acetylcholinesterase (ACHE) was also present in neural crest cells at the onset of migration. Transverse sections of quail and chick embryos at various stages of development were processed for histochemical and cytochemical demonstration of ACHE activity. Results obtained at the optical level showed that ACHE activity was present in cephalic as well as truncal neural crest cells before and during their migration. Enzyme activity was first histochemically detectable at the time of closure of the neural tube, in cells of the neural fold. Activity increased thereafter and maximum staining occurred at the time of individualization of the neural crest. The histochemical reaction then diminished in the migrating crest cells. Identical results were obtained with both quail and chick embryos although the intensity of the reaction was somewhat stronger in crest cells of the latter. Intracellular distribution of the cytochemical reaction product was also studied. Strong ACHE staining was found mainly in perinuclear cisternae and smooth endoplasmic reticulum of crest cells, before and during their migration. Reaction product was sometimes observed in the Golgi apparatus but was never found associated with cell membranes or cell processes. A small proportion of the migrating crest cell population appeared totally devoid of ACHE activity.

These findings indicate that neural crest cells from each level of the neural axis possess the enzyme responsible for ACH degradation well before they start migrating. Moreover it appears that they retain this characteristic while migrating. Thus, it seems that neural crest cells develop some traits of cholinergic metabolism at an early stage of development. This phenomenon could represent the expression of an early differentiation of presumptive cholinergic neuroblasts or be an intrinsic property of all developing neuroblasts. Alternatively the metabolism of ACH might be related to the migration process.

This work was supported by the NIH and the CNRS, France.

250.4 EFFECTS OF TUNICAMYCIN ON NEURITE OUTGROWTH, GLYCOCONJUGATE SYNTHESIS AND LECTIN BINDING IN GOLDFISH RETINA EXPLANTS. Anne M. Heacock. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

Tunicamycin (TNI), a blocker of glycoprotein synthesis via the dolichol pathway, inhibits outgrowth of neurites from explanted goldfish retina (Heacock, Neurosci. Abst. 5, 756 (1979). Dolichol sugars participate in the sequential addition of carbohydrate units to long chain isoprenoid alcohols which act as glycosyl carriers for ultimate transfer of core oligosaccharide to protein-bound asparagine residues. TNI (gift of G. Tamura) interferes with an early step in this pathway, the synthesis of N-acetyl glucosaminyl pyrophosphoryl dolichol, thus preventing formation of dolichol oligosaccharides. This report further characterizes the effects of TNI on goldfish retina explants and on intact retina in vitro.

Tunicamycin at 1, 5 or 10 μg/ ml inhibited neurite outgrowth by 32, 61 and 80%, respectively. The neurites which did grow out had an appearance similar to controls. In order to determine if the neurite membrane assembled in the presence of TNI was deficient in glycoproteins, the lectin binding ability of these neurites was compared to that of untreated cultures. Fluorescence photomicrography, following labeling with rhodamine Concanavalin A, indicated a decreased binding in the TNI-treated cultures.

The mode of action of TNI was examined by labeling studies with various precursors. Maximal inhibition of ^{3}H-glucosamine and ^{3}H-mannose incorporation into glycoprotein (46-54%) occurred with 5 μg/ml TNI. Protein synthesis was inhibited only 13% under these conditions. Because of the limited amount of tissue in the explants, further studies were carried out with intact retinas incubated in vitro. A 4-6 h preincubation with TNI was necessary for maximal inhibition of glycoprotein labeling (40-50%). The residual labeling may at least partially represent more distal attachment of carbohydrate to pre-existing, incomplete glycoproteins. SDS gel electrophoretograms of ^{3}H-glucosamine-labeled homogenates from control and TNI-treated retinas were very similar except for a low molecular weight gel region where labeling was almost abolished by TNI. Material in this region, which was also labeled with ^{3}H-mannose and ^{3}H-glucose, was extractable in $CHCl_3$:MeOH:H_2O (10:10:3) and appears to represent dolichol oligosaccharide. The magnitude and concentration dependence of the TNI effect on the synthesis of this presumptive dolichol oligosaccharide closely resembled that for neurite outgrowth. SDS PAGE revealed at least one ^{3}H-leucine-labeled protein band which was present in TNI-treated but absent in control retinas. (Supported by NS 13743.)

250.5 ANALYSIS OF ABUNDANT mRNAS IN THE DEVELOPING RAT CEREBELLUM. M. Morrison* and W.S.T. Griffin. (SPON: A. Ehle). Dept. of Neurology and Cell Biology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, Tx 75235.

Although many brain functions are dependent on the regulated synthesis of specific proteins, little is known about the synthesis and utilization of their messenger RNAs (mRNAs) during development. We have used two-dimensional gel electrophoresis to compare both the pattern of abundant steady state proteins and the steady state levels of their cerebellar mRNAs. At various stages of postnatal development there were reproducible increases in the steady state levels of several proteins with pIs between 4.5 and 7.0. Of particular interest was a decrease in the levels of the more basic tubulin subunits with age. In order to determine whether these changes were a consequence of alterations in cerebellar mRNA levels, total cytoplasmic RNAs were isolated and compared by two dimensional gel analysis of their in vitro synthesized translation products. During early postnatal development (day 2-6), the mRNA profiles are relatively similar. By day 14, at a time when cell division is slowing and synaptogenesis and myelination are occurring, the mRNA pattern characteristic of adult cerebellum is emerging. By 30 days, this pattern is dominant and remains almost unchanged thereafter. Quantitation of synthesized proteins show that there is a 65% decrease in the synthesis of the tubulin subunits. This decrease is especially marked for the mRNAs coding for the more basic tubulin subunits, T_{β}. Their ratio relative to the mRNAs for the acidic subunits, $T_{\alpha 1}$ and $T_{\alpha 2}$ falls from 1.17 at day 2 to 0.57 at day 90. There is also a 61% decrease in the relative amount of actin mRNA as well as in the mRNAs coding for several as yet unidentified proteins (e,q,s,v,5). Although there is a dramatic decrease in the levels of protein s mRNA between days 2 and 6 and in the levels of protein 5 mRNA between days 6 and 14, most changes occur somewhere between days 14 and 30. The steady state levels of other mRNAs increase with age (p,k,1). Each of these shows different developmental patterns with 1 mRNA increasing steadily throughout development, p mRNA being dramatically induced between 14 and 30 days and k mRNA only being substantially increased after 30 days. This study supports the idea that periods of rapid brain development are accompanied by precisely regulated steady state levels of specific mRNAs.

Support for this work was provided by NIH AI 14663 and a grant from the Leland Fikes Foundation.

250.6 THE DEVELOPMENT OF GLUTAMIC ACID DECARBOXYLASE (GAD) AND CHOLINE ACETYLTRANSFERASE (ChAT) IN THE VISUAL CORTEX AND DORSAL LATERAL GENICULATE NUCLEUS OF THE RAT. J.K. McDonald, S.G. Speciale* and J.G. Parnavelas. Depts. of Cell Biology and Psychiatry, Univ. Tx. Health Sci. Ctr., Dallas, TX 75235.

Although the mammalian visual system is anatomically and electrophysiologically one of the most extensively investigated systems in the brain, there are surprisingly few accounts of its neurochemistry. It is in this regard, that we sought to investigate the activity of glutamic acid decarboxylase (GAD) and choline acetyltransferase (ChAT), the enzymes responsible for the synthesis of γ-aminobutyric acid and acetylcholine respectively, in the visual cortex and the lateral geniculate nucleus of the adult and developing rat.

Sprague-Dawley albino rats of both sexes 0,4,6,8,14,18,21,24 days and 5 months old were used in this study. The animals were decapitated, their brains were rapidly removed and frozen on dry ice and 300 μm coronal sections were cut with a cryostat. Punches of 500 μm in diameter were taken from the visual cortex and lateral geniculate nucleus of adult and developing rats, using 50 μm stained sections as guides, and analyzed for GAD and ChAT (Fonnum et al., Brain Res. 20:259-275 1970; Fonnum et al., Biochem. J. 115:465 472, 1969). Protein determinations were performed with a modified technique of Lowry et al. (J. Biol. Chem. 193:265-275, 1951).

GAD activity in the visual cortex was very low during the first few days of postnatal life and showed a gradual increase between days 4 and 8. Significant increases were observed between days 8 and 14 (4-fold) and days 14 to 18 (2-fold) and after this time only slight increases were observed until day 24. Adult rats showed slightly lower GAD activity in the visual cortex compared to the 24 day animals. GAD activity in the dorsal lateral geniculate nucleus exhibited a similar developmental pattern as in the visual cortex with significant increases occurring between days 8 and 18.

At birth, rats had very low ChAT activity in the visual cortex. This activity showed a moderate increase until day 14 and a dramatic increase between days 14 and 21 of postnatal life. ChAT activity in the dorsal lateral geniculate nucleus displayed a similar developmental pattern as in the visual cortex with the most significant increases occurring between days 14 and 18 and between days 21 and 24. These results will be discussed in the light of existing morphological studies of the visual cortex and the lateral geniculate nucleus of adult and developing rats.

The support of the Biological Humanics Foundation and NIH grant EY02964 to JGP are acknowledged.

250.7 MORPHOLOGICAL DEVELOPMENT AND FUNCTIONAL RESPONSIVENESS OF SUBCORTICAL NE AND DA SYSTEMS IN FETAL RAT BRAIN. M. Schlumpf and W. Lichtensteiger, Inst. Pharmacol. Univ. Zürich, CH-8006 Zürich, Switzerland.

In the rat fetus, the responsiveness of central catecholamine (CA) systems to cholinergic action closely follows the morphological development of these neuron systems. Ascending and descending CA projections develop during the last gestational third. When studied with a modified histofluorescence technique, subcortical target regions (hypothalamus, preoptic and septal areas) are found to be reached by CA fibers around ED 15 (ED 1 = 24 hr after the 2 hr mating period). By then intensely fluorescent fibers of the descending CA systems are also seen in the marginal layer of the spinal cord.

The functional capacity of fetal norepinephrine (NE) and dopamine (DA) systems was analyzed by measuring turnover rates in different brain regions. Nicotine which had been found to affect DA levels in fetal brain (Schlumpf et al., Neurosci. Abst. Vol. 5, 177, 1979), was used to influence the functional state of CA systems. In the adult rat, this drug increases firing in zona compacta of substantia nigra (Lichtensteiger et al., Brain Research 117, 85, 1976) and in locus coeruleus (Svensson and Engberg, Acta physiol. scand., in press). Experiments were conducted mainly at ED 19 3/4.

The mother rat was anesthetized with chloral hydrate Fetal brains were dissected before and 25-75 min after α-methylparatyrosine (α-MT, 250 mg/kg s.c.). NE and DA were determined by a COMT assay in 4 regions of the fetal brain: lower brainstem, midbrain, anterior thalamus + hypothalamus and rostral forebrain with caudate-putamen. According to our preliminary observations, turnover rates of NE and DA of fetuses from untreated mothers differed only moderately, at ED 19 3/4, from adult values. Nicotine increased NE turnover in male and female rat fetuses while its effect on DA turnover varied with the brain region studied.

Our data indicate that certain types of input to central CA systems can be activated in the prenatal period when these systems are still developing.

250.8 REGIONAL CHANGES IN NOREPINEPHRINE CONTENT IN THE MOUSE BRAIN FOLLOWING NEONATAL 6-HYDROXYDOPAMINE TREATMENT. K. L. Lovell. Pathology Dept., Michigan State Univ., E. Lansing, MI 48824.

Previous studies demonstrated that 6-hydroxydopamine (6-OHDA) injected systemically into newborn rats permanently destroys norepinephrine (NE) fibers in the cortex. In the brainstem and cerebellum, NE content and ^{3}H-NE uptake are initially reduced after 6-OHDA treatment but show recovery, with the final levels achieved dependent on dose and age of the rats during injection. The present study was designed to characterize the short-term effects of 6-OHDA and to analyze the dynamics of recovery of NE concentrations in the mouse brain. Mice were injected subcutaneously with 6-OHDA (34, 50, 68 or 100 mg/kg) on various schedules beginning on day 1 after birth. NE content, assayed by a radioenzymatic method, was measured in the cerebellum, cortex and brainstem 24 hr after the last injection or at 18 days of age. For animals given 3 or 5 injections between days 1 and 5, increasing the dose or number of injections above 3 injections of 50 mg/kg did not increase NE depletion measured on day 6 in the cerebellum, but did increase NE depletion in the cortex. Thus these regions show different dose-response characteristics. To investigate variables regulating recovery of the NE system in mice, neonates were given 6-OHDA injections of 50 mg/kg on day 1, days 1 and 3, or days 1, 3, and 5. In the cerebellum NE content was reduced to 20-30% of control values 1 day after the last injection for all groups. There was a significant difference among the groups in the extent of recovery at 18 days of age, with NE content 180%, 132% and 85% of control values after 1, 2 and 3 injections, respectively. In the brainstem, however, the extent of recovery was similar for all schedules of injection, with recovery to 175-200% of control values at 18 days of age. In contrast to studies in rats showing similar short-term cortical depletion, some recovery was seen in the mouse cortex, with initial depletion up to 10% of control values and recovery to 50-60% of control values on day 18. Thus peripheral 6-OHDA treatment in mice produces regional variations in NE depletion and recovery of NE content, dependent on dose and time of treatment, but dose-response relationships and subsequent development of the NE system are different in some respects from those in rats. Association of differences between species with the stage of development or other factors can help delineate variables regulating growth and regeneration of NE fibers. (Supported by the NIH Biomedical Research Support Grant of the College of Osteopathic Medicine, MSU)

250.9 FUNCTIONAL ONTOGENY OF DOPAMINE PRESYNAPTIC RECEPTOR REGULATION
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Dopamine (DA) presynaptic and autoreceptors exert inhibitory feedback control on DA biosynthesis and neural activity. Interruption of impulse flow along afferent DA pathways with lesions or by pharmacological blockade with drugs such as gamma-butyrolactone (GBL) results in an increase in tyrosine hydroxylase activity and DA content in the terminal regions of these pathways, an effect which is attenuated by pretreatment with DA agonists such as apomorphine. This effect is thought to be mediated through presynaptic receptors that function to regulate DA biosynthesis. It has also been suggested that the behavioral sedation induced by low doses of apomorphine is due to preferential stimulation of these inhibitory presynaptic receptors.

In a previous report (Shalaby and Spear, Soc. Neurosc. Abst., 1979) we observed that while high doses of apomorphine were able to increase the behavioral activity of rat pups as early as 7 days postnatally, presumably due to stimulation of DA post-synaptic receptors, low dose apomorphine-induced sedation was apparent no earlier than 4 weeks of age. It was suggested that this effect might be due to late ontogeny of DA autoreceptors. In the present report, the ontogeny of DA presynaptic receptor regulation was examined neuropharmacologically in 7, 14, 21, 28 and 35 day old rats. Animals were injected with either GBL (750mg/kg) alone, apomorphine (1mg/kg) alone, a combination of apomorphine and GBL, or saline alone. 40 minutes later, animals were sacrificed and the striata and olfactory tubercles were extracted and assayed for DA content. As early as 7 days of age GBL significantly increased DA levels in striata, an effect attenuated by apomorphine pretreatment. However, no increase in DA was observed after GBL in the olfactory tubercles until animals were 21 days of age, when apomorphine was also able to attenuate the increase in DA. These results suggest that the DA presynaptic receptors in mesolimbic brain regions mature several weeks later than the early maturing striatal brain regions. Results will be discussed with respect to possible connections with previous behavioral findings on ontogeny of DA presynaptic receptors.

250.10 GANGLIOSIDE DISTRIBUTION IN THE NEONATAL RAT HIPPOCAMPUS.
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The cellular localization of specific gangliosides during neural differentiation remains unclear. Therefore, we have studied the subcellular and subregional distribution of the major gangliosides present at early stages of morphogenesis in the hippocampus. Gangliosides were isolated by a miniaturized procedure (Irwin&Irwin. 1979. Analyt. Biochem. 94:335) from intact hippocampal formations, specific subfields (CA1, CA2-3, and area dentata) of the hippocampus, or subcellular fractions of each of these, and analyzed by thin-layer chromatography. Previous results (Irwin&Irwin. 1979.Dev. Neurosci. 2:129)showing an essentially mature ganglioside pattern at birth except for low levels of GM1 and GD1a were confirmed. Our present results showed this to be true even for the area dentata, prior to extensive synaptogenesis. However, the relative amount of a slowly migrating band tentatively identified as a tetrasialoganglioside, was conspicuously lower in this less differentiated subfield of the hippocampus. Also, some membrane subfractions yielded less GD3 - a ganglioside characteristic of early neurogenesis - than did unfractionated tissue. These results suggest that in the rat hippocampus, the ganglioside most closely correlated with synaptogenesis is a tetrasialoganglioside, and that GD3 may have a subcellular localization separate from other gangliosides or be more labile to preparative and isolation procedures. (Supported by NIH grant 1R01NS15924 and a grant from the Simmons College Fund for Research.)

250.11 DIFFERENCES IN PHOSPHORYLATION BETWEEN THE TWO LARGE SUBUNITS OF BRINE SHRIMP NA^+,K^+-ADENOSINE TRIPHOSPHATASE.
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In brine shrimp Na,K-ATPase, there are two large subunits which function as catalytic sites. Previous observations indicated that both large subunits were specifically phosphorylated from ATP in the presence of Na^+ and Mg^{++} and dephosphorylated in the presence of K^+ [Peterson et al. (1978) JBC 253: 4762]. More recently, Peterson et al. (in preparation) have demonstrated that both subunits have similar peptide maps. In spite of their striking similarities in peptide maps, these two subunits migrate at different positions in Na^+ dodecyl sulfate gels and have different ouabain binding characteristics [Churchill et al. (1980) J. Supramol. Struc., Suppl. 4: 100a]. The presence of two large subunits and similar differences in ouabain binding characteristics have also been observed in mammalian brain Na,K-ATPase [Sweadner (1979) JBC 254: 6060]. In the process of studying the differences in ouabain binding, differences in phosphorylation were also observed between the two subunits. The phosphorylation of the lower large subunit isolated on a pH 7.4 gel was very small at low ATP concentrations (0.8 and 0.08 μM); however, when 10^{-3} M ouabain was present, the inhibition of K^+ dephosphorylation made the phosphorylation of this subunit at 0.08 μM ATP more demonstrable. The phosphorylation at higher ATP concentrations (8 and 80 μM) was easily observed for both large subunits. Since the phosphorylated intermediates are more stable at a lower pH, we analyzed phosphorylation of the two subunits after isolation in a pH 2.4 gel [Avruch and Fairbanks (1972) PNAS USA 69: 1216]. In this gel system, the proportion of the two subunits shifted so that the lower large subunit was more prominent. (In the pH 7.4 gel, the upper large subunit is more prominent for the same preparation of enzyme from 12 h nauplii.) Also, the phosphorylation of the upper large subunit was not observed at 0.08 μM ATP unless ouabain at 10^{-3} M inhibited the K^+ dephosphorylation, but was easily observed at 80 μM ATP. These results suggest that the upper and lower large subunits have charge differences that cause them to migrate to different positions at different pHs. Since the concentration of the lower large subunit at pH 7.4 decreased during development, while the concentration of the upper large subunit at pH 7.4 increased, the synthesis of the two subunits was analyzed to determine if there was a precursor-product relationship (Peterson et al., in preparation). The pulse-chase labeling showed that the lower subunit was not a precursor. Since the lower subunit at pH 7.4 has a more labile phosphorylated intermediate and is less sensitive to ouabain, it may be the catalytic site of an isozyme during early development of brine shrimp nauplii.

250.12 TURNOVER OF TUBULIN IN DEVELOPING RAT BRAIN.
June L. Dahl and Victor J. Weibel*, Department of Pharmacology, University of Wisconsin Medical School, Madison, WI 53706.

The difference between protein synthesis and degradation rates must, to a great extent, control the growth of the brain during its critical stages of development. While it is generally agreed that rates of protein synthesis are higher in the developing brain than they are in the adult animal, there have been conflicting reports regarding the contributions of changes in rates of protein degradation. Previous results from our laboratory (Biochem. J., 180:423, 1979) suggested that a reduced rate of protein degradation is an important factor contributing to the overall increase in protein content in neonatal rat brain. To further examine the role of protein degradation in determining the protein content of developing brain, we have measured the degradation rate of tubulin, the microtubule subunit protein. Tubulin is particularly interesting because its concentration decreases with increasing developmental age.

Five-day-old rats were injected intraperitoneally with 1 mCi of (^{14}C)$NaHCO_3$ per 100 g body weight. Groups of five animals were killed at intervals up to 13 days later. Brains were rapidly removed and individually homogenized. A portion of each homogenate was centrifuged at 100,000 x g to prepare a soluble protein fraction. Tubulin was isolated from the remainder by $(NH_4)_2SO_4$ fractionation and DEAE-cellulose chromatography. Purity of tubulin preparations was monitored by SDS polyacrylamide gel electrophoresis; tubulin content was measured by a colchicine binding assay.

Our results show that tubulin is degraded more rapidly in neonatal than in adult brain. Furthermore, whereas soluble proteins have shorter half-lives than tubulin in adult brain ($t_{½}$ ~ 3 days compared with $t_{½}$ ~ 9.8 days), in the neonatal animal, there is little difference between their degradation rates. These results provide further evidence for the importance of the degradative process in regulating the protein content of developing brain.

(This work was supported by National Institutes of Drug Abuse Grant No. 00697.)

250.13 SYNAPTIC MEMBRANE ANTIGENS IN RAT BRAIN CORTEX DURING POSTNATAL DEVELOPMENT. S.P. Mahadik, A.Korenovsky*, V.Ciccarone*, H.Laev* and M.M. Rapport, Div. of Neuroscience, N.Y. State Psychiatric Inst. and Dept. of Biochemistry, Columbia Univ. N.Y.,N.Y.,10032.

Using an antiserum to a highly purified synaptic plasma membrane (SPM) fraction from rat brain cortex, we have recently detected six polypeptide antigens (56K,58K,62K,63K,64K,66K). The concentrations of the first 5 are higher in SPM than in other subcellular fractions of adult rat cerebrum (1,2). We have now determined the quantities of these 6 antigens in frontal cortex at 6 stages of postnatal development: 0-1,5,14,28,60 & 180 days. Antigenic content was determined in a total membrane fraction (145,000xg pellet) to avoid selective losses, particularly in immature tissue. Polypeptides in the pellet were separated on SDS-polyacrylamide slab gels and stained with Coomassie Blue. Stained bands, representing polypeptides of determined size,were then examined for antigenic content by measuring rocket areas obtained by rocket immunoelectrophoresis. Results are tabulated:

	Rocket area (cm^2) for 250 µg protein from the total membrane fraction					
Postnatal Age (Days)	56K	58K	62K	63K	64K	66K
0-1	0.23	0.71	0.16	<0.05	<0.05	<0.05
5	0.20	0.68	0.14	<0.05	<0.05	<0.05
14	0.55	0.75	0.35	0.09	<0.05	<0.05
28	0.66	2.28	0.71	0.19	0.16	<0.05
60	0.27	1.09	0.91	0.19	0.10	0.08
180	0.24	1.43	1.65	0.57	0.11	0.06

Only three antigens (56K,58K,62K) were present at birth; the other antigens (63K,64K,66K) were only detected at 14,28 and 60 days of age, respectively. Three antigens (56K,58K,62K) increased rapidly during the period of maximal synaptogenesis (12 to 28 days). Two of these (56K,58K) then decreased considerably in the 28 to 60 day period. Two antigens (62K,63K) increased continuously from birth to 180 days, the 62K antigen with differential incremental rates at different developmental periods and the 63K antigen at a relatively constant rate. The contrasting changes in the content in rat brain frontal cortex of these 6 SPM antigens during postnatal development suggest that antigens whose rapid increase coincides with the rapid phase of synaptogenesis could be related to synapse formation. The others may reflect either proteins concerned with synaptic stabilization or proteins in synaptic connections of diverse types.

1. Mahadik et al (1980) J. Neuroscience Methods, 2, 169.
2. Mahadik et al (1980) Trans. Amer. Soc. Neurochem. 11,226,227.

250.14 DEVELOPMENT OF SEROTONIN RECEPTORS IN THE CHICK SPINAL CORD. W. Prozialeck*, A. Pylypiw*, W. Chmelewski* and L. Ross. Department of Anatomy, The Med. Coll. of Penn., Phila., PA 19129.

The avian spinal cord receives a substantial serotonergic input from supraspinal levels. Serotonin-containing fibers originating in the brainstem descend via the anterior and lateral funiculi and terminate in the ventral horn, the dorsal horn, and in the nucleus of Terni, where cell bodies of the pre-ganglionic sympathetic neurons are located. Although it is known that these descending serotonergic fibers establish their first synaptic contacts with neurons in the spinal cord at about 10 days in ovo, the functional development of this system is much less clear. We have therefore characterized and examined the ontogeny of serotonin receptors in this region. The binding of tritiated serotonin to washed membrane preparations of chick thoraco-lumbar (T-L) spinal cord was measured by the method of Bennett and Snyder (Mol. Pharm. 12: 373-389, 1976). Specific binding was defined as the amount of radiolabeled serotonin that could be displaced by an excess (5 µM) of non-labeled serotonin. Receptor density (Bmax) and the apparent dissociation constant (Kd) were calculated from Scatchard plots. Results on T-L spinal cords from 3-4 week old chicks showed that serotonin bound specifically to two distinct types of sites, a high affinity site, Kd=5nM, Bmax= 4.8fmoles/mg tissue, and a lower affinity site Kd=25nM, Bmax= 6.8fmoles/mg tissue. The high affinity binding site displayed pharmacological properties which are characteristic of serotonin receptors. For example, bromo-LSD and non-labeled serotonin were 100 times more potent than propranolol, phentolamine, or dopamine at inhibiting the specific, high affinity binding of serotonin. Studies aimed at characterizing the lower affinity binding site are in progress.

During development, high affinity binding sites were present as early as eight days in ovo (Bmax$\sim$2.0fmoles/mg tissue), the youngest age examined. From day 8 to day 12 the density of receptors increased to $\sim$4.6fmoles/mg tissue and remained constant until day 21 when a marked increase was seen (Bmax$\sim$6.5fmoles/mg tissue). Receptor density remained at this peak level for the first week after hatching and then gradually fell off to $\sim$4.8 fmoles/mg tissue by day 30. The Kd for the high affinity binding of serotonin remained $\sim$4-7nM throughout development.

Since serotonin receptors are present at least two days before descending serotonergic neurons form synaptic contacts with target cells in the spinal cord, it appears that the initial development of serotonin receptors does not require a neuronal input. The role of these serotonin receptors in the development and maintenance of synapses is under investigation.

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251.1 ORGANIZATION OF THE RETINOGENICULATE PROJECTION IN POND TURTLES, PSEUDEMYS AND CHRYSEMYS. P. S. Ulinski. Dept. of Anatomy, The Univ. of Chicago, Chicago, IL, 60637.

The dorsal lateral geniculate nucleus (DLGN) in turtles is an elongate structure which is situated dorsomedial to the optic tract and is bordered rostrally by nucleus ovalis. It is known to receive a direct retinal projection and to project to the dorsal cortex. However, the organization of the retinogeniculate projection is unknown. Accordingly, I examined the retinogeniculate projection by making a series of restricted retinal lesions and studying the resulting degeneration which was visible in Fink-Heimer preparations of DLGN after 14 to 28 days of survival. The experiments demonstrated that the retina projects topographically to DLGN and nucleus ovalis. Thus, lesions of the temporal edge of the retina produced degeneration in the caudal segment (Rainey, 1979, Neurosci. Abstr., 5:804) of DLGN. Lesions situated at successively more nasal points on the retina produced degeneration at successively more rostral levels of DLGN. Lesions of the nasal edge of the retina produced degeneration in nucleus ovalis. Thus, the horizontal meridian of the retina is represented along the rostrocaudal axis of the DLGN-ovalis complex. Since these turtles have a well developed visual streak, consisting of a line of densely packed ganglion cells oriented along the horizontal meridian of the retina (Peterson and Ulinski, 1979, J. Comp. Neurol., 186:17), this result suggests that the retinogeniculate projection is dominated by a magnified representation of the visual streak. (Supported by PHS Grant NS-12518).

251.2 ORGANIZATION OF THE TECTAL PROJECTION TO NUCLEUS ROTUNDUS IN POND TURTLES. W.T. Rainey. Department of Anatomy, University of Chicago, Chicago, IL 60637.

In most reptiles and birds, the optic tectum projects to the thalamic nucleus, nucleus rotundus, which projects in turn to the telencephalon. Two types of evidence suggest that the tectorotundal projection is non-retinotopic in the pond turtles Pseudemys scripta elegans and Chrysemys picta bellii. First, large, non-total injections of HRP into the optic tectum result in anterogradely-filled, tectorotundal axons that are scattered throughout all of rotundus. Primary axonal shafts in the tectothalamic tract give off primary collaterals that enter the caudolateral sector of rotundus. These collaterals produce sparsely-branched, conical arborizations that are often oriented caudolateral to rostromedial in much of the nucleus. Preterminal branches in these arbors bear thin collaterals that have varicosities along their lengths. Although the total spread and complete shape of a primary collateral's arborization is unknown, many arbors probably extend the full length of rotundus and spread fairly widely across the width of the nucleus. Since rotundal neurons have dendritic fields that span one-third to one-half of the dimensions of the nucleus (Rainey, J. Morph. 160:121), the domain of an arbor would intersect the dendritic fields of many rotundal neurons. Second, EM-degeneration analyses provide evidence that tectal synapses are distributed along much of the length of rotundal dendrites. Degenerating boutons have asymmetrical active zones and vesicle sizes that are somewhat larger or smaller than the size of round vesicles in normal boutons with asymmetric active zones. Degenerating boutons are observed on thin dendritic profiles, less than 0.5μ in diameter, medium-sized profiles and large profiles, greater than 1.5μ in diameter. Therefore, they are probably distributed along all segments of rotundal dendritic arbors, but tectal boutons are more common on the distal two-thirds of these arbors. In conclusion, the tectorotundal projection is non-retinotopic and highly convergent upon single neurons. Furthermore, each arborization of a primary collateral will provide a divergent projection to a large number of rotundal neurons. (Supported by PHS Grants GM-00094 and NS-12518).

251.3 RECIPROCAL TECTO-GENICULATE CONNECTIONS IN A SNAKE (Thamnophis sirtalis). D.M. Dacey* (SPON.: R. Tarr). Dept. of Anatomy, The University of Chicago, Chicago, IL 60637.

In addition to retinal input, the lateral geniculate complex of snakes receives a massive tectal projection (Ulinski, J. Comp. Neurol., 173: 1977). This raises the possibility that there is a major interaction between two retinofugal pathways in the lateral geniculate. However, there is no information about the organization of either the retinal or tectal input to the lateral geniculate, so that the precise relationship between the tectal and retinal afferents is unknown. In the present study, restricted injections of HRP were placed in the tectum to examine the organization of the connections between the tectum and the lateral geniculate. There are two major findings. First, there is a discrete projection from both the dorsal and ventral segments of the geniculate to the tectum. Second, connections between the tectum and geniculate are reciprocal and topographically organized.

A single, punctate injection of HRP into the tectum, anterogradely filled tecto-thalamic axons and retrogradely filled geniculate cells. Labelled cells formed a small cluster in both the dorsal and ventral segments of the lateral geniculate. Each cluster was precisely overlapped by a patch of fine terminal arbors. Multiple, non-overlapping tectal injections produce multiple, non-overlapping clusters of labelled cells.

Cobalt enhanced, DAB reacted material provided information about the morphology of the labelled elements. Thus, geniculate cells projecting to the tectum ranged from large, multipolar to small, fusiform types. Tectothalamic axons enter the geniculate by either descending in the axial optic tract or by travelling rostroventrally along the medial face of the marginal optic tract. Axial fibers give off a dense, beaded collateral network, which extends horizontally into both the dorsal and ventral clusters of labelled cells. Marginal fibers turn medially into the geniculate, to form a highly branched terminal arbor, which is flattened in the horizontal plane.

This report provides the first description of a projection from the dorsal segment of the lateral geniculate to the optic tectum. In addition, the tecto-geniculate connections are reciprocal and topographically organized. Thus, in Thamnophis, there is a strong interaction between two retinofugal visual structures in which retinotopy may be preserved. (Supported by PHS Grants GM-00094 and NS-12518).

251.4 VISUAL AND INFRARED INTERACTIONS IN THE OPTIC TECTUM OF RATTLESNAKE: CROSS-MODALITY SUMMATION, ENHANCEMENT AND DEPRESSION. Eric A. Newman* and Peter H. Hartline (SPON: Janice I. Gepner). Eye Research Institute of Retina Foundation, Boston, MA 02114.

We have investigated interactions between the visual and infrared (IR) systems in the tectum of the pit viper (Crotalus viridis) using single unit recordings. Separate, precisely repeated IR stimuli (directed at the heat-sensitive pit organ) and visual stimuli were interleaved with simultaneously presented visual-IR stimuli. By comparing responses (spike counts and histograms) to these three stimulus conditions we evaluated modality summation characteristics. Of the 135 units thus far characterized, 80 were influenced to some degree by both visual and IR modalities. 20 responded well to either stimulus presented alone. These units summed to give a greater response to simultaneously presented stimuli, some summing more, and others less, than linearly. Another 33 cells were driven reliably by only one of the two stimulus modalities (the primary stimulus), but gave enhanced responses when a stimulus in the other modality (the secondary stimulus) was presented simultaneously. In 12 of these units the primary stimulus was visual; in 21, the primary stimulus was IR. In these cells, the secondary stimulus enhanced the response (number of spikes) to the primary stimulus by as much as 300%. Another 3 units responded poorly or not at all to either stimulus alone, but responded reliably when both stimuli were presented simultaneously. Finally, in 24 bimodal cells, the response to a stimulus in one modality was depressed by a stimulus in the other modality. In some cases, this depression abolished the responses completely. In 12 of these units, the primary (excitatory) stimulus was visual; in 12, the primary stimulus was IR.

The cross-modality interactions described here indicate that the rattlesnake tectum is an important center for the integration of visual and IR information. Among the possible functions that might be attributed to these interactions are: 1) cross-modality 'feature-detection' of biologically significant stimuli and 2) enhancement of detection and localization of weak, dual-modality stimuli. Our use of combined stimulation and quantitative comparison of responses revealed multimodal interactions in many units that otherwise would have been classified as unimodal. These techniques, which have not been applied to the tecta and superior colliculi of other animals, might reveal the presence of similar types of multimodal interactions in many species. This research was supported by the Charles A. King Trust, Boston, MA, NIH grant EY 07028 and by NSF grants BNS-7824162 and BNS-7817084.

251.5 VISUAL SYSTEM OF ZEBRAFISH OPTIC TECTUM. P. Sajovic and C. Levinthal, Dept. Biological Sciences, Columbia University, New York City, New York 10027.

We have carried out studies of the visual system in the optic tectum of the zebrafish with the objective of correlating the physiology, morphology, and synaptic interactions of particular cell types. The laminar organization of the tectum is similar in essential respects to other teleosts. Rapid Golgi stains show morphological cell types similar to other fish as well; periventricular layer (SPV) cells have a "pyriform" morphology.

Our physiological studies utilize a computer system for stimulus control and data recording. Single units are recorded using extracellular electrodes plated with silver for marking. Both retinal fibers and tectal cells can be recorded in the tectum; the two are distinguished by an optic-nerve-shock latency test. Retinal units of the most common type give phasic on and off responses to small spots over receptive fields (RF's) about 15 degrees in diameter. There is no evidence of concentric organization.

Tectal cells responsive to visual stimulation fall into four classes, with many cells exhibiting intermediate characteristics. The recording sites for all four response types are usually in the SPV, indicating that cells of the pyriform morphology can exhibit different behaviors. Type I cells show low spontaneous activity and give phasic on and off responses to small spots. The RF's are large (50 degrees) and are often composed of disconnected areas; there is no concentric organization. The excitatory response is briefest where it is strongest, suggesting that it is cut short by an inhibitory process. Response to a large stimulus covering the RF is greatly reduced in comparison to a standard spot. These and other lines of evidence suggest that tectal cells of type I receive inhibition from near neighbor cells of the same type.

Type T cells show tonic firing in the dark. Pure inhibitory RF's can be observed as well as excitatory areas. The phasic excitatory responses are more prolonged than those of type I cells and the reduction of response produced by a large whole-RF stimulus is much less pronounced.

Type S cells are similar to type I except that responses to light on are sustained rather than phasic. Finally there are tectal cells of type B which exhibit spontaneous bursting in the dark; light stimulation may increase or decrease the burst rate.

The results of these studies indicate: 1) that cells of a single layer and common morphological type can exhibit a number of different physiological properties; and 2) some tectal cells appear to derive important properties from inhibitory connections with their near neighbors of similar response type.

This work was supported by granst from the National Institutes of Health (NS 09821 and RR-00442).

251.6 SUBCORTICAL AFFERENTS TO THE SUPERIOR COLLICULUS IN THE HAMSTER. R. L. Van Buskirk. Dept. of Psychology, University of Wyoming, Laramie, WY 82071.

The deep layers of the superior colliculus (S.C.) have been implicated in orienting and attentional functions such as visual fixation, auditory location and lordosis, and respond to somatosensory, auditory and visual stimuli in a variety of mammalian species (Stein, 1975; Chalupa & Rhoades, 1977). Anatomical demonstrations of deep S.C. subcortical afferents have largely been restricted to the cat (Grofova, et al., 1978; Edwards, et al., 1979). This study demonstrates deep S.C. afferents in the hamster using horseradish peroxidase (HRP, Sigma VI, .02 - .05 μl). After 72 hours the animals were sacrificed and the brains reacted with the TMB chromogen (Mesulam, 1978). In the hamster, most somatosensory afferents to the deep S.C. were contralateral to the injection site, including the lateral cervical nucleus and cervical dorsal horn, spinal trigeminal caudalis and oralis, n. gracilis and cuneatus, trigeminal n. principalis and n. intertrigeminalis. Potentially somatosensory afferents were found bilaterally in the external n. inferior colliculus and n. intercollicularis. Auditory afferents were either ipsilateral (medial and lateral superior olives) or bilateral (ventral nucleus of the lateral lemniscus and the nucleus of the inferior collicular brachium). Visually-related afferents included the ipsilateral n. pretectalis profundum and ventral lateral geniculate nucleus, the contralateral deep S.C., and bilaterally the parabigeminal, peri-parabigeminal and pretectal thalamic nuclei. Visual-motor afferents were strictly contralateral in origin (abducens and peri-abducens), whereas cerebellar and related motor afferents were either contralateral (n. dentatus and lateral reticular nucleus) or bilateral (n. reticularis tegmenti pontis and the dorsal-laterally adjacent area). Reticular and nonspecific afferents to the deep S.C. included the dorsal raphe' nucleus, the ipsilateral central gray lateralis, substantia nigra, zona incerta and n. posteriori commissuralis, contralaterally the cervical reticular formation lateral to the dorsal horn and n. prepositus hypoglossi, and bilaterally n. gigantocellularis, n. reticularis pontis caudalis and oralis, n. cuneiformis and subcuneiformis and the ventral central gray. Larger injections which spread into the central gray and n. cuneiformis led to labelling ipsilaterally in the dorsal-medial and lateral hypothalamus and n. lateralis thalami, contralaterally in n. supragenicularis pontis, and bilaterally in locus ceruleus and n. trapezoid. This multiplicity of inputs undoubtedly provides the basis for the sensory, reticular and motor interactions underlying the attentional and orientation functions of the deep superior colliculus.

251.7 THE MORPHOLOGY OF SINGLE AXONS INNERVATING THE HAMSTER'S SUPERIOR COLLICULUS. George M. Sachs* and Gerald E. Schneider (SPON: R. Held). Dept. of Psych., M.I.T., Cambridge, MA 02139

The superior colliculus of the hamster receives partially overlapping, retinotopic projections from the contralateral retina and the ipsilateral visual cortex. Retinofugal axons terminate heavily throughout the stratum griseum superficiale (SGS) whereas the cortex projects mainly to the deepest 1/3 of this layer. In order to visualize single axons of these projections, we made small injections of horseradish peroxidase (HRP) into the brachium of the superior colliculus in adult hamsters. Filled axons and their terminal arbors were followed through serial parasagittal sections and traced with the aid of a drawing tube.

A majority of the filled axons took a straight course within the stratum opticum (SO), turned dorsally into the SGS, sometimes as a pair of branches, and through a series of bifurcations formed a dense terminal arbor in the upper portion of this layer. Their terminal fields were confined to the superficial 2/3 of the SGS and consistently extended 100-150 μm along the antero-posterior (AP) axis of the tectal surface. Less numerous but commonly filled were thicker axons which terminated in deeper portions of the SGS. These axons often divided into several branches within the SO. Some collaterals ascended directly to the terminal field while others continued further caudally only to loop back before terminating. Thereby, despite widespread branching, single axons of this sort also showed terminal fields confined to 100-150 μm in the AP direction. Occasionally, fibers with diffuse terminal fields or irregular trajectories were observed. Some of these gave rise to a few widely separated branches which distributed terminals over a distance of 600 μm along the AP axis.

To investigate the source of these axons, adult hamsters underwent eye removal or transection of posterior cortical efferents 1-3 wk prior to injection. The thinner, superficially projecting axons survived the cortical lesion but were absent after removal of the contralateral eye. Neither cortical lesion nor eye removal alone eliminated all of the thick, deeply projecting fibers.

Previous studies of degenerating retinal projections have noted that terminal degeneration appears initially in the deep 1/3 of the hamster's SGS with more superficial terminals degenerating later. The thinner superficial fibers seen in the present study probably account for the later phase of degeneration. Although alternative sources must still be tested, those thicker, deeply projecting fibers which survive cortical lesions may belong to the deep portion of the retinal projections.

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251.8 A GOLGI IMPREGNATION STUDY OF CELL MORPHOLOGY IN THE HUMAN SUPERIOR COLLICULUS, T.W. Robertson* and T.L. Hickey. School of Optometry/The Medical Center, University of Alabama in Birmingham, Birmingham, Alabama 35294.

The morphology of cells in the superficial (0-1000 micrometers), intermediate (1000-2000 micrometers) and deep (2000-5000 micrometers) regions of the human superior colliculus has been studied in Golgi impregnated material.

The superficial region is composed of the stratum griseum superficiale (SGS; 0-500 micrometers) and the upper half of the stratum opticum (SO; 500-1000 micrometers). Cells located in the SGS tend to have either vertically or radially oriented dendrites. Neurons with vertically oriented dendrites are characterized by eccentrically placed cell bodies (within the dendritic field) from which several dendrites extend either toward the surface of the colliculus (wide- and narrow-field vertical cells) or down toward the intermediate region (marginal cells). Other cells in this region exhibit medium caliber dendrites that extend radially from a more centrally placed cell body (stellate cells). The upper half of the SO is characterized by neurons with horizontally oriented dendrites and centrally placed perikarya. For most of these cells, the total horizontal extent of the dendritic tree is between 250 and 500 micrometers, although a few cells exhibit dendritic spreads of up to 1 millimeter.

The intermediate region of the human superior colliculus is composed of the lower half of the SO and contains cells with vertically oriented dendrites and cells with radially oriented dendrites. Vertically oriented dendrites can be seen extending either above or below the perikaryon for some cells and both above and below the perikaryon for other cells. The total dendritic spread of these latter cells can reach over 1 millimeter. The radially organized stellate cells seen in this region exhibit either smooth or spine laden dendrites and tend to be larger than the stellate cells seen in the superficial region.

The analysis of cell morphology in the deep region of the human superior colliculus is less complete since the Golgi impregnation technique used tends to stain few cells below 2000 micrometers from the colliculus surface. So far two cell types have been studied in this region: large cells with vertically oriented dendrites and large cells with horizontally oriented dendrites. Although these cell types resemble those seen in the superficial and intermediate regions, the cells in the deep region tend to have even larger perikarya and dendritic spreads.

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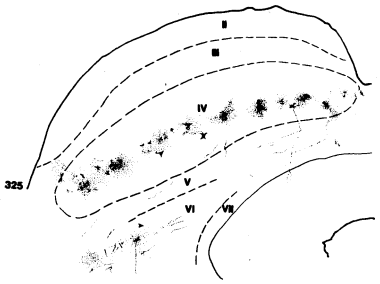
251.9 THE TRIGEMINOCOLLICULAR PATHWAY IN THE CAT: PATCH-LIKE TERMINATIONS. Michael F. Huerta. Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706

HRP studies have shown that cells within the cat's trigeminal complex project to the superior colliculus (SC). In order to determine the distribution of trigeminocollicular axons, either of two anterograde tracers were used; i.e., ^{3}H-proline or HRP liganded to wheat germ agglutinin (HRP-WGA).

Following an injection of ^{3}H-proline into the magnocellular alaminar subdivision of the spinal trigeminal nucleus, discrete patches of label are present contralaterally within the middle of the dorsal-ventral dimension of the stratum griseum intermediale (SGI). Patches of transported protein are also found in the stratum griseum profundum (SGP), but they are fewer in number and less discrete than those in the SGI. In the SGI, patches of label: (1) typically measure 330μ by 250μ, with their long axis parallel to the collicular surface, (2) are separated by 150μ label-free zones, (3) span the entire medial-lateral expanse of the SGI and (4) are present throughout most of the SC, but not present in the caudal pole.

Injections of HRP-WGA which involve the entire trigeminal complex, as well as adjacent structures, result in a distribution and pattern of anterograde label which is identical to that just described. In addition, isolated groups of retrogradely labeled SC cells lie in close proximity to many of the patches of anterogradely transported HRP-WGA (see fig.). The intimate association of retrogradely labeled cells with the anterogradely transported HRP-WGA is especially apparent in the SGI. Approximately half of the backfilled cells in the SC are between 25μ and 40μ in diameter (i.e., medium sized), the remainder have diameters which range from 16μ to 49μ. (Supported by EYO 1277).



251.10 DISCRETE PATCHES OF RETINOTECTAL ARBORS IN NORMAL FROGS (XENOPUS AND RANA). Susan B. Udin. Div. of Neurobiology, Dept. of Physiology; SUNYAB; Buffalo, NY 14214.

The retinotectal projection of amphibians has previously been thought to cover the tectum uniformly. However, filling of cut optic axons with horseradish peroxidase now reveals a very striking pattern of "puffs" of terminals in the stratum zonale, the most superficial of the retinal-recipient laminae of the tectum of Rana pipiens. Cut optic nerves were exposed for 2-6 days to gelfoam or filter paper soaked with horseradish peroxidase. Brains were cut at 25 μ in the transverse or horizontal plane or tecta were removed, flat-mounted, and sectioned tangentially at 25 μ. Filled axons were stained with benzidine dihydrochloride.[1]

The patchy distribution is most visible in the rostral region of the tectum, where the stratum zonale is thickest. The arbors occupy approximately the middle 60% of the lamina, as seen in cross section. When cut tangential to the surface, the puffs are seen to be 25-50 μ in diameter and are round or oval in shape.

In Xenopus laevis, the same methods reveal patchiness, but the segregation is far less distinct than in Rana. Rather than forming discrete puffs of terminals, Xenopus retinotectal arbors are distributed in patches of greater and lesser density in the superficial tectum. The patches of greater density are roughly the same size as the puffs in Rana.

In neither species do the patches form obvious rostrocaudal rows. In this respect they differ from the bands formed by the ipsilateral retinotectal projection of cats[2] and monkeys[3], as well as from the bands observed in three-eyed Rana pipiens[4]. Moreover, the puffs in normal Rana are smaller in size than the bands in three-eyed Rana.

The stratum zonale, the lamina where this patchiness is found in normal frogs, receives not only direct input from the contralateral eye, but also receives an indirect input from the ipsilateral eye, relayed via the nucleus isthmi.[5] The patchy pattern of termination thus may underlie an ocular dominance arrangement of visual inputs to the tectum.

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251.11 THE DUPLEX TECTO-ISTHMO PROJECTION OF THE FROG: AN ANATOMICAL STUDY. R. Leventhal*, S. Glasser, and S.C. Sharma. (Spon: M. Romeskie). Dept. Ophthal., N.Y. Med. Col., Valhalla, N.Y. 10595.

The rostral tectum of the frog receives input from both contralateral and ipsilateral retina.

Fibers from the contralateral retina project directly through the optic chiasm to the tectum. Ipsilateral retinotectal input is relayed first through contralateral tectum and then nucleus isthmi (Keating and Gaze, 1970; Glasser & Ingle, 1978). Fibers from nucleus isthmi, however, terminate in caudal (monocular) as well as rostral (binocular) tectum (Gruberg & Udin, 1978; Grobstein et al. 1978). Destruction of nucleus isthmi not only abolishes ipsilateral units contralaterally, but also produces a significant increase in spontaneous activity throughout both tecta (Glasser & Ingle, 1978). The binocular component of the (isthmotectal) projection arises from ventral and medial rim cells of the nucleus. Fibers from medullary neurons of nucleus isthmi terminate throughout the entire contralateral tectum, (Grobstein et al.1978) and therefore may be responsible for modulating overall tectal activity.

To determine the location of tectal cells projecting to each region of nucleus isthmi, horseradish peroxidase was locally iontophoresed into either rim cell or medullary regions. After survival of 2-14 days and sectioning, the brains were reacted with benzidine dihydrochloride. Neurons projecting to the rim cell region of nucleus isthmi were found at the layer 6-7 border, whereas cells giving rise to the medullary component of the tecto-isthmo projection were found deeper in layer 6.

The implications of these findings will be discussed

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251.12 FOURIER ANALYSIS ON FEATURE EXTRACTION. E. Tzanakou, and A.N. Gentile*. Physics Department, Syracuse University, Syracuse, New York 13210.

In research done in the past, the Receptive Field (RF) of a visual neuron is viewed as a spatial filter capable of processing three independent parameters - spatial frequency, phase and orientation. It has also been shown that a local two-dimensional Fourier-like transform domain provides a good first approximation to the pattern space in which cortical units could be processing stimuli.

We analyze visual RF's in the frog tectum by using a two-dimensional Fast Fourier Transform (FFT). The RF's are mapped by a scanning technique previously described. The intensity patterns that describe the RF's of the individual neurons are then analyzed by a FFT, and the neurons are classified according to their low or high frequency components.

A clustering algorithm segregates multiple, repetitive pattern elements into homogeneous brightness levels as a function of pattern element form, configuration and intensity. Performing Fourier analysis to the RF as a whole and as a separate region as defined by our clustering algorithm, one is able to find the influence at different spatial frequencies from regions surrounding the center of the RF where proximity and similarity are viewed as quantitative parameters in terms of Fourier analysis that relates space grouping to pattern element configurations.

The feature extraction done by our response feedback method (ALOPEX) adds another parameter into our analysis - time. As the pattern approaches the optimal stimulus and therefore the maximum sensitivity of the neuron, the Fourier components segregate.

251.13 METABOLIC MAPPING OF STIMULUS-INDUCED NEURAL ACTIVITY IN THE BASS OPTIC TECTUM: COMPARISON OF ^{14}C-2-DEOXY-D-GLUCOSE WITH ^{14}C GLUCOSE. Kim Traub*, D.P.M. Northmore*, and Leslie C. Skeen (SPON: S. Barnes). Department of Psychology and Institute for Neuroscience, University of Delaware, Newark, DE. 19711.

Recent studies indicate that ^{14}C glucose may provide a number of advantages over ^{14}C-2-deoxy-D-glucose for mapping stimulus-induced patterns of neural activity in the brain (Hawkins & Miller '78). Therefore, we compared glucose and deoxyglucose for functional mapping in the optic tectum of the large mouth bass and explored various modifications of the standard X-ray film autoradiographic method in efforts to improve resolution.

Fish that had been enucleated in one eye 7 days earlier were injected with glucose (S.A. 301mCi/mM, NEN) or deoxyglucose (S.A. 318mCi/mM, NEN) in the dorsal musculature (0.1 µCi/g in 10 µl fish Ringers) and placed in a transparent water-filled cylinder that was surrounded by rotating vertical stripes whose direction alternated every 2 minutes. After 50 min. of visual stimulation one of two procedures was followed: 1) The brain was removed and immediately frozen, or 2) The brain was fixed in 2.5% glutaraldehyde-1% paraformaldehyde by transcardial perfusion and then removed and frozen. Sections were then cut on a cryostat (30µm), dried, and exposed to X-ray film for autoradiography.

Regional optical density measurements from the autoradiographs of all cases showed consistently greater uptake of label in the tectum contralateral to the stimulated eye. Comparisons of these differences between the stimulated and unstimulated optic tecta showed that stimulus-induced labeling was much greater for glucose than for deoxyglucose, and that these differences were further enhanced by perfusion. Autoradiographic resolution was assessed in these same cased by measuring the density gradients across the layers of the stimulated optic tecta. All cases displayed a broad band of increased optical density in the central layers (SFGS & SGC), a drop in density over a deeper fiber layer (SAC), and then a narrow band of increased density over the innermost cell layer (SPV). Comparisons of these tectal density gradients showed that the autoradiographic resolution was greater for glucose than for deoxyglucose and, again, was further enhanced by perfusion. Finally, intertectal density differences and measures of resolution were both improved in the glucose cases by washing the sections with an ethanol/ether solution.

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251.14 THE MORPHOLOGY AND ULTRASTRUCTURE OF TECTAL NEURONS WITH KNOWN EFFERENT PROJECTIONS. Paul J. May and William C. Hall. Dept. of Anatomy, Duke University, Durham, N. C. 27710

Neurons in stratum griseum intermediale of the superior colliculus are of special interest since they project to contralateral paramedian reticular regions known to play an important role in the control of eye movements (Holcombe & Hall, '79). We devised a technique which homogeneously fills the soma and dendrites of neurons with HRP reaction product. This procedure allows us to correlate our light and electron microscopic examinations of these neurons in the grey squirrel (Sciurus carolinensis). To label the neurons a mixture of HRP in 10% saponin is injected into their axons where they cross the midline in the predorsal bundle. After a survival period of 2 to 4 days, the animals are perfused and their brains are sectioned with a vibratome. The tissue is then reacted using an adaptation of the ortho-tolidine technique (Somogyi et al., '79).

The neurons we have labelled exhibit medium-sized (10 x 20µm) somas with several large primary dendrites which typically branch into 2 or 3 smaller secondary dendrites. Fine, beaded tertiary dendrites are also present. The sparsely-branching dendritic field extends up to 200µm from the cell body and is almost always confined to stratum griseum intermediale. Similar neurons can also be observed among Golgi impregnated cells of this layer.

For the electron microscopic experiments, 80µm sections which have been reacted with O-tolidine are osmicated and flat-embedded in araldite. Single labelled cells are cut from the araldite wafer and thin-sectioned. In electron micrographs, the label appears as a darkening of the cytoplasm in the somas and dendrites of filled neurons. The filling with HRP does not seriously disrupt the ultrastructure of either the labelled cells or their synaptic contacts. Synaptic terminals, containing either round or pleomorphic vesicles, make contacts on both the somas and the dendrites of the labelled cells. Often these profiles entirely surround the darkened dendritic shafts for short distances. Thus, with the use of this relatively simple technique, the ultrastructure and afferent connections of single neurons with known efferent projections can be studied.

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251.15 VISUAL WULST NEURONS PROJECTING TO THE OPTIC TECTUM AND THALAMUS IN PIGEONS: RECEPTIVE FIELD PROPERTIES L.R.G. Britto, R. Golfetti* and M.T.R. Perez* Dept. Physiology and Biophysics, UNICAMP, 13100 Campinas, S.P., Brazil.

The hyperstriatal area known as visual Wulst (W) gives rise to fiber systems directed to the optic tectum (TeO), thalamic dorsolateral complex (DLC), pretectum (PT) and other structures. Those W efferents modulate activity at the level of TeO, DLC and PT, primary visual relays in birds, making possible a close comparison of such a projection with mammalian corticofugal systems. The present experiments aimed to identify the W neurons projecting to TeO and DLC, and to characterize their visual response patterns. In anesthetized (Urethane) or paralyzed, artificially ventilated and only locally anesthetized pigeons, stimulating electrodes were placed at TeO and DLC, and in some experiments, at both optic tracts as well. Conventional procedures were used to record extracellular unitary activity of 145 W efferent neurons, identified by antidromic invasion from TeO (latencies ranging 3.2-7.1 msec) or from DLC (2.6-5.2 msec). The main results can be summarized as follows:1. The great majority of W efferent cells could be activated from both ipsi- and contralateral optic tracts, with latencies ranging, respectively, 24-53 msec and 32-81 msec.2. All of them showed anteriorly located fields, approximately rectangular, covering areas from 15 to 380 deg^2, what corresponds to 4-20 deg in their longer axes.3. Those receptive fields appeared highly organized, with several "ON" and "OFF" regions and, accordingly, their responses depended on the form, dimension and orientation of visual stimuli projected on a screen.4. The most effective photic stimuli were moving spots or bars, and all neurons had directional preferences. Further, many were sensitive to specific scan velocities.5. Ipsi- and contralateral receptive fields were similar, but we could observe some degree of dominance, when considering magnitude of responses in terms of total number of spikes elicited. These results agree with anatomical studies which indicate that W projections could subserve some functions in binocular processing and suggest that W neurons convey complex information back to DLC and TeO.

Supported in part by FAPESP (MTRP) and CAPES (RG) fellowships.

251.16 ALTERATION OF CORTICAL ACTIVITY AFTER ABLATION OF SUPERIOR COLLICULUS IN RABBIT, Lex C. Towns, Ph.D., Department of Anatomy, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501

It is well established that the superior colliculus sends axons to terminate in those thalamic nuclei, principally the dorsal lateral geniculate nucleus and lateral posterior nucleus, which project to visual cortex. In the present experiment, a 2-Deoxy-D-glucose (2DG) technique adapted from that described by Sokoloff and his associates (Sokoloff, et al., J. Neurochem, 28, 897-916; 1977) was used to assess alternations in visual cortical activity which accompany removal of collicular input to the visual thalamus.

In each of five 1.5-2.0 kg rabbits, the superior colliculus was ablated unilaterally under Nembutal anesthesia. One week later 2-Deoxy-D-1-[^{3}H]-glucose was injected i.v. (500,µCi/kg) and each animal was immediately placed in a visual stimulating cylinder and stimulated for 45 minutes. Upon completion of visual stimulation, the rabbit was killed, briefly perfused with saline followed by fixative and the brain was rapidly removed, blocked and frozen by slow immersion in a dry ice/acetone bath. Coronal sections of the whole rabbit brain were cut on a cryostat and rapidly dried onto coverslips. Arrays of coverslip-mounted sections were placed in contact with a film sensitive to the low energy beta particle (Ultrofilm, LKB) and exposed for 4 weeks. A qualitative comparison of these autoradiograms revealed a reduction in functional activity of the visual cortex on the ablated side when compared to the intact side. The decrease in activity was particularly apparent in layer IV of striate cortex but there was a generalized decrease throughout both striate and occipital cortex. In addition to the alteration of cortical neuronal activity, there was a consistent decrease in the neuronal activity in the dorsal lateral geniculate nucleus on the ablated side. These results suggest that visual cortex function, at least as that function is related to glucose metabolism, is partially determined by input relayed from the superior colliculus. Supported by NIH EY02285.

252.1 IMMUNOCHEMICAL STUDIES ON ELECTROPHORUS ELECTRICUS ACETYLCHOLINE RECEPTOR USING MONOCLONAL ANTIBODIES. S.J. Tzartos*, D.E. Rand*, and J.M. Lindstrom. Receptor Biology Lab., The Salk Institute for Biological Studies, P.O. Box 85800, San Diego, California, 92138.

Forty cell lines producing monoclonal antibodies (mAbs) against Electrophorus electricus acetylcholine receptor (eel AChR) have been established and characterized. In a previous report (1) mAbs to Torpedo AChR disclosed the existence of a main immunogenic region on Torpedo AChR located on the α subunit, distinct from the toxin binding site and exposed on the extracellular surface of the membrane. We also described immunochemical similarities between α and β as well as between γ and δ Torpedo AChR subunits as revealed by two mAbs. We now have extended our library of anti-AChR mAbs with 33 mAbs derived from rats immunized with Triton X-100 solubilized eel AChR and 7 mAbs from rats immunized with SDS-denatured eel AChR subunits.

These 40 mAbs have shown some characteristic similarities with the 17 anti-Torpedo AChR mAbs (1). Thirty bound detectably to SDS-denatured eel AChR subunits, the majority of which (24) were directed against the α' subunit. Three others were anti-β' and one anti-δ'. Two mAbs crossreacting with two subunits each were also found (one with α' - β' and another with γ' - δ').

Most of the anti-α' mAbs crossreacted with native Torpedo AChR, but usually with lower affinity. Half of the anti-α' also crossreacted with fetal calf muscle AChR, generally with very low affinity. The mAb #35, however, had a titer for fetal calf AChR comparable to that for eel AChR (3 μM against 15 μM). The group of six mAbs directed to other than α' subunits were absolutely species-specific.

Using a technique described previously (1), pairs of antibodies were tested for their capacity to bind simultaneously on Sepharose-bound AChR or compete with each other for the same region on the receptor. We found that a main immunogenic region also exists on eel AChR, when injected in rats, that is located on the α' subunit and is rather homologous to the Torpedo AChR main immunogenic region. Indeed, all 10 anti-α' mAbs which so far have been studied were found to compete with each other for the same region on eel AChR. These mAbs also compete with anti-Torpedo mAb #6 which binds to the main immunogenic region of Torpedo AChR (1) and crossreacts with eel AChR. However, in contrast to the mAbs directed against the Torpedo AChR main immunogenic region, the above 10 mAbs crossreact well with SDS-denatured α' subunit. (Supported by grants to J.M.L. from NIH and MDA, and a MDA fellowship to S.J.T.)

(1) Tzartos, S.J. and Lindstrom, J.M., PNAS, 77, 755-759, 1980.

252.2 BINDING OF ANTI-ACETYLCHOLINE RECEPTOR ANTIBODIES TO DETERGENT-SOLUBILIZED AND MEMBRANE-BOUND RECEPTORS. S. C. Froehner* (SPON: N. G. Bacopoulos). Dept. of Biochemistry, Dartmouth Medical School, Hanover, NH 03755.

The accessibility of antigenic sites on detergent-solubilized and membrane-bound Torpedo acetylcholine receptors (AChR) has been investigated with two classes of antisera. Antisera to native AChR (anti-nAChR) or to SDS-denatured AChR (anti-dAChR) were raised in rabbits. Both animals injected with native AChR developed muscle weakness while the two injected with denatured AChR did not. Antibody activity determinations with ^{125}I-AChR showed that all four antisera preferred native to denatured antigen. Titers of anti-nAChR were 2-4 fold higher than those of anti-dAChR when native ^{125}I-AChR was used. Reaction of antisera with the subunits of AChR separated by SDS gel electrophoresis indicated that all four antisera recognized determinants on each of the subunits.

The ability of these antisera to react with membrane-bound AChR was determined by incubating a constant amount of antiserum with various concentrations of AChR-rich membrane vesicles. After removal of the membrane-bound antibodies by centrifugation, the supernatant was assayed for anti-AChR activity. In the presence of an excess of AChR-rich membranes, approximately 70-80% of anti-nAChR was bound while only 10-20% of anti-dAChR reacted. Control experiments indicated that rabbit anti-α-bungarotoxin (anti-αBuTx) antibodies did not bind to the membranes. Thus, the anti-nAChR sera are directed predominantly against sites exposed in the membrane, while anti-dAChR sera primarily recognize antigenic sites that are not accessible in the membrane. Alkaline extraction of the AChR-rich membranes, which removes a polypeptide of 43,000 mol. wt., causes a 4-6 fold increase in their ability to bind anti-dAChR. Treatment of the membranes with saponin to allow access of antibodies to the interior of the vesicles also enhances anti-dAChR binding. Neither alkaline-extraction nor saponin treatment promotes non-specific binding of anti-αBuTx antibodies. Thus, these treatments expose antigenic sites on membrane-bound AChR which are normally inaccessible to antibodies. Supported by NIH grant NS 14871 and the Muscular Dystrophy Association.

252.3 Cationic sodium substitutes block endplate channels at the amphibian motor endplate. J.F. FIEKERS* (SPON: G.D. WEBB) Dept. Anatomy and Neurobiology, Univ. of Vermont College of Medicine, Burlington, Vermont 05405.

Previous studies demonstrated that complete substitution of TRIS or Glucosamine (GLU) ions for sodium in normal frog Ringers' solution (NFR) produces a voltage-dependent reduction in the amplitude of endplate currents generated by iontophoretically applied (EPCs) or spontaneously released (MEPCs) acetylcholine (ACh). (Fiekers, J.F. and Henderson, E.G. Biophys. J. 21:177A, 1978. This was manifested by a nonlinear peak current-voltage plot (I-V) in which peak current at negative membrane potentials was markedly reduced, but peak current at +40mV was approximately equal to control. The present experiments were undertaken to investigate the mechanism of blockade produced by these cationic substitutes. MEPCs and EPC fluctuations produced by iontophoretically applied ACh were obtained in single voltage clamped fibres from a monolayer preparation of the cutaneous pectoris muscle (Rana pipiens). Mean channel lifetime (τ), single channel conductance ($\bar{\gamma}$), MEPC amplitude and time constant of decay (τMEPC) were determined and the variation of these parameters as a function of voltage and concentration of each substitute was analyzed. In NFR, totally substituted, and partially substituted solutions MEPCs decayed as a single exponential function of time and the spectra were adequately fit by a single Lorentzian. The reversal potential was obtained by the actual reversal of mean EPC. With total substitution of TRIS or GLU for sodium in NFR, MEPC amplitude at hyperpolarized potentials was markedly reduced and measurement of $\bar{\gamma}$ was difficult. In four endplates $\bar{\gamma}$ was 2.3±1.4pS @ -80mV (19°C). Conversely $\bar{\gamma}$ estimated at +30mV was unaltered from values obtained in NFR (23.2±4.8pS). At a constant membrane potential (-80mV) $\bar{\gamma}$ decreased and τ_{noise} increased with increasing concentrations of TRIS and GLU. Partial substitution (50%) yielded values of $\bar{\gamma}$ of 6.3±1.3pS (TRIS) and 4.8±0.4pS (GLU) at -80mV (90°C); however, at +22mV $\bar{\gamma}$ was 24.5±2.1pS (TRIS) and 23.4±2.7pS (GLU). MEPC amplitude decreased and τ_{MEPC} increased with increasing concentrations of each substitute. τ_{MEPC} approximated τ_{noise} when measured in the same endplate. In the substituted solutions, $\bar{\gamma}$ increased and τ decreased as the membrane became more positive. These observations suggest that these cationic substitutes interact with endplate channels to reduce cation permeation. The dependence of this block on the value of membrane voltage suggests that these ions bind to a site within the endplate channel. These data provide an explanation for the nonlinear I-V plots obtained in the presence of these substitutes. Supported by PHS HL-22269-02.

252.4 FREEZE FRACTURE EVIDENCE SUGGESTS THAT AGGREGATED ACETYLCHOLINE RECEPTORS ON CULTURED EMBRYONIC MUSCLE LIE IN MEMBRANE REGIONS LOW IN CHOLESTEROL. P. C. Bridgman*. (SPON: Y. Nakajima) Dept. Biol. Sci., Purdue Univ., West Lafayette, IN 47907

Several sterol-specific cytochemical agents have been recently introduced for the freeze-fracture localization of cholesterol in cell membranes (1,2,3). These agents include the polyene antibotic filipin, the glycosylated sterol digitonin and the surface active agent saponin.

I have used all three of these sterol-specific agents on cultured noninnervated and innervated Xenopus embryonic muscle cells and the distribution of their sterol complexes was very similar. The appearance of these complexes, however, was markedly different. Filipin-sterol complexes were recognizable as 25-30 nm protuberances on the P-face of the replica and seemed to provide the best resolution and specificity for detecting cholesterol.

Freeze-fracture of 1 day and 7 to 8 day old muscle cell cultures revealed that sterol complexes were present on most of the membrane, but were absent from shallow depressions on the plasma membrane which probably represent coated pits (3). Complexes were also absent from long streaks of membrane usually found on thin extended processes of muscle cells. Occasionally areas of the plasma membrane which presumably underlie the nucleus also contained a low density of complexes. Cross-fractures revealed the absence of complexes from nuclear membranes which are known to be low in cholesterol content. The most striking observation, however, was the virtual absence of complexes from tight aggregates of large intramembrane particles characteristic of acetylcholine receptors (AChR's). This was true in both large clusters of aggregates which may represent AChR "hot spots" and in the area surrounding smaller isolated aggregates which are occasionally found on the membrane.

Treatment of 2 day old nerve-muscle cocultures again revealed the absence of complexes from areas of tightly aggregated large particles in the postsynaptic membrane.

Based on this evidence it appears that aggregated AChR's in embryonic muscle lie in membrane regions of low sterol content. A difference in the lipid content of the membrane containing high densities of AChR's may aid in restricting their dispersion. This may be one factor responsible for the stability of such AChR aggregates.

(Supported by USPH Grant NS-10457 and 5-T32-GM07211)

1. Elias, P. M., et al., (1978) J. Cell Biol. 78:577
2. Andrews, L. D. and Cohen, A. I., (1979) J. Cell Biol. 81:215
3. Montesano, R., et al., (1979) PNAS 76:6391.

252.5 THE HILL COEFFICIENT OF THE ACETYLCHOLINE RECEPTOR DOSE-RESPONSE RELATION IS INDEPENDENT OF MEMBRANE VOLTAGE AND TEMPERATURE.
H.M. Hoffman* and V.E. Dionne* (SPON: S. Varon). Div. of Pharm., University of California at San Diego, La Jolla, CA 92093.

A rapid equilibrium dose-response method has been developed which allows effects of environmental factors on this relationship to be examined. Experiments were performed on the acetylcholine receptors of garter snake (sp. Thamnophis) twitch fiber neuromuscular junctions. These compact endplates were observed with Nomarski optics and voltage clamped with two microelectrodes, allowing agonist-induced changes in membrane conductance to be monitored. Agonists were applied iontophoretically from a point source located a measured distance form the endplate; this distance was chosen so that variation of source-receptor separation for different receptors on the endplate surface was $\leq$ 5%. Agonists were released from the source with a 5-8 second ramp of iontophoretic current such that the release rate of agonist ions increased linearly with time. The agonist concentration at the receptor surface was computed from the diffusion equation assuming the iontophoretic pipette transfer number was constant for currents above a critical value at which just-detectable release occurred. By plotting measured endplate current against predicted surface concentrations, an uncalibrated dose-response curve was obtained; on a log-log plot these curves were linear with nominal slope values near two for acetylcholine and carbamylcholine. Accurate evaluation of the slope -- termed the Hill coefficient, n_H -- depends on several factors, not all of which were measured in these experiments. We examined, however, the effects of temperature and voltage on n_H by recording as many as 24 separate, rapid dose-response curves from individual cells as these extrinsic variables were changed. In these experiments temperature was varied between 6-20°C and membrane voltage from -40 to -120 mV without effect; neither variable produced a significant systematic change in n_H. The average temperature dependence of n_H (normalized to unity) was -.0091 ± .0030 per °C, and the dependence on voltage was -.0010 ± .0018 per mV (mean ± S.D.).

This work was supported by USPHS grant NS 15344 from the NIH.

252.6 PURIFICATION AND CHARACTERIZATION OF A NICOTINIC ACETYLCHOLINE RECEPTOR FROM *DROSOPHILA MELANOGASTER*.
T. Schmidt-Glenewinkel*, T.R. Venkatesh* and L.M. Hall, Dept. of Genetics, Albert Einstein College of Medicine, Bronx, NY 10461.

The central nervous system of *Drosophila melanogaster* contains an α-bungarotoxin binding component with the properties expected of a nicotinic acetylcholine receptor(Schmidt-Nielsen, B.K., J.I. Gepner, N.N.H. Teng and L.M. Hall, J. Neurochem., 29:1013, 1977). The isolation of this receptor from *Drosophila* has been approached with the underlying concept that the receptor in this species would be amenable to sophisticated genetic manipulation and analysis. The receptor was solubilized from a membrane preparation from heads of *Drosophila* in the presence of 0.5 M sodium chloride and 1% Triton-X-100. The solubilized form was initially purified by passage over an affinity column, composed of lectins from *Lentil culinaris* covalently linked to Biogel A-15m. It was eluted from this column with 2% methyl α-D-mannopyranoside in the presence of 1% azolectin. Further purification was achieved by two passages over a second type of affinity column prepared from α-cobratoxin from *Naja naja siamensis* linked to Sepharose 4B. Binding of α-bungarotoxin was determined with a modified DEAE-filter assay which allows the quantitation of the binding component in both its membrane-bound and solubilized form. Purification was monitored by one- and two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis and isoelectric focusing, using a sensitive silver staining procedure for the detection of proteins. The receptor binding properties of α-bungarotoxin derivatized with 4-methylazidobenzoimidate have been studied and the electrophoretic behavior of the receptor after cross-linking to α-bungarotoxin has been investigated. The acetylcholine receptor from *Drosophila* is a glycoprotein with a molecular weight in the range of 250,000-500,000. Data will be presented for its subunit composition, the possible acetylcholine binding site, and its pharmacological properties in the crude and purified extracts.
(Supported by grant 1126A from The Council for Tobacco Research-U.S.A., Inc. and NSF grant BNS-7929435. L.M.H. is a McKnight Scholar in Neuroscience).

252.7 BLOCKADE AND BINDING OF QUINUCLIDINYL BENZILATE AND α-BUNGAROTOXIN TO HELIX ACETYLCHOLINE RECEPTORS.
J. C. Hancock, D. B. Hoover and N. S. Talley, Department of Pharmacology, East Tennessee State University, College of Medicine, Johnson City, Tennessee 37601.

Quinuclidinyl benzilate (QNB) binds with great specificity to muscarinic receptors and α-bungarotoxin (αBT) binds with great specificity to nicotinic receptors in vertebrate systems. We have studied the effects of QNB and αBT on the electrophysiological response to acetylcholine (ACH) on identifiable cells in the nervous system of the snail Helix aspersia and the binding of ^{3}H-labeled QNB and ^{125}I-labeled αBT to subesophygeal ganglion preparation. Standard binding and electrophysiological techniques were used. Specific binding of QNB (1nmole) was obtained at a level of 17 fmole/μg protein. QNB binding to whole brain preparations was blocked by atropine (1μmole) but not by d-tubocurine (d-TC:50μmole) or hexamethonium (C_6:50μmole).α-BT (2.8nmole) bound specifically at the level of 0.39fmole/ug protein to brain homoginates. αBT binding was blocked by nicotine (100μmole). Its blockade by atropine, d-TC and C_6 have not been determined. Electrophysiological studies show several different types of responses to ACh. We have studied the effect of QNB and αBT on two of these responses. On inhibitory responses where the hyperpolarization is mediated by an increase in conductance to chloride, QNB (100μmole) blocked the hyperpolarization caused by ACh (5μmole). This response was also blocked by atropine (5-100μmole), d-TC(10-100μmole) or C_6 (10-100μmole). On excitatory responses, where the depolarization is mediated by an increase in sodium concentration, the minimum concentration of QNB that would block the depolarization caused by 5μmole ACh was 1mmole QNB. Soaking for 1 hr. in 10μmole αBT did not alter the hyperpolarization caused by 5μmole ACh. Atropine (5-100μmole) and d-TC(10-100μmole) blocked the ACh-induced depolarization of these cells. The results indicate that, while QNB and α-BT bind specifically to cholinergic receptors of snail brain, these agents are not highly specific for the cholinergic receptor types tested as demonstrated by the high concentrations necessary to block.

Cholinergic receptors of gastropods cannot be classified as distinctly nicotinic or muscarinic as evidenced by their blockade by either atropine or d-TC. This lack of differentiation may account for the failure of QNB and α-BT to prevent pharmacological responses to ACh. (Supported by Biomedical Research Development Grant 1-S08-RR 09171-01).

252.8 Effects of Physostigmine and Mecamylamine on the Response to Acetylcholine in Aplysia.
M.G. Filbert,* J.T. Moffitt,* P.G. Sokolove, D.O. Carpenter and M.J. McCreery.* (Spon: B. Hackley) USA Biomedical Laboratory, APG, MD 21010; Dept Biol, UMBC, Catonsville, MD 21228 and Bethesda, MD 20014.

It is widely accepted that the effects of physostigmine (physo) can be attributed to the accumulation and prolonged action of acetylcholine (Ach) due to inhibition of Acetylcholinesterase (AChE). However, evidence also suggests a direct action of physo on postsynaptic membranes (1,2). It has been shown that physo blocks binding of ^{125}I-α-Bungarotoxin (α-Btx) to ganglionic homogenates of aplysia and also blocks inhibition by α-Btx of the electrophysiological response to Ach (3). Recently, mecamylamine (meca), a nicotinic blocker was observed to inhibit α-Btx binding to Aplysia homogenates as well. The I_{50}'s for the inhibition of α-Btx binding by physo and meca were comparable to that of d-tubocurarine. (4)

We have examined the effects of these cholinergic agents on the electrophysiological responses of Aplysia neurones to iontophoretic application of Ach and carbechol (Carb). Iontophoresis of physo onto the cell just before Ach or Carb almost always resulted in a reduced response whereas its perfusion sometimes caused an increase in the amplitude and duration of the response to Ach as expected with inhibition of AchE. The sensitivity of the Ach and Carb response to inhibition by physo was dependent upon the conductance change elicited: $g_{Na^+} > g_{Cl^-} >> g_{K^+}$. Iontophoresis of meca resulted in diminution of the response irrespective of whether the voltage change was due to an increase in conductance to Na^+, Cl^- or K^+.

These results suggest that the classic anti-AChE physo can also interact directly with the ACh receptor. At low concentrations of physo, enzyme inhibition predominates leading to potentiation of the ACh response. Higher concentrations of physo results in its binding to the ACh receptor which reduces the ACh response amplitude and offsets the effects due to enzyme inhibition. Only a reduction in the response to the nonhydrolysing substate Carb was observed with physo. These data are consistent with the binding curves for physo with AChE (I_{50}=.25μm) and for its inhibition of α-Btx binding (I_{50}=5.0μm)(3). The ACh response is reduced by meca which has no enzyme inhibiting activity.

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252.9 USE OF (^{3}H)BROMOACETYLCHOLINE TO LABEL NICOTINIC ACETYLCHOLINE RECEPTORS FROM THE CENTRAL NERVOUS SYSTEM. T.H. Large*, R.G. Siman*, and W.L. Klein. (Biological Sciences, Northwestern University, Evanston, IL 60201)

We report here the first use of (^{3}H)bromoacetylcholine (BAC) to quantify nicotinic acetylcholine (ACh) receptors in the central nervous system. High specific activity (^{3}H)BAC was synthesized from (^{3}H)choline and bromoacetylbromide and purified by TLC (butanol:water:ethanol:acetic acid::4:3:2:1). Previous work has shown that (^{3}H)BAC reacts covalently with reduced nicotinic ACh receptors from electrophlax (Damle, V.N., et al., Biochem. Biophys. Res. Comm. 84: 845, 1978). Similarly, using dithiothreitol-reduced membranes prepared from retinas of 1 day old chicks, we have found that 3 x 10^{-9} M (^{3}H)BAC specifically reacts with 1100 fmols of nicotinic sites per mg membrane protein. Labeling is complete within 90 minutes at 23°. Binding in the presence of 10^{-3} M nicotine is taken to be nonspecific. The IC_{50} of ACh for blockade of labeling by 3 x 10^{-9} M (^{3}H)BAC is 10^{-6} M. A ten-fold higher concentration of (^{3}H)BAC labels the same number of sites as the lower concentration but in a period of less than 30 minutes. The concentration of specifically labeled sites in membranes from 16 day old embryonic chick retinas is only 15% that measured in membranes from 1 day old animals, correlating with a similar difference in specific (^{125}I)alpha bungarotoxin binding sites between the two ages. Additionally, we have found that unlabeled BAC at concentration of 10^{-8} to 10^{-7} M irreversibly blocks 50% of specific (^{125}I)alpha bungarotoxin binding to chick retina, optic tectum, and skeletal muscle preparations. If membranes are not reduced with dithiothreitol, BAC reversibly blocks binding of 10^{-8} M (^{125}I)alpha bungarotoxin with an IC_{50} of 10^{-5} M. Not all of specific toxin binding can be irreversibly blocked by BAC, even at concentrations adequate to reversibly block binding 100%. The ability of BAC to block toxin binding and to covalently react with reduced sulfurs in both skeletal muscle and central nervous system preparations suggests considerable homology between muscle cell and nerve cell nicotinic ACh receptors. (Supported by NIH grant 5 RO1 NS15299-02 to WLK.)

252.10 ANALYSIS OF CHANNELS ACTIVATED BY ACETYLCHOLINE AND GLUTAMATE IN CRUSTACEAN MUSCLES. C. Lingle and A. Auerbach. Biology Dept., Brandeis U., Waltham,MA 02254; Lab. Neurobiol., San Juan, PR 00936

Striated muscles in the decapod crustacean foregut are innervated by either cholinergic or glutamatergic excitatory motor neurons. Some muscles that receive glutamatergic innervation also display ACh receptors extrajunctionally. Thus, it is possible to characterize both cholinergic and glutamatergic synaptic conductances on similar muscles and, further, to compare channels activated by either ACh or Glu on the same muscle fibers. Towards this end, ACh-induced currents were analyzed by two methods on cholinergic gm1 muscles of Cancer and Panulirus. First, current noise generated by ACh application to voltage-clamped regions of fibers was examined with fluctuation analysis. Second, decays of synaptically evoked currents were examined by averaging of focal extracellular synaptic potentials at cholinergic junctional sites. ACh application to cells evoked stable mean current increases and an increase in current noise variance. Power spectra of current fluctuations were fit to single Lorentzians from which values for both duration(τ) and amplitude(γ) of elementary events were obtained. At 12°C, τ(noise) was about 11.5 ms at -60 mV and about 16.5 ms at -100 mV. At -60 mV, τ(noise) was prolonged to 25 ms at 5°C and shortened to about 4 ms at 21°C. Single channel conductances were calculated assuming a reversal potential of 0 mV. In single fibers γ was voltage dependent varying between about 4.5 pS at -120 mV to about 8 pS at -40 mV. Average γ at 5°C was 2.7 pS (n=3); at 12.5 °C, 6.2 pS (n=14); and,at 21°C, 13.5 pS (n=13). The Q_{10} of both τ and γ is about 3. Decays of extracellular focal currents from single junctional sites were single exponentials over most of their time course. The voltage dependence of τ(decay) varied slightly among fibers, but at 13°C was about 10 ms at -60 mV and 15 ms at -100 mV. At -60 mV, τ(decay) was about 25 ms at 6°C and 5.5 ms at 21°C. The correspondence of the voltage and temperature dependence of τ obtained by both methods supports their validity and indicates that τ(decay) of synaptic currents at a crustacean cholinergic synapse is probably determined by channel closing rate. ACh responses of these muscles are pharmacologically similar to ganglionic nicotinic receptors (Marder and Paupardin-Tritsch, J. Exp.Biol. 1980, in press). It is interesting that the properties of τ of these muscle ACh channels also bear similarities to the ganglionic channel τ. In addition, we have examined both ACh and Glu current fluctuations on muscle gm6b, which receives glutamatergic innervation while displaying extrajunctional ACh receptors. Glu opens channels of larger conductance (20-30 pS) and different voltage dependence than those activated by ACh on this muscle.

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253.1 ATTENUATION OF THE PRESSOR RESPONSE TO CENTRALLY ADMINISTERED PROSTAGLANDIN E_1 BY PROGESTERONE. K. M. Skoog* and Nancy J. Kenney, Dept. of Psychology, University of Washington, Seattle, WA 98195.

Progesterone attenuates the pressor effect of various peripherally administered hypertensive agents, e.g., norepinephrine and desoxycorticosterone. Progesterone's effect on centrally mediated pressor responses has not yet been reported.

In this study, changes in mean arterial blood pressure (MABP) following intracerebroventricular (IVT) injection of two well-known pressor agents, angiotensin II (AII) and prostaglandin E_1 (PGE_1) were studied in ovariectomized (ovx) and ovx,progesterone-treated Long-Evans rats. Progesterone (5mg/rat/day) or its seseme-oil vehicle (0.1ml) were injected subcutaneously for 10 days beginning the day after ovariectomy. Blood pressure changes following IVT injections of either 100ng PGE_1 or 5ng AII and their respective carrier solutions were measured by means of indwelling aortic catheters on the last two days of progesterone or oil treatment.

Baseline MABP was unchanged by progesterone treatment. Average basal MABP was 101.1 $\pm$ 2.9 mm Hg for the ovx, progesterone-treated rats and 100.0 $\pm$ 0.8 mm Hg for the oil-treated controls. Progesterone treatment had no effect on the increase of MABP to IVT AII. Ten minutes following the AII injection MABP for the progesterone-treated rats had an average of 9.2 $\pm$ 0.8 mm Hg over baseline levels while that of controls had increased an average of 11.2 $\pm$ 3.1 mm Hg ($t(5)=0.396$, $p > .20$).

The pressor response following IVT PGE_1 injection was markedly attenuated by progesterone treatment. MABP of control animals increased an average of 17.0 $\pm$ 1.5 mm Hg 10 min following the PGE_1 injection while that of progesterone-treated rats rose only an average of 1.6 $\pm$ 4.3 mm Hg ($t(5)=2.920$, $p < .05$).

Progesterone has been reported to block the effects of the E prostaglandins in various peripheral organs. Since progesterone readily crosses the blood-brain barrier, this steroid may act either centrally or peripherally to attenuate the hypertensive response resulting from increases in central PGE levels.

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253.2 EVIDENCE THAT THE VENTRAL SURFACE OF THE BRAIN STEM IS THE SITE WHERE MUSCIMOL ACTS TO PRODUCE HYPOTENSION AND BRADYCARDIA. D.J. Williford, B.L. Hamilton, K. Yamada,* and R.A. Gillis. Departments of Pharmacology & Anatomy, Georgetown University, School of Medicine & Dentistry, Washington, D.C. 20007.

Earlier reports from this laboratory have indicated that stimulation of GABA receptors in the CNS with muscimol (M) causes withdrawal of sympathetic outflow resulting in dose-related reductions in blood pressure (BP) and heart rate (HR). M produces this effect when administered into the 4th ventricle (4V), but no effect is observed when M is restricted to the lateral and 3rd ventricles. M injected into the 4V could be acting in any one of 3 general brain areas: 1) the dorsal surface of the brainstem, 2) the ventral surface of the brainstem, or 3) the spinal cord. The present study was designed to locate the brain area(s) in which M acts to lower BP and HR. Experiments were performed in 6 anesthetized cats and consisted of selectively perfusing either the cisterna magna or the spinal cord with M. M produced no effect on BP or HR when the spinal cord was perfused with a dose of 32 ug M over 10 minutes. A similar perfusion of the cisterna magna however, produced falls in BP and HR of -55 $\pm$ 8 mmHg and -40 $\pm$ 6 beats/min, respectively. These results are comparable to those observed following 4V injection of a dose of 16 ug M (-58 $\pm$ 12 mmHg and -44 $\pm$ 9 beats/min). In each experiment perfusion of the cisterna magna with dye indicated that the ventral surface of the brainstem was heavily stained and no flow of dye occurred from the cisterna magna into the 4V. We conclude that M appears to lower BP and HR by acting in the region of the ventral surface of the brainstem and that BP and the HR changes obtained with injection of this agent into the 4V were most probably due to flow of drug through the Foramen of Lushka to the ventral surface.

253.3 CENTRAL CARDIOVASCULAR EFFECTS OF THE GABA MIMETIC AGENT, THIP. D. W. Snyder, L. J. Macklem* and W. H. Severini*. Squibb Institute for Medical Research, Princeton, New Jersey 08540.

Intracerebroventricular (ICV) administration of the GABA agonist 4,5,6,7-tetrahydroisoxazolo [5,4-c]-pyridin-3-ol (THIP) produced dose related decreases in arterial blood pressure in chloralose anesthetized cats. ICV infusion of THIP in total doses of 4, 15 and 40 µg/kg over a 10 min period lowered mean arterial pressure 18±7%, 37±10% and 55±6%, respectively. The onset of the fall in blood pressure occurred within 2 min, and was maximal at the end of the infusion. ICV infusion of THIP also caused changes in heart rate and renal nerve discharge. Similar to the blood pressure response, a fall in heart rate was evident within 2 min of the initiation of infusion at all doses of THIP. The maximal response in heart rate was seen 10 to 15 min after termination of the infusion. The magnitude of the fall in heart rate (15-25% reduction) appeared to be related to the level of resting heart rate prior to THIP administration. Renal nerve discharge was inhibited at the highest dose of THIP (40 µg/kg). At lower doses, effects on renal nerve discharge were variable. Recovery from the effects of THIP was dose related. With the highest dose of THIP only a slight return toward control was seen 60 min following the administration of THIP, whereas all parameters had returned to control within 40 min following the end of administration of the lowest dose of THIP. The data suggest that THIP, like other GABA receptor agonists, lowers blood pressure and heart rate through central inhibition of sympathetic nervous discharge, possibly by activation of central GABA-ergic neurons.

253.4 SUPPRESSION OF BRADYCARDIA INDUCED BY GIGANTOCELLULAR RETICULAR NUCLEUS BY CLONIDINE AND MORPHINE IN THE CAT. Julie Y. Hwa and Samuel H.H. Chan. Department of Life Sciences, Indiana State University, Terre Haute, IN 47809.

A cardioinhibitory mechanism was recently identified in the gigantocellular reticular nucleus (GRN) in the medulla oblongata of the cat. Electrical activation of this medullary mechanism elicited a significant reduction in heart rate (HR), accompanied mostly by hypotension. Parallel studies in our laboratory suggested that the antihypertensive agent clonidine may enlist this same medullary mechanism to induce bradycardia and vasodepression. Furthermore, microinjection of morphine directly into the GRN elicited a significant inhibition of the dental pulp evoked jaw-opening reflex, an experimental pain index. Since clonidine and morphine share a common neural substrate in exerting their respective pharmacologic actions and both agents possess suppressive effect on the cardiovascular system, the present study was undertaken to investigate their possible interactions with the GRN-induced bradycardia.

Experiments were performed on chloralose-urethane anesthetized cats. Intravertebral injection of clonidine (0.5-4.0 µg/kg) produced a dose-dependent decrease in HR and arterial blood pressure (ABP). Superimposed on its inherent circulatory action, clonidine also elicited a drastic depression of the GRN-induced bradycardia. For any given stimulus pulse frequency, clonidine blocked the cardioinhibitory effect of GRN in a dose-related manner. On the other hand, the amount of suppression of GRN-induced cardiodepression by clonidine, at any dose, was inversely related to the reticular stimulus pulse frequency and intensity.

Intravertebral injection of morphine (1.0-4.0 mg/kg) promoted a dose-dependent decrease in ABP and no significant change in HR. It is interesting to note that, superimposed on its cardiovascular effect, morphine also induced a suppression of the GRN-induced bradycardia. The degree of blockage was again directly proportional to the dose of morphine and inversely related to the intensity and pulse frequency of the reticular stimulus train.

It should be mentioned that clonidine and morphine, delivered intravertebrally at the same doses, exerted no influence on the cardioinhibition and vasodepression induced by direct stimuli to the cardiac end of a severed vagus nerve, suggesting that the observed blockage by these two pharmacologic agents on GRN-induced bradycardia should take place in the brainstem.

We conclude that clonidine and morphine may utilize the GRN to exert cardiovascular suppressions. Their activation of the GRN may render the reticular neurons less excitable upon subsequent electrical stimulation, resulting in the reduced degree of bradycardia. (Supported in part by the American Heart Asso., Indiana Affiliate).

253.5 Kainic acid-elicited tachycardia: role of dopaminergic, glutamatergic, and peptidergic systems. A. Y. Deutch, L. S. Clark, and L. J. Peacock. Dept. Psychology, Univ. Georgia, Athens, GA.

We have previously reported that kainic acid (KA) administrato the lateral septum (LSN) effects a short latency tachycardia, the occurence of which is dependent upon glutamatergic afferents. We have extended these findings and attempted to characterize the neuronal systems subserving heart rate changes secondary to KA administration. KA (4.7 nM in 1.0 µl buffered saline; barbiturate-anesthetized rats) effected a short latency tachycardia upon administration to the nucleus accumbens (ACB) and nucleus interstitialis stria terminalis (NIST); vehicle had no effect. Since these sites receive dopamine (DA) afferents from the A10 cell group, we examined a possible role for mesolimbic projections in KA-elicited tachycardia. KA administration to the central amygdaloid nucleus and ventral tegmental area (mesolimbic terminal field and origin, respectively) did not effect significant heart rate changes.

The glutamatergic (Glu) projection system originating in the hippocampus/subiculum innervates the LSN, ACB, and NIST, the three sites at which KA elicits tachycardia. In light of the finding that knife cuts of the Glu projection to the LSN block the heart rate increase subsequent to septal KA administration, we administered KA to the mammillary body (MB), which also receives a Glu projection via the fornix from the subiculum. No heart rate changes were noted upon KA injection of the MB, suggesting that Glu afferents, like DA afferents, do not by themselves subserve the tachcardia induced by KA administration.

We are currently assessing a possible peptidergic mediation of the heart rate changes. Thyrotropin releasing hormone (TRH) has been reported to be localized to the LSN, ACB, and NIST, but not the central amygdala, ventral tegmental area, or the MB. Preliminary data indicate that TRH (50µg) administration to the LSN results in a short latency tachycardia, similar to that observed following KA administration. The tachycardia does not reflect emergence of the animal from anesthesia; delivery of noxious mechanical stimulation did not induce transient shortening of the interbeat interval. Wet dog shakes were noted to occur following administration of TRH, with onset at approximately 8 minutes following initiation of administration of TRH. It is interesting to note that intraventricular administration of TRH at 8-10 minutes following initiation of KA administration to the ventricle (i.e., after both tachycardia and seizure onset) did not attenuate the epileptiform discharge (as recorded electocorticographically) that results from KA administration. This indicates that TRH may not potentiate the anticonvulsant properties of barbiturates, as has been previously suggested.

253.6 INDUCTION OF CARDIAC ARRHYTHMIAS BY TRANSIENT BLOOD PRESSURE ELEVATION DURING SLEEP IN CATS. R.B. Trelease*, G.C. Sieck*, R.E. Hall, and R.M. Harper. Depts. of Anatomy, Physiology, and The Brain Research Institute, UCLA Sch. of Med., Los Angeles, CA 90024.

Episodes of sleep apnea in humans, resulting from upper airway obstruction, are accompanied by transient increased arterial blood pressure, slowing of heart rate, and occasional cardiac arrhythmias. We have attempted to simulate the hemodynamic consequences of these phenomena by transiently increasing blood pressure during sleep and waking states in cats. Cats were surgically implanted with electrodes for monitoring physiological parameters and identifying waking and sleep states. Arterial and venous catheters were inserted into the descending aorta and inferior vena cava. Recording sessions began after the animals had recovered for at least one week. At the time of recording, the cat was placed in a chamber in which it could move freely. EEG, EOG, EKG, respiratory activity, and arterial blood pressure were continuously monitored. Respiratory movements were detected using a piezoelectric stain gauge fastened around the lower thorax. During each state, arterial pressure was transiently elevated by infusion of 30 ug phenylephrine (0.3 ml over 2 seconds) through the venous catheter. Possible non-specific drug effects were controlled by transiently elevating arterial pressure with inflation of a balloon-tipped (Swan-Ganz) monitoring catheter placed in the descending aorta. Various cardiac arrhythmias accompanied periods of increased blood pressure. While the fundamental pattern of reflex bradycardia was obtained with all stimuli in every state, responses to elevated pressure could be divided into four types based on the accompanying cardiac rhythm changes. The first type (normal response in most cases) consisted of a transient sinus bradycardia accompanied by state specific changes in the magnitude of respiratory-related heart rate variation. The second type of response included a transient pulsus alternans (paired long-short interbeat intervals) of sinus origin. A third type was an A-V nodal escape arrhythmia, which apparently occured when sinus rate fell below the rate of spontaneous A-V nodal pacemaker discharge. Transient ventricular arrhythmia characterized the fourth type of response to elevated arterial pressure. Although arrhythmias could be occasionally elicited during waking, the incidence of these arrhythmias was higher during sleep states. We conclude that transient elevation of blood pressure during sleep, such as that associated with obstructive sleep apnea, can cause cardiac arrhythmias.

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253.7 CARDIOVASCULAR RESPONSES TO TRANSIENT BLOOD PRESSURE ELEVATION DURING SLEEP AND WAKING IN CATS. R.M. Harper, R.E. Hall, G.C. Sieck*, and R.B. Trelease*. Depts. of Anatomy, Physiology, and The Brain Research Institute, UCLA Sch. of Med., Los Angeles, CA 90024.

A variety of somatic reflexes are attenuated during sleep. The activity of visceral reflexes, such as the sinoaortic baroreflex, may not be affected in a similar fashion. It has been suggested that baroreflex activity is maintained across sleep and waking states, serving to "buffer" cardiovascular alterations, in particular those associated with REM sleep. The purpose of this study was to compare baroreflex gain during different sleep and waking states. Cats were surgically instrumented with electrodes for recording EEG, EOG, EKG, hippocampal and lateral geniculate activity. Arterial and venous catheters were introduced via the femoral vessels and advanced to the descending aorta and inferior vena cava. Recordings were initiated following one week of recovery from surgery. Recordings were obtained while the animals were unrestrained in a sound attenuated chamber. Sleep states were assessed using standard physiological criteria. During each state, arterial pressure was transiently elevated by bolus injection of phenylephrine (ca. 10 ug/kg, 0.3 ml in 2 sec.) through the venous catheter. To elevate blood pressure nonpharmacologically, the balloon of a double-lumen (Swan-Ganz) catheter was inflated in the descending aorta. Baroreflex gain was calculated in two ways: Absolute gain ($\Delta HR/\Delta BP$) and relative gain ($(\Delta HR/\overline{HR})/(\Delta BP/\overline{BP})$). Mean heart rate and systolic blood pressure values were derived from 5 second intervals immediately preceeding pressure elevation. Periods which included cardiac arrhythmias were excluded from the calculations. Elevation of arterial pressure pharmacologically or by balloon inflation produced progressive bradycardia in all states. The pressure increase in response to phenylephrine was comparable between states, although this elevation was sustained for a longer period of time during quiet sleep. Both absolute and relative baroreflex gains were slightly decreased during sleep states as compared to wakefulness. Therefore, unlike somatic reflexes, the sinoaortic baroreflex is not as markedly influenced by sleep states.

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253.8 INCIDENCE OF CARDIAC ARRHYTHMIAS IN NORMAL AND DECEREBRATE RATS AFTER ACUTE SUBARACHNOID HEMORRHAGE. Priti S. Lacy* and A. M. Earle. Dept. of Anatomy, Univ. of Nebraska Med. Center, Omaha, NE 68105.

An anatomical and functional relationship exists between the supramedullary vasomotor regions of the cortex, hypothalamus, mesencephalon and the medullary vasomotor center. The present study was undertaken to determine the role that supramedullary vasomotor regions and the brainstem play in generating different types of electrocardiographic abnormalities after an acute experimental subarachnoid hemorrhage was induced in rats. Rats were divided into two groups and in both groups a subarachnoid hemorrhage was experimentally induced by the introduction of 0.2-0.5 ml of heparinized rat blood into the subarachnoid spaces over the region of the circle of Willis through a burr hole in the frontal bone. The femoral artery was cannulated for measurement of systemic arterial pressure and EKG was monitored from lead II. Both parameters were recorded in both groups of rats before inducing a subarachnoid hemorrhage and group II was then electrolytically lesioned at the midcollicular level. Blood pressure and EKG were recorded after lesioning animals in group II. Animals of both groups were subjected to a subarachnoid hemorrhage when bradycardia and a variety of electrocardiographic abnormalities were seen. Systemic arterial blood pressure either rose slightly or not at all in both groups of animals. After bradycardia ensued, blood pressure decreased and continued to decrease till death in decerebrate rats. Thirty-eight (38) percent of the control rats of group I showed premature ventricular contractions which failed to occur in decerebrate rats. Bradycardia, junctional rhythms, and changes in the shape and size of T waves were of common occurrence in both the control and decerebrate rats which suggests that a sustained elevation of intracranial pressure (Lacy and Earle, Anat. Rec. 196:106A, 1980) after a subarachnoid hemorrhage may through ischemia or deformation activate the vasomotor center in the brain stem. The premature ventricular contractions that failed to occur in decerebrate rats were probably mediated through supramedullary vasomotor centers in control rats of group I.

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254.1 ELECTROCHEMICALLY STIMULATED (ECS) LUTEINIZING HORMONE (LH) RELEASE AND NUCLEAR CATECHOLAMINE (CA) CONTENT AFTER HYPOTHALAMIC KNIFE CUTS. C.P. Phelps, S. Saporta and D.M. Nance, Dept. of Anatomy, College of Medicine, Univ. of South Florida, Tampa, Florida 33612

Retrochiasmatic knife cuts that interrupt frontal (FC) or lateral frontal (LFC) neural connections of the rat mediobasal hypothalamus (MBH) respectively reduce or abolish the increases in plasma LH seen in sham (S) cut animals after ECS of the medial preoptic area (MPOA). However, these cut effects on LH release appear to be time dependent, for when the length of the postoperative (postop.) survival period is increased from 21 to 75d LH response to ECS of MPOA was improved in LFC rats (Phelps and Sawyer, Brain Res. 131:335). The present report concerns an examination of female rat response to ECS of MPOA 180d after FC, LFC or S cuts and high performance liquid chromatography with electrochemical detection to assay selected brain regions at 7,21,60 and 180d postop. for norepinephrine (NE), dopamine (DA) and epinephrine (E). Vaginal smears were monitored before and after cuts. At 170d postop. rats were ovariectomized (OVX) and 1 wk later received estradiol benzoate (EB,1µg/75gbw/day at 10:00 hr) x2d. Animals subsequently received pentobarbital (35mg/kg IP) 76 hr after the first EB injection, followed by bilateral ECS 1 hr later in MPOA (20µA anodal DC x 60s, bipolar concentric electrodes, anode positive). Blood was removed by venipuncture at 0,40,80,120 and 160 min after ECS for estimation of plasma LH by RIA. Sham surgeries had no effect on estrous cycles, FC produced constant estrus and polyfollicular ovaries, while LFC rats had variable vaginal cytology and atrophic ovaries. Both FC and S rats showed similar significant increases in plasma LH levels (FC,353±117; S,259±58ng/ml), at 40 min after ECS, which remained elevated at 80 and 120 min. There was no significant change in LH at 40 min (56±37ng/ml) or later in LFC rats. Hypothalamic nuclear NE levels (pg/µg protein) in MPOA, arcuate (ARC) and ventromedial (VMN) nuclei of EB-primed, OVX rats increased 2 and 3-fold above preoperative levels at 7 and 21d after FC and S, but returned to preop. levels at 180d after surgery. LFC resulted in a sharp decline in NE content of the paraventricular nucleus (preop., 38.1±10.8; 21d:4.7±0.3; 180d: 2.8±1.3pg/µg) while E was approximately doubled (7d) and then returned to preop. levels (180d). LFC also increased DA content of ARC from 10.2±2.8pg/µg to 33.9±5.3pg/µg (7d postop.) and 25.4±4.1 pg/µg (180d). Histological examination revealed that all cuts were complete. We conclude that hypothalamic knife cut effects on ECS release of LH and hypothalamic CA content will vary not only with cut location and dimension, but also with survival time.

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254.2 IMMUNOCYTOCHEMICAL ANALYSIS OF CHANGES IN HYPOTHALAMIC LHRH DISTRIBUTION FOLLOWING NEONATAL ADMINISTRATION OF MONOSODIUM GLUTAMATE (SPON: E. Boyd), C. A. Bennett-CLARKE*, M. A. Romagnano, S. A. Joseph* and W. H. Pilcher*. Neuroendocrine Unit, University of Rochester, Rochester, N.Y. 14642.

The immunocytochemical distribution of LHRH was examined in control and MSG lesioned mice, using the unlabeled antibody enzyme method of Sternberger. MSG was administered to neonatal mice, in increasing doses according to the injection protocol of Pilcher et al. (1980), while control animals received similar injections of 0.9% saline. Experimental and control mice were intracardially perfused with Zamboni's fixative. Frontal and sagittal sections of brain were serially sectioned at 50µ on an Oxford Vibratome and cytochemically stained for LHRH with antiserum B4305 (supplied by Dr. Sternberger). Control dishes of the same brain were stained with anti-ACTH antiserum, 531 (supplied by Dr. Joseph). Following incubation in primary antiserum, the sections were stained according to previous described immunocytochemical procedures. Correlative radioimmunoassay, to determine content of these peptides in brain, was performed on lesioned and control animals. Neonatal administration of MSG has been shown to destroy 80-90% of the neurons of the arcuate nucleus including those cell bodies which form the bed nucleus of the opiocortin system. In this study, the extent of the MSG-induced arcuate nucleus lesion was determined by routine nuclear staining as well as by *ACTH immunocytochemistry*.

The distribution of LHRH neurons and fibers in both experimental and control animals was similar to that previously described in thick vibratome sections in the rat (Bennett-Clarke & Joseph, 1980) with the exception of the area of the arcuate nucleus. No LHRH neurons were observed in this area in either the lesioned or control animals, however, LHRH fibers were observed projecting along the ventral portion of the third ventricular wall and through the arcuate nucleus along the course of tanycyte processes to the portal capillaries in the control mice. In MSG lesioned animals, the similar portion of the ventricular wall as well as the arcuate nucleus was devoid of immunoreactive LHRH fibers.

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254.3 THE DISTRIBUTION OF SOMATOSTATIN IN THE MOUSE BRAIN FOLLOWING NEONATAL ADMINISTRATION OF MSG. M.A. Romagnano, C.A. Bennett-Clarke*, S.A. Joseph* and W.H. Pilcher*. Neuroendocrine Unit, University of Rochester, Rochester, NY 14642.

The distribution of somatostatin (SRIF) was examined using the unlabeled antibody enzyme method of Sternberger on brains of control and monosodium glutamate (MSG) treated mice. Neonatal administration of MSG was given in increasing doses according to the injection protocol of Pilcher et al. (submitted to Nature, 1980) which produced a 80-90% cell loss within the arcuate nucleus of the hypothalamus. Control animals were injected with 0.9% saline. Adult mice were perfused through the heart with Bouin's fixative. Brains were removed and 50µ coronal and sagittal sections were cut on a Vibratome. Sections were incubated in Joseph's anti-SRIF "Charlie 8" at a dilution of 1:1000. Only animals with complete lesions as determined by staining for the ACTH component of the opiocortin system were used for the experimental animals. We have shown that the bed nucleus of ACTH-containing cells in the region of the arcuate nucleus is destroyed by the neonatal administration of MSG (Pilcher et al.'80). In addition, the presence or absence of cell bodies in the arcuate nucleus was examined in counterstained cresyl violet sections.

The distribution of hypothalamic and extrahypothalamic SRIF-containing cells and fibers in control animals was similar to that described for the rat (Bennett-Clarke et al., Br. Res., 188:473, 1980). It was noted that the arcuate nucleus contained SRIF fibers throughout its entire extent. Mice with with complete arcuate lesions contained SRIF cells and fibers in the same areas as the control animals with one important exception, the arcuate nucleus in the lesioned animal was devoid of fibers. Correlative RIA data has been performed on control and experimental animals. The loss of SRIF fibers from the arcuate nucleus subsequent to MSG administration raises questions concerning the role of SRIF in the arcuate nucleus during development.

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254.4 MONOSYNAPTIC PROJECTIONS OF THE AVIAN HYPOTHALAMUS TO BRAINSTEM AND SPINAL CORD. Judith A. Finkelstein and Mitchell L. Berk, Dept. Anat., N.E. Ohio Univs. Col. Med., Rootstown, Ohio 44272.

The mammalian paraventricular nucleus (PVN) of the hypothalamus has direct connections to brainstem and spinal cord autonomic nuclei. We explored the possibility of a similar hypothalamic projection in birds by the use of the autoradiographic and horseradish peroxidase (HRP) techniques.

In the pigeon, iontophoretic injections of ^{3}H-leucine were placed in n. periventricularis magnocellularis (PVM), the presumed homologue of PVN of mammals. Heavily labeled fibers from PVM course ventrolaterally in the lateral hypothalamic area (LHA), approach the supraoptic decussation, and then turn ventromedially to densely innervate both layers of the median eminence. Another prominent bundle of fibers proceeds caudally through LHA, stratum cellulare externum, and then enters the midbrain tegmentum lateral to n. ruber. These fibers continue posteriorly in the lateral mesencephalic reticular formation. At pontine levels, some of the fibers leave the lateral bundle, travel in a dorsomedial direction and appear to terminate lateral to locus coeruleus. The remaining fibers in the bundle continue caudally in the ventrolateral pons, lateral to the superior olivary nucleus, and proceed into the ventrolateral medulla. Some fibers leave the bundle, course dorsomedially, and densely innervate n. intercalatus, which lies ventral to the dorsal motor nucleus of the vagus (DMX). At this level, fibers also surround DMX and lie within the dorsal, ventral, and lateral margins of n. solitarius. At the level of the central canal, the medial part of n. solitarius is most densely labeled. Also, fibers are still present in the ventrolateral bundle at this caudal medullary level.

Additional PVM projections course to the medial and periventricular preoptic areas, hypothalamus, lateral septum, and nucleus of the septal commissure. Other fibers densely innervate the periventricular part of the dorsomedial thalamus. Fibers also extend into the midbrain periventricular gray adjacent to the cerebral aqueduct.

In other pigeons, HRP was injected into the spinal cord at the level of T1. Labeled cells are observed throughout PVM, many with processes extending into the lateral hypothalamus. Unlike HRP injections into the mammalian spinal cord, very few labeled cells are seen in the lateral hypothalamus. Based on all of the above information, the avian PVM is indeed the homologue of the mammalian PVN. Supported by NIH Grant 5 Rol NS 14344, NSF Grant BNS 77-19302 and NIH Fellowship 1 F32 NS06186.

254.5 THE SUPRACHIASMATIC HYPOTHALAMIC NUCLEUS: ULTRASTRUCTURE OF RELATIONS TO OPTIC CHIASM. J.P. Card, J.N. Riley and R.Y. Moore. Department of Neurology, SUNY at Stony Brook, NY 11794.

Recent studies have demonstrated a retinohypothalamic input to neurons other than those of the suprachiasmatic nuclei (SCN) via dendrites which project into the optic chiasm or tracts. Since dendrites of SCN neurons are also known to project into the optic chiasm, these findings have raised the possibility that the SCN itself may receive a more widespread retinal input than previously demonstrated with the autoradiographic and HRP techniques. As a first step toward the resolution of this question we have conducted a systematic analysis of the interrelation of the SCN and the optic chiasm throughout the rostrocaudal extent of the nucleus. Adult female rats were perfused with buffered aldehyde solutions, the brain removed and 100 μm Vibratome sections cut throughout the rostrocaudal extent of tissue containing the SCN and optic chiasm. Following processing of tissue by conventional procedures for ultrastructural analysis, thick and ultrathin sections were cut from the anterior surface of each Vibratome section. This provides a means for quick and reliable sampling of representative areas of the nucleus at standardized levels to allow comparison among animals. Extensive interrelations of the SCN and optic chiasm throughout the rostrocaudal extent of the nucleus are characterized by projections of SCN neuropil well into the dorsal one-half of the chiasm. Longitudinally cut dendrites are evident coursing from the nucleus proper into the densely packed, myelinated axons of the chiasm. These dendrites often extend into large pockets of neuropil deep within the confines of the chiasm. Numerous axon terminals establish synaptic contacts with intrachiasmatic dendrites. Some of these terminals exhibit lucent mitochondria and other fine structural criteria which have been associated with optic boutons in previous TEM studies while others contain large neurosecretory-like dense-core vesicles. Dendrodendritic synapses are also present between SCN dendrites coursing into the chiasm. The extent of the SCN-chiasm interrelationship varies throughout the rostrocaudal extent of the nucleus. At rostral levels, interdigitation is confined primarily to the ventrolateral aspect of the SCN. As one progresses caudally, intermeshing of the SCN neuropil and chiasm becomes more extensive in this ventrolateral region and further interdigitation is evident throughout the mediolateral extent of the SCN-chiasm interface. The present study has demonstrated a consistent and extensive interrelationship between the SCN and optic chiasm. Further studies are in progress to determine if this relationship is indicative of a more widespread retinal input to the nucleus. Supported by USPNS Grants NS-06247 and NS-16304.

254.6 THE SUPRACHIASMATIC HYPOTHALAMIC NUCLEUS: NEURONAL ULTRASTRUCTURE. R.Y. Moore, J.P. Card and J.N. Riley. Department of Neurology, SUNY at Stony Brook, NY, 11794.

The present study was undertaken to analyze the fine structural characteristics of suprachiasmatic nucleus (SCN) neurons. Adult female Sprague-Dawley rats were perfused with buffered aldehyde solutions. In order to provide a means of sampling representative levels throughout the rostrocaudal axis of the nucleus, 100 μm Vibratome sections of fixed tissue were cut through the extent of the optic chiasm prior to processing of tissue. Thick and ultrathin sections were cut from the anterior surface of each specimen thereby providing a means of determining the exact location within the nucleus and standardizing the comparison among rats. At least two morphologically distinct cell types are found at all levels of the SCN. The first of these is characterized by an elongated perikaryon in which the dominant feature is the cell nucleus. The nucleus of these cells filled the majority of the cell body and sometimes exhibited one or two fingerlike invaginations. A dense homogeneous cytoplasmic matrix exhibiting a paucity of organelles forms a thin rim of cytoplasm on either side of the nucleus in the short axis of the cell. Mitochondria, Golgi cisternae, polyribosomes and sparcely distributed individual cisternae of rough endoplasmic reticula (RER) accumulate in the cytoplasm at each pole of the cell. Few axosomatic contacts were noted on the soma which is either ensheathed by fibrous astrocytic processes or directly opposed to the membrane of an adjacent neuron. These neurons are densely packed along the dorsal and medial aspect of the SCN and are separated from the optic chiasm by the second cell type. This, too, is characterized by a nucleus which fills the majority of the cell body. However these cells differ in that the perikaryon is more spherical and the cell nucleus highly invaginated. Additionally, the cytoplasmic matrix is lighter than that of the first cell type and filled with a richer collection of cellular organelles. RER were more numerous, although still scattered throughout the cytoplasm as individual cisternae. This cell type is concentrated in the lateral and ventral SCN and is often observed in aggregations with direct membrane apposition or adjacent to the endothelium of capillaries. Few axosomatic contacts are observed. In conclusion, the present study has demonstrated two distinct populations of neurons in the SCN of the adult rat. Whether either of these cell types can be correlated with the immunocytochemically distinct cell populations (vasopressin and vasoactive intestinal peptide containing neurons) which have been demonstrated in the SCN is presently under investigation. Supported by USPNS Grants NS-06247 and NS-16304.

255.1 DECREASED TSH RESPONSE TO TRH IN MANIA COMPARED TO SCHIZOPHRENIA. I. Extein, A.L.C. Pottash, M.S. Gold. Fair Oaks Hospital, Summit, NJ 07901, and Yale Univ. School of Medicine, New Haven, CT.

We report here a follow-up to our initial observation of a significant decrease in the release of thyroid-stimulating hormone (TSH) from the pituitary after administration of thyrotropin-releasing hormone (TRH) in mania compared to schizophrenia (Extein, I., et al, Am J Psychiat, in press). The ability to differentiate mania from schizophrenia has important treatment implications for the choice of lithium versus neuroleptics. Because manic patients can be psychotic, differentiating mania from schizophrenic psychosis by clinical criteria alone can be difficult. Our results from a large series of hospitalized patients suggest that the TRH test may be a laboratory diagnostic test useful in differentiating these two disorders.

The TRH test was performed in 76 consecutive newly admitted inpatients who met Research Diagnostic Criteria for mania (n=25), schizoaffective disorder, manic type (n=18), or schizophrenia (n=33; 27 undifferentiated, 6 paranoid; all actively psychotic). Patients with thyroid disease, drug or alcohol abuse, OBS, or recent lithium use were excluded. Patients at bedrest after an overnight fast were administered 500 ug of synthetic TRH via an indwelling venous catheter. Blood samples were taken before and 15, 30, 60, and 90 minutes after TRH infusion for measurement of serum TSH in duplicate by radioimmunoassay. Maximum TSH response (Δ TSH) was calculated for each patient by subtracting the baseline TSH from the peak TSH after TRH infusion. Mean Δ TSH ± SEM of 6.47 ± 0.64 in mania was significantly lower than that of 9.57 ± 0.84 in schizophrenia ($p < .01$ by 2-tailed t-test), and this was not explainable by differences in age, sex, baseline thyroid function or neuroleptic use. The mean Δ TSH of 8.50 ± 1.25 in the schizoaffective group did not differ significantly from either other group. 15 of the manics (60%),9 of the schizoaffectives (50%), and 10 of the schizophrenics (30%) had a Δ TSH ≤ 7.0 *(mania different from schizophrenia, $p < .05$ by Chi-Square)*

Release of the tripeptide TRH from the hypothalamus is inhibited by serotonin and stimulated by norepinephrine and dopamine. Thus the blunted TSH response to TRH in mania may reflect changes in monoaminergic transmission, or reflect changes in endogenous TRH that have been hypothesized to play a role in mood disorders (Prange, A.J.,et al, Lancet 2:999, 1972). The findings reported here suggest that in cases in which it is difficult to distinguish mania from schizophrenia clinically, the TRH test may aid in diagnosis and choice of treatment. Further research is needed to determine if the results of the TRH test predict outcome or response to lithium versus neuroleptics in manic,schizoaffective, and schizophrenic patients

255.2 OPIATE - ENDORPHIN TEST DYSFUNCTION IN MAJOR DEPRESSION. M.S. GOLD, A.L.C. Pottash, D.A. Martin* and I. Extein. Fair Oaks Hospital and Psychiatric Diagnostic Laboratories of America, Summit, NJ 07901, and Yale Univ. Sch. of Medicine, New Haven, CT 06510

There are anecdotal reports from the pre-psychopharmacology era of the efficacy of opiates in periodic and idiopathic depression. The discovery of stereospecific opiate receptors and endogenous morphinomimetic opioid peptides in the brain have again suggested the possible involvement of opioid systems in major affective disorders. The limbic and hypothalamic distribution of opiate receptors and endorphins and the suggestions of a neuromodulatory role of endorphins for noradrenergic and dopaminergic systems suggest that opioid peptides are widely distributed in areas and systems which are involved in the pathophysiology and pharmacotherapy of depression.

As a preliminary in vivo test of the hypothesis that major depression may be accompanied or etiologically-related to a functional decrease in limbic opioid peptide activity, we utilized the opiate-induced serum prolactin (PRL) elevation. We administered 5 mg of morphine sulfate intravenously through an indwelling venous catheter to fifteen patients with a major depressive disorder by RDC criteria and ten normal, age - and sex-matched controls. All subjects gave written informed consent and were medication-free for at least one week prior to the provocative test. All were at bed rest after an overnight fast for the placement of an indwelling catheter (800h) and intravenous administration of morphine (900h) over 60 seconds. Blood was taken through the catheter at 0,30,60,90,120, and 180 minutes for the measurement of serum PRL in duplicate by RIA (1). The maximum PRL response ΔPRL was calculated by subtracting the baseline from peak PRL.

There were no significant differences between groups for age, sex, or baseline PRL. The mean PRL response was significantly decreased at all time points for the depressed group. Mean ΔPRL levels were significantly different ($p < 0.01$) between groups.

Endogenous and exogenous opiates have been demonstrated to be potent stimulators of PRL secretion in man, nonhuman primates,and rodents possible by activation of opiate receptors on dopaminergic neurons (1). The blunted PRL response reported here may be consistent with an opiate receptor abnormality, presence of an endogenous opiate antagonist, or may be another manifestation of deranged hypothalamic-pituitary function in patients with major affective illnesses. As endorphins and ACTH-corticosteroids have a common precursor these endorphin test response data may relate to the vast body of ACTH-cortisol abnormality data reported for patients with major depression. These data offer some support for a hypothesized role for endorphins in pathological mood states and the reported antidepressant efficacy of B-endorphins.

MS Gold, et al. Endocrinology 105:284, 1979.

255.3 HYPOPHYSECTOMY INDUCED HYPERSENSITIVITY TO APOMORPHINE: SUPPRESSION BY ESTROGEN. K.O. Perry*, B.I. Diamond, J.H. Gordon, Dept. of Pharmacology. The Chicago Medical School, Chicago, IL 60612.

Recent reports have suggested that the ability of estrogen to suppress dopamine agonist potency was dependent upon an intact or functioning pituitary. The present series of experiments was designed to test this hypothesis utilizing both apomorphine-induced stereotypy and ^{3}H-Spiroperidol (^{3}H-SPIRO) binding to striatal membranes. Hypophysectomized (HYPOX) or sham HYPOX (SHAM) and ovariectomized rats were purchased from Hormone assay labs, Chicago. The HYPOX and the SHAM animals received either estradiol benzoate (EB; 10 µg/kg/day x 3) or Sesame oil (0.25 ml/kg/day x 3). All animals were challenged with apomorphine on the 4th day of the experiment, 20-24 hours after the last dose of EB or Oil, and the intensity of the stereotypy scored during the 10-20 min following the apomorphine injection. The ED_{50} for apomorphine-induced stereotypy was calculated following probit-log dose transformation. The HYPOX + oil were more sensitive to apomorphine than the SHAM + oil (ED_{50} = 0.24 and 0.45 mg/kg respectively). Treatment weth EB resulted in a significant shift to the right of the dose response curves for both the HYPOX and the SHAM groups. The ED_{50} of the HYPOX + EB = 0.62 mg/kg and SHAM + EB = 0.93 mg/kg. The intensity of the apomorphine-induced stereotypy in the various groups was indistinguishable at doses of 1.6 mg/kg or greater. The separation of the dose response curves was readily apparent as doses below 1.2 mg/kg.

All animals received an injection of EB or oil on day 4 of the experiment following the behavioral test. Animals were sacrificed 24 hours later and the striatum utilized for ^{3}H-SPIRO binding. No significant change in the K_D was noted in any of the experimental groups. The B_{max} values were indistinguishable for the SHAM + oil, SHAM + EB and HYPOX + EB groups, however, the B_{max} of the HYPOX + Oil group was significantly increased (54%) relative to all other groups, at 7 days post-HYPOX. By 28 days post-HYPOX the B_{max} of the HYPOX animals was increased by 94% relative to the SHAM animals. The HYPOX induced sensitivity could be enhanced by chronic haloperidol treatment and withdrawal (193% increase in B_{max}). The haloperidol induced increase in apomorphine stereotypy and ^{3}H-SPIRO binding could be antagonized by EB treatment during the withdrawal from the chronic haloperidol treatment. The data indicate that the pituitary can modulate the sensitivity of the animal to dopamine agonists, but that the presence of the pituitary is not required for estrogen to suppress the efficacy of dopamine or dopamine agonists, thus indicating the probability of a direct CNS effect of estrogen on dopaminergic mechanisms. (This work was supported in part by USPHS Grant RR-5366 and BNS-782-0025.)

255.4 MODULATION OF DOPAMINE AGONIST POTENCY BY ESTROGEN: DOSE AND TIME DEPENDENT EFFECTS. J.H. Gordon, K.O. Perry* and B.I. Diamond. Dept of Pharmacology, The Chicago Med. School, Chicago, IL 60612

Previous reports have presented data which indicates that estrogen can either suppress or enhance the potency of dopamine and/or dopamine agonists.

Female Sprague-Dawley rats were ovariectomized (OVX) or sham operated under ether anesthesia and were allowed two weeks to recover prior to behavioral testing. All rats received either oil (sesame oil) or estradiol benzoate (EB) for three days between 8 and 10 a.m. Three doses of EB were administered to separate groups of rats (10, 50 and 100 µg/kg/day). Animals were tested for apomorphine-induced stereotypy at 24, 48 and 96 hours after the last dose of EB or oil.

At 24 hours after the last dose of EB the results of the apomorphine-induced stereotypy test were indicative of a dose related suppression of apomorphine potency by estrogen. The stereotypy scores of the 10 µg/kg/day dose of EB were suppressed at 24 hours, but were indistinguishable from controls (OVX+oil or sham+oil) during the 48 and 96 hour tests. At 48 hours after the last dose of EB the apomorphine-induced stereotypy, following a 1.0 mg/kg dose of apomorphine, was increased over control values in the OVX animals that had received 100 µg/kg/day of EB. During the 96 hour test both the 50 and 100 µg/kg/day treated animals displayed an enhanced response to the behavioral effects of apomorphine.

These data indicate that estrogen can suppress dopamine and/or dopamine agonist potency 24 hours after the last dose of EB, thus indicating the possibility that the role of endogenous estrogen is to suppress dopaminergic transmission. However, the data are also indicative of an enhanced sensitivity to apomorphine when the drug sensitivity is determined at 48 hours or more after the last dose of EB. The time delay and the dose dependence of the enhanced response to apomorphine following EB is indicative of a "withdrawal" phenomenon. Thus if one uses pharmacologic doses of estrogen (i.e., greater than those needed to maintain uterine weight or treatment for a prolonged period of time), then the withdrawal or discontinuation of these types of treatments will result in a rebound supersensitivity similar to that seen with chronic neuroleptic treatment and withdrawal.

In conclusion, both suppression and enhancement of dopamine and/or dopamine agonist potency can be seen following estrogen treatment, however the data support the conclusion that endogenous estrogen acts to suppress dopaminergic function.

This work was supported in part by USPHS Grant RR-5366 and BNS-782-0025.

255.5 EFFECTS OF ESTROGEN ON THE DEVELOPMENT AND EXPRESSION OF DOPAMINE RECEPTOR SUPERSENSITIVITY. J.Z.Fields, M.F. Callahan*and John H. Gordon. Dept. Pharmacol.,Chicago Med. Sch.,Chicago IL 60612

Dopamine receptor(DAr) supersensitivity has been implicated in tardive dyskinesia, L-DOPA induced dyskinesias and schizophrenia by both direct and indirect evidence and in both humans and animal models. Agents that reverse or prevent the development of DAr supersensitivity may prove useful in either treating these disorders, or in yielding clues as to their underlying pathophysiological mechanisms. One such agent, we have observed, is estradiol benzoate(EB).

When rats were treated with EB(8 to 10 µg/kg/day) during the period of withdrawal from chronic haloperidol(HAL: 0.5 mg/kg X 3 weeks), the appearance of the characteristic DAr supersensitivity was inhibited. This inhibition affects both the increase in apomorphine(APO: 0.25 mg/kg) elicited stereotype behavior and the increase in specific, striatal (3H)spiroperidol binding to DAr.

For HAL treated animals, the cumulative scores, based on a hierarchy of stereotype activities (locomotion, rearing, head bobbing, sniffing, gnawing) increased over 200% relative to saline treated controls; the same APO dose response curves indicated that about one half as much APO was needed to elicit any particular level of stereotypy. The stereotypy score decreased almost to control levels when EB was given during withdrawal. Similarly, the number of striatal (3H)spiroperidol binding sites increased about 30%(high affinity site) after HAL, and decreased to control values when EB was used. No changes in affinity were observed.

The molecular mechanisms of this down-regulation of DAr are not clear since co-administration of EB with HAL(only saline given during withdrawal) did not inhibit the development of DAr supersensitivity. Subsequent experiments suggested that the hypothalamic/pituitary axis is not involved. The possibilities that chronic HAL can produce supersensitive limbic DAr and that EB can down-regulate limbic DAr are currently being studied.(Supported by grants from N.I.H.(RR-5366) and NSF(BNS-782-0025)).

255.6 FLUROTHYL-INDUCED CONVULSIVE SHOCK DELAYS ONSET OF SEXUAL MATURATION IN THE FEMALE RAT. M. Wilkinson and J. A. Pincock*. Dept. of Physiology and Biophysics and Dept. of Chemistry, Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Canada.

Electroconvulsive shock therapy (ECT) is an empirical but effective treatment for refractory depressive symptoms. At present there is no satisfactory explanation for its effects. Recent studies emphasize the importance of central monoamines, and in this regard ECT has something in common with other anti-depressants such as MAO inhibitors. Chronic anti-depressant drugs or convulsive shocks (CS) decrease noradrenaline-stimulated adenylate cyclase as well as the density of β-adrenergic receptors in rat brain. These changes in synaptic activity are possibly widespread within the nervous system and it would be surprising if adverse effects of CS were not observed on the hypothalamic control of endocrine status in laboratory animals and in man. We report here our initial studies on one such neuroendocrine control system, sexual maturation in female rats. The functional integrity of monoaminergic synapses is crucial to this developmental process.

Expt. 1: CS was given once per day to immature Sprague-Dawley female rats (age 25 days; weight 60 gm) by exposing them to the convulsant Flurothyl (F). F was used in preference to ECT since this ether gives results identical to ECT but does not reduce body weight. After 10 days, 60% of control rats exhibited vaginal opening (VO) ("puberty") whereas only 20% of CS rats had VO.

Expt. 2: Using animals of the same age but with lower body weights (45 gm), CS was given on consecutive days until all the control rats showed VO. Rats were inspected daily for VO. When VO was observed, ovaries, uterus, adrenals and pituitary were dissected and weighed. After 18 days all control rats had VO (mean age VO 39.0 ± 1.0 days) whereas none of the CS rats had ovulated. There were no differences in body weight between treated and control rats. CS rats were killed at day 42; organ weights, mg per 100 gm body weight, were: ovaries 17.3 ± 0.8 (control, C: 30.4 ± 1.7; $p < .001$); uterus 79.4 ± 13.5 (C: 124.4 ± 10.7; $p < .02$); pituitary 3.8 ± 0.2 (C: 4.1 ± 0.4; N.S.); adrenals 25.5 ± 0.6 (C: 26.1 ± 1.0 N.S.). We conclude that maturation of the female reproductive system is sensitive to CS treatment and therefore in clinical terms, could well be susceptible to disruption by other, routine, anti-depressant treatments.

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258.1 EVIDENCE OF TWO CONTROL SCHEMES FOR MOTOR UNITS.
C.J. De Luca, A.P. Xenakis*, R.S. LeFever* Dept. of Orth. Surg., Children's Hosp. Med. Ctr., Harvard Med. Sch., Boston, MA. 02115.

The discharge pattern of concurrently active motor units has been investigated in the first dorsal interosseous and deltoid muscles of 13 normal adult subjects. Both constant-force and force-varying (positive ramp followed by a negative ramp) contractions were studied. The subjects were asked to visually track a constant line and a triangle with the force generated by their muscle. The firing patterns were obtained by a technique reported at the 8th Annual Meeting of the Society for Neuroscience (R.S. LeFever and C.J. De Luca).

When the constant-force contractions were executed, the force output of both muscles was not constant. They contained random fluctuations (greater than 1 Hz) about the desired value. The concurrent firing rates of the motor units contained similar fluctuations. Correlation values of 0.65 were obtained between the force output and the firing rate of one motor unit. Furthermore, the fluctuations of the firing rates of all the concurrently detected motor units (ranging from 3 to 8) were highly correlated; at times with a correlation value of 0.85. When the triangular force patterns were executed, it was observed that the firing rates of the motor units would begin to decrease before the force output would reverse (at the peak of the triangle). It was also noted that the earlier recruited, faster firing, motor units began decreasing their firing rates before the later recruited, slower firing, motor units. It is well known that the earlier recruited motor units tend to have relatively long time duration twitches with progressively recruited motor units at higher force having shorter time duration twitches. Therefore, it appears that the observed scheme is necessary to provide precision in the force output of a muscle. These observations were consistent in both muscles of all the 13 subjects.

We postulate that there exists at least two control schemes for motor units. Compensatory force variations consisting of relatively rapid (greater than 1 Hz) low amplitude fluctuations are accomplished by a simultaneous change in the firing rates of most (if not all) the motor units. Slower, higher amplitude intended force variations, that determine the accuracy with which a muscle may generate a desired force value, are regulated by an elegant control scheme which acts individually upon each active motor unit (or pools of motor units). This latter scheme implies the existence of a direct relationship between the CNS and each motor unit (or pools of motor units) in a muscle. (Supported in part by NIAMDD Grant #AM 19665, and The Insurance Institute for Highway Safety).

258.2 CONTROL OF FOREARM POSITION: INFORMATION TRANSMITTED BY THE ACTIVITIES OF THE MUSCLES ACTING ON THE ELBOW JOINT.
B. Sakitt, F. Lestienne* and T. Zeffiro, Department of Psychology, Massachusetts Institute of Technology, Cambridge, MA 02139.

We applied the concepts of Information Theory to visually triggered extension and flexion movements of the forearm. Human subjects were required to rotate the forearm about the elbow joint so as to point to a visual stimulus, without sight of the limb. The trajectory, final position, and electromyographic (EMG) activities of the triceps and biceps were recorded. The number of visual targets in each session was fixed at either 2, 3, 5, 9, 16 or 17. On each trial only one target was lit and presentation was random with replacement. An Information Theory (Shannon, 1948) analysis of the data yielded the result that the arm could only transmit slightly more than 3 bits of Information. This is equivalent to the forearm having only 9 independent positions, even though the range tested covered about 75% of the physiological limits. These results lend support to the hypothesis that one of the processes underlying voluntary movements involves a shift in the equilibrium between the agonist and antagonist muscles (Fel'dman, 1966; Bizzi et al, 1976). Such a motor program would be feasible with either a lookup table or a computation since the numbers involved are surprisingly small.

The Information transmitted by the ratio of the EMGs of the triceps and biceps was almost the same as that transmitted by forearm position. In contrast, the Information transmitted by either the triceps or biceps individually was consistently less than that transmitted by the ratio. Since the variance of independent quantities is additive, this suggests that the ratio of the alpha innervations to the agonist and antagonist muscles is programmed more directly than either innervation separately.

The ratio of the EMGs has previously been shown to play a specific role with regard to final position. Lestienne, Polit, and Bizzi (1978) found that for movements which varied with speed and direction, the final position correlated with the ratio of the EMGs. Sakitt (1980) using an equilibrium spring model of the arm predicted that the ratio of the alpha innervations usually determines joint angle. The present results show that the quantitative relationship between joint angle and the ratio of the EMGs is in good agreement with the theoretical prediction for the range of joint angles tested. Taking these together, this provides evidence that the equilibrium final position is programmed by setting the ratio of the alpha innervations to the agonist and antagonist muscles.

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258.3 INTENDED REACTIONS TO EXTERNAL DISPLACEMENT STIMULI IN THE LABIAL MUSCULATURE OF MAN, J.H. Abbs* and K.C. Cole* (SPON: T. Imig) Speech Motor Control Labs., Univ. of Wisconsin, Madison, Wisc. 53706

Several recent studies have revealed short-latency (50-70 msec.) "intended" responses to displacements applied to the limbs. These responses have been interpreted as pre-programmed motor subroutines released by appropriate sensation of movement and developed through learning. The mechanisms underlying this response capability have been suggested variously to involve spindle, cutaneous, and/or joint afferents apparently mediated through cerebellar-cortical pathways. The major controversy in these studies has been in separating the observed responses from long loop "reflexes," inasmuch as the displacement stimuli often have involved loading or unloading a contracting muscle in a manner that disturbs the intended limb position.

In the present study, the intended reaction paradigm was modified in an attempt to (1) reveal the potential independence of these response mechanisms from segmental, brain stem, or long loop reflexes, and (2) determine if motor systems lacking spindles and joint receptors (Viz. the lips) are endowed with comparable response capabilities. Unanticipated displacements were applied to the corner of the mouth with the lips pre-postured for generation of the vowel "ah." Upon sensation of the stimulus, subjects were instructed to respond "as soon as possible" with a rapid, forceful lip closure, as in the generation of a "b" consonant. This configuration provided a situation where the displacement stimulus (25 msec. duration) did not constitute a disturbance to the ongoing sustained gesture nor to the requested response. Further, this stimulus did not elicit a perioral reflex, hence greatly simplifying data interpretation re: potential reflex contamination. EMG was observed in m. orbicularis oris, a muscle that is quiescent during "ah" and phasically active in generating bilabial closure. Between 400 and 700 responses were obtained for each of four young adult male subjects. Interstimulus intervals were determined randomly under on-line computer control from a table of 11 values.

Minimal response latencies, measured from stimulus onset to muscle contraction onset in individual response records, ranged from 45-55 msec. for all subjects. As reported in limb studies of intended reactions, the labial responses showed latency and waveform variability of reaction time-like responses while at the same time displaying certain characteristics that indicated "automatic" or unconscious mediation.

258.4 RECRUITMENT ORDER AND FIRING PATTERNS OF IDENTIFIED CAT HINDLIMB MOTOR UNITS DURING UNRESTRAINED TREADMILL WALKING.
J. A. Hoffer, M. J. O'Donovan*, C. A. Pratt and G. E. Loeb. Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Physiological and histochemical studies of muscles, done mostly on cat hindlimb extensors, have demonstrated distinct motor unit types. We are investigating whether the pattern of activation and usage of such units during normal activity are consistent with distinctly different functional roles for the unit types. Using chronically implanted electrodes (Hoffer et al., Neurosci. Abstr. 5:1248, 1979) we recorded firing patterns from intact L5 ventral root fibers in normal cats walking on a treadmill. Muscles of destination were identified from spike-triggered, averaged records of EMG. Axonal conduction velocity was determined from averaged unitary records on two adjacent sets of femoral nerve cuff electrodes. Mechanical parameters, and hence unit type, could sometimes be determined from averaged records of tension obtained from an implanted strain gauge on the patellar ligament, elicited by microstimulating the axon in isolation through the recording microelectrode.

Records obtained from 80 motoneurons, of which 22 projected to quadriceps or sartorius, can be summarized as follows:

1) Individual motor units were reliably recruited when the rectified, smoothed parent muscle EMG crossed a reproducible level for each step.

2) Once recruited, most units exhibited appreciable modulation of their firing rates. The frequencygram for a given unit closely resembled the envelope of the parent muscle EMG. Mean firing rates for individual units were higher at faster walking speeds. Peak rates typically reached 25-60 pps. This is in contrast to earlier observations in decerebrate cats (Severin et al., Biofizika 12:660, 1967; Zajac & Young, Neural Control of Locomotion, 1976, p.789; Jordan et al., Neurosci. Abstr. 5:2445, 1979) where unit firing rates were reported to plateau at fairly low values.

3) Initial doublets (interspike interval < 25 ms), reported to occur commonly in decerebrates, were rare in normal cats. Occasional doublets occurred during fast walking and trotting.

4) EMG bursts during both stance and swing in sartorius demonstrated the complex mechanical action of this muscle. In contrast, individual sartorius motor units fired either during stance or during swing, indicating that sartorius is comprised of 2 or more functionally different populations of motor units.

5) Identified units active during walking were all type S (slow twitch) or type FR (fast twitch, fatigue resistant).

258.5 PROGRAMMED RESPONSES OF ELBOW EXTENSORS AFTER JUMPING. P.A. Reback*and J.L. Smith. (SPON: R.D. Lindsay). Neuromotor Control Lab., UCLA, Los Angeles, CA 90024

During landing from a fall, hindlimb extensor EMG is not predetermined centrally on the basis of a prediction of height of fall (Prochaska, et al, J. Physiol. 272:705, 1977). During voluntary jump downs, motor programming is necessary and smooth landing may depend more on an interaction between peripheral reflexes and central programming.

Five adult cats were trained to jump down onto a force plate from a platform placed at heights of 1.0, 0.8 and 0.6 m. Ground reactive forces and EMG of the lateral triceps (LT) were coordinated with 16mm high speed film (100 fr/s).

The figure below illustrates the typical three burst pattern and ground reaction forces of landing. Contrary to the hindlimb fall data, the onset latency of the initial burst is related to jump height. After the forepaw leaves the platform the onset latency, on average, is 206 ms at 1.0 m, 185 ms at 0.8 m and 135 ms at 0.6 m. Peak extension velocity (arrow c) occurs soon after. The duration of the initial burst, 40-50 ms, is unrelated to jump height, and it is followed by a short (14-20 ms) reduction or pause (arrow a). The second burst precedes landing by 30-40 ms, and the peak amplitude of the rectified-averaged EMG is correlated with the height of the jump. The second burst is also followed by a period of reduction (arrow b) that coincides with an unloading of the reactive force. This pause occurs 15-20 ms after landing prior to which the torque at the elbow is maximal. This period of inhibition, similar to that described by Laursen et al (Acta Physiol. Scand. 102:462, 1978), may be due to homonymous tendon organ (Ib) inhibition. Up to maximal elbow flexion (arrow e), the EMG during the remainder of the landing is less than the prelanding or contact EMG, and there is little evidence for a landing stretch reflex of the magnitude of that observed by Prochaska, even during peak flexion velocity (arrow d).

During landing the angular displacement and peak flexion velocity at the elbow joint was nearly constant for a given animal across the three jump heights. This data suggest that muscle stiffness was preset and depended on jump height.

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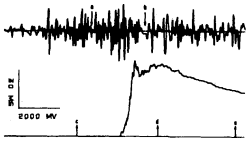


258.6 BIDIRECTIONAL NEURONAL DISCHARGE IN CEREBELLAR NUCLEI AND MOTOR CORTEX DURING SLOW HOLD-RAMP-HOLD TRACKING. Marc H. Schieber & W. Thomas Thach. Dept. Anat. & Neurobiol., Wash. U. Sch. Med., St. Louis, MO 63110.

Trained monkeys made flexion and extension wrist movements guided by a visual pursuit display to track a slow, predictable, hold-ramp-hold target trajectory. Parameters of the trajectory were varied between blocks of trials as extracellular neuronal activity was recorded. EMG activity of the muscles producing these movements was related to load, wrist position, and, although minimally correlated with movement velocity, was clearly related to movement direction. During the movements, agonist and antagonist muscles did not cocontract (Soc. Neurosci. Abstr. 5:585, '79).

In motor cortex, during flexion and extension slow tracking movements, some wrist-related units discharged in a directionally reciprocal manner. In contrast, other wrist-related units were bidirectional: their discharge frequency increased sharply during slow tracking movements in both directions, flexion and extension. The firing frequency of directionally reciprocal units studied thus far was related to load and/or to wrist position, but not to movement velocity. The firing frequency of bidirectional units, however, was related to load, was unrelated to position, but usually increased with increasing velocity in both directions.

In the cerebellar dentate and interposed nuclei, almost all units related to slow tracking movements were bidirectional. In one monkey, several features confirmed that bidirectional neurons were related to movement at the wrist: i) directionally reciprocal activity related to self-paced, alternating movements; and, in interpositus only, ii) reciprocal responses to oppositely directed torque pulses and iii) activity coupled to a fortuitously present tremor. The discharge of these bidirectional neurons was not systematically related to load or position, but did increase with the magnitude of velocity in both directions.

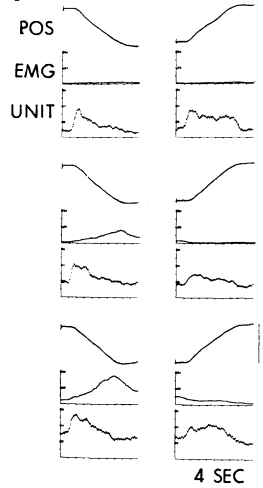
Spindle afferents from forearm muscles were also active bidirectionally, and from their activity we inferred that α-γ linkage was flexible (see companion abstract). These results suggest that, during slow precisely controlled movements, there is functional dissociation of two parallel motor subsystems. One subsystem consists of some motor cortex neurons and of α motoneurons whose discharge remains reciprocal to produce oppositely directed movements. The other subsystem consists of cerebellar nuclear neurons and of other motor cortex neurons, whose discharge under these conditions becomes bidirectional.This subsystem may receive spindle information, control γ motoneurons, or both. It may maintain the constancy of spindle feedback in the face of different conditions (Kuffler, Hunt, & Quilliam, J. Neurophysiol. 14:52,'51): this constancy may be needed for control of slow, precise movement.

Supported by NIH GM 07200 and by 5 R01 NS 12777.

258.7 BIDIRECTIONAL SPINDLE AFFERENT DISCHARGE DURING SLOW TRACKING MOVEMENTS: EVIDENCE FOR ALPHA-GAMMA DISSOCIATION. W. Thomas Thach & Marc H. Schieber. Dept. Anat. & Neurobiol. Wash. U. Sch. Med., St. Louis, MO 63110.

A trained monkey made flexion and extension wrist movements guided by a visual pursuit display to track a slow, predictable, hold-ramp-hold target trajectory. Parameters of the trajectory were varied between blocks of trials as extracellular neuronal activity was recorded from the C7 and C8 dorsal root ganglia.

Spindle afferents thus far studied (N=5) from forearm muscles discharged bidirectionally. The figure shows activity of a spindle afferent from the wrist extensors: the left column shows extensions (parent muscle shortening); and the right, flexions (parent muscle lengthening). In the top row, flexors are loaded (parent muscle relaxed); in the middle row, no load; and in the bottom row, extensors are loaded (parent muscle active). Can spindle stretch and α-γ coactivation explain the results shown? Spindle stretch might explain afferent discharge during all flexions (right column); but even greater discharge might have been expected during flexion under extensor load (bottom right) if the linkage of γ and α motoneuron activity were rigid. Coactivation might explain spindle discharge when extensors were active both under load (bottom row) and during unloaded extension (middle left), yet it does not explain the different time/amplitude profiles of spindle discharge and EMG. But neither stretch nor coactivation can explain the spindle discharge when the parent muscle was relaxed and passively shortening (extension under flexor load, top left). For these reasons we infer, in addition to stretch and coactivation, a third factor: that α-γ linkage is adjustable to the point that γ MN's may drive spindle afferent discharge without coactivation of homonymous α MN's.



Gamma activation of spindles in a quiescent muscle could retrieve information that would otherwise be lost. Furthermore, the observed bidirectional activity of spindle afferents could account for bidirectional units found in motor cortex and cerebellar nuclei (see companion abstract). These higher level neurons may receive spindle feedback, control gamma motoneurons, or both.

Supported by 5 R01 NS 12777 and NIH GM 07200.

258.8 EFFECTS OF A SENSORY CONFLICT ON HEALTHY HUMAN STAIR DESCENT. R. L. Craik and W. Freedman. Rehabilitation Engineering Center #2, Moss Rehabilitation Hospital, Philadelphia, PA 19141.

The occurrence of lower limb muscle activity (emg) prior to step contact marked the beginning of investigations into the motor programs used during stair descent (Melvill Jones and Watt, 1971). Subsequent studies have focused on the role that various sensory systems play in the initiation or modification of this anticipatory muscle activity. Healthy human subjects participated in this experiment designed to characterize the manner in which altered visual input modifies the voluntary motor behavior seen during stair walking.

Fourteen subjects walked down three steps whose height was randomly varied to be 20.3, 30.5, or 40.6 cm. The steps were surrounded by a room 2.77 m high and 0.7 m wide which was connected through a servo system to the vertical position of the subject. Subjects were asked to walk down the three steps while a surface electrode placed over the gastrocnemius-soleus complex recorded muscle activity, a potentiometer measured sagittal plane ankle motion, switches registered bilateral foot-ground contact and release, an accelerometer recorded vertical acceleration and a transducer in the second step measured the force with which it was hit. Anticipatory muscle activity and the ease of landing were examined only on the first two steps. The emg signal was integrated within 10 ms bins over the entire first swing interval (toe off to toe strike) and ease of landing was described by the angular velocity which occurred at the ankle and the rate at which load was applied with contact on the second step. Control performance was compared to that recorded under the following conditions: blindfolded with audition masked and unaware of stair height; aware of stair height and wearing a collar which masked stair height during descent; aware of stair height and wearing a collar while the room moved up, down, or not at all.

Anticipatory muscle activity was diminished and landing was harder for each of the experimental conditions but performance was significantly different between test conditions. These results imply that there is adequate time for a sensory perturbation which occurs during the swing phase to alter the subsequent landing. These data suggest that the visual information predominates when there is a conflict between visual and other somatosensory information as evidenced by the manner in which the subjects contacted the second step. To date, the visual, vestibular and tactile sensory systems have each been demonstrated to modify the anticipatory muscle seen during human stair descent. Additional research is necessary to examine the interaction among these systems.

(Supported by NIHR #23-P-55518/4 and NINCDS #NS14133-02.)

259.1 AFFERENT CONNECTIONS OF THE PARVOCELLULAR RETICULAR FORMATION IN THE RAT. W. R. Mehler and J. A. Rubertone* NASA-Ames Research Center, Moffett Field, CA 94035 and Dept. of Anatomy, Univ. of Calif., San Francisco, CA.

The parvocellular reticular formation (PC) is a bilateral cylindroid of grey matter in the dorsal lateral tegmentum of the brain stem extending from the level of the pons through the medulla oblongata into the upper cervical spinal cord. Nauta method studies of regional lesions in PC revealed extensive bilateral ascending and descending pathways that interconnect trigeminal, facial, glossopharyngeal, vagal and hypoglossal motor nuclei which in various combinations participate in cardiovascular control, food intake and expulsion (emetic) mechanisms and concomitant control of respiration (Holstege and Kuypers, Brain 100:239, 1977).

Extensive bilateral corticobulbar connections were the first PC afferents to be described. In 1963 Mehler found a system of collaterals from degenerating axons of the mesencephalic root of the trigeminus (Mes. V) that descended in the ipsilateral PC and issued terminal branches into PC from MoV levels to the obex. Comparative studies of cerebellofugal fibers (Mehler, '67) revealed a unique, ipsilateral descending branch of the brachium conjunctivum in the rat that also distributed throughout PC. Cajal ('03) had discovered this pathway in the rat but his contemporaries, working on non-rodents, rejected the idea. Silver studies also showed an extensive commissural system between the two PC regions.

Iontophoretic injections of horseradish peroxidase (HRP) in different regions of PC in rats (see Rubertone and Mehler, this volume) labels cells in both MesV and, primarily the dorsolateral "hump" of the interpositus crebellar nucleus ipsilaterally confirming the earlier studies cited. Scattered labeled cells appear in the upper PC region and in the contingent supratrigeminal and parabrachial regions ipsilaterally and in greater numbers in the contralateral PC. The latter cells appear at, or caudal to, the level of the injection. Some HRP+ cells also appear in the ipsilateral spinal trigeminal nucleus. Labeled cells in the central nucleus of the amygdala appear to confirm Hopkins and Holstege's (1978) anterograde evidence of an amygdalo-PC connection. The results of larger injection and longer survival times, relative to the location of the cells of origin of corticobulbar-PC afferents will be discussed.
NASA-Task 199-05-02-07.

259.2 INDEPENDENCE OF ASCENDING SOMATIC SENSORY PATHWAYS IN THE CAT AND MONKEY. K.J. Berkley. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

Double-labeling experiments using autoradiographic and degeneration orthograde tracing methods in the cat indicate that fibers from the dorsal column nuclei (DCN), lateral cervical nucleus (LCN) and spinothalamic tract (ST) form their densest terminations on different groups of neurons in the ventrobasal complex (VB) and surrounding regions of the thalamus. In the monkey, the same relative terminal arrangements exist; that is, although the precise locations of the terminations of the three pathways in the monkey's thalamus differ from those in the cat, the DCN, LCN and ST fibers form their densest terminations on different small groups (or clusters) of neurons that often are adjacent to each other, particularly within VB. Thus, individual clusters of neurons within VB and its surrounding regions in both the cat and monkey appear to receive their input predominantly from one of the three ascending pathways and relatively little input from either or both of the other two pathways.

Retrograde tracer experiments using the transport of horseradish peroxidase and ^{3}H-inactivated horseradish peroxidase in the cat indicate that the neurons within DCN which project to the thalamus form a morphologically homogeneous population. Neurons within LCN that project to the thalamus, however, form a morphologically heterogeneous population. When these two retrograde tracers are used together in a double-labeling paradigm, it also becomes evident that the DCN neurons which project to the thalamus do not appear to send collateral fibers to the tectum, whereas many of the LCN neurons which project to the thalamus do send collaterals to the tectum. These results suggest that the functional properties of the DCN neurons which project to the thalamus may be different from those of the LCN neurons which project to the thalamus.

The results of these orthograde and retrograde tracing experiments are consistent with the conclusion that individual clusters of neurons within VB (and surrounding regions) may receive input from fibers in different pathways with different response properties. When this conclusion is considered together with experiments by other investigators which have demonstrated a precise and discrete topography in the connections between clusters of neurons in VB and the cerebral cortex, it appears that there exists a structural mechanism from the periphery to the cortex for the maintenance within the somatic sensory system of multiple, and possibly functionally independent, lines of projection.

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259.3 SEGREGATION OF NEURONS HAVING SPECIFIC SUBMODAL PROPERTIES WITHIN SOMATOTOPICALLY ORGANIZED REGIONS OF THE THALAMIC VPL AND VPI NUCLEI OF THE SQUIRREL MONKEY (Saimir; Sciureus). R.W. Dykes, M. Sur, M.M. Merzenich, J.H. Kaas and R.J. Nelson. Depts. of Surgery, Neurology and Neurosurgery, McGill University, Montreal; Psychology & Anatomy, Vanderbuilt University, Nashville, Tenn.; Physiology & Otolaryngology, Univ. of California, School of Medicine, San Francisco, Calif.

While studying the detailed topology of the body representation in the ventroposterior (VP) thalamus of the squirrel monkey, we observed restricted regions within this nucleus which received a predominant input from specific kinds of receptors.

Adult squirrel monkeys were anesthetized with ketamine hydrochloride, their skulls fixed in a stereotaxic device and a craniotomy performed. Platinum-iridium microelectrodes were advanced through the thalamus along all three cardinal axes of the Horsey-Clark coordinate system. At 50 μm intervals, multiunit responses were characterized in terms of (i) receptive field (RF) location, (ii) modality (skin or deep), and (iii) probable receptor type: slowly adapting (SA), rapidly adapting (RA) and Pacinian. Histological sections stained with cresyl violet were searched to identify the electrode tracks and to relate the functional data to particular thalamic regions.

Modality changes were closely correlated with nuclear boundaries. The deep responses stopped abruptly at the upper border of VP. Upon entering VPL, neuronal responses were elicited by cutaneous stimuli and this remained the adequate stimulus mode until the ventral border was crossed. Within VPL the first responses encountered were frequently cutaneous RA responses which may have been activated by convergence from several cutaneous RA fibers. Below this, cutaneous SA responses were encountered. These regions appeared to receive a predominant input from cutaneous slowly adapting afferents. In the ventral portion of VPL, cutaneous RA responses were often encountered. In this region the responses suggested a predominant input from non-Pacinian cutaneous RA fibers.

As the electrode left the VP region and entered the VPI nucleus the neurons became most responsive to Pacinian-like stimuli and the neurons in the VPI appeared to receive a predominant input from this receptor class.

Further, we observed (i) abrupt shifts of RF locus, (ii) long sequences of RF's serving one body site and (iii) regular, gradually-shifting sequences of RF's within VP. These data suggest that there are discrete, submodality-specific regions within VP which are topographically organized in a manner far more complex than earlier studies have suggested.
(Supported by NIH, Bethesda and MRC, Ottawa)

259.4 RECEPTIVE FIELD SEQUENCES IN SOMATOSENSORY CORTEX OF THE CAT. D. Sretavan* and R.W. Dykes (SPON: J. Commissiong). Microsurgical Laboratory, Departments of Surgery and Physiology, McGill University, Montreal, Quebec, Canada.

Recently, electrophysiological mapping experiments have demonstrated the presence of several representations of the body within primary somatosensory cortex of cats and monkeys. Despite detailed characterization, a general principle of organization underlying these maps is still to be defined. One description of the internal organization of the maps proposes that within each map, separate body regions such as the digits, wrist and forearm are each served by a distinct island of cortex and that each representation of the body consists of the aggregate of these islands or 'composites'. An essential requirement for this 'composite' organization is that RF loci should shift abruptly at the edge of the composite as the RF locus jumps to the next body part. The identification of the abrupt RF shift at the edge between two composites is therefore crucial to this theory.

An alternative view is that there are few or no boundaries within each map and that each map is a continuum or smooth sequence of gradually shifting RF loci. This latter hypothesis has certain genetic and developmental advantages over one with many boundaries at the edges between cortical maps. We chose to test these two alternatives in nembutal anesthetized mongrel cats with glass microelectrodes. We made vertical and tangential penetrations through one of the several maps in somatosensory cortex and carefully recorded RF size and locus at 50 μm intervals. Thionin-stained 50 μm frozen sections were used to identify each electrode trajectory and relate it tothe cytoarchitecture.

Results from vertical penetrations document the degree of variability in RF locus found within a vertical path through the cortex and set the degree of variability in RF locus which can be found at a single cortical site. Any shift in RF locus which is to be taken as the boundary of a composite must be larger than the variability found in vertical penetrations. During penetrations tangential to the cortical surface, the sequence of RF loci encountered reflects both the variability seen at any one cortical site plus the shift due to the lateral displacement of the electrode towards another cortical site. Results from tangential penetrations show that the progression of RF was smooth and continuous.

In a few experiments however, abrupt shifts in RF locus were found. The histological data and submodality changes showed that these abrupt RF shifts usually occurred at the edges between cortical maps and not within boundaries of a given map. These results suggest that in the cat, the body surface is represented as a continuum within each cortical map.
(Supported by the Medical Research Council of Canada)

259.5 QUANTITATIVE ANALYSES OF THALAMOCORTICAL SYNAPSES ON IDENTIFIED NEURONS IN MOUSE SmI CORTEX. E.L.WHITE, M.P.ROCK* AND S.M.HERSCH*. Dept. Anatomy, Boston U. Sch. Med, Boston, MA. 02118.

A broad variety of neuronal types in mouse SmI cortex (specifically in posteromedial barrel subfield cortex) synapse with thalamocortical (TC) axon terminals (White,E.L., J.Comp.Neur. 181:627, 1978). Although preliminary data suggest these cell types differ in the amounts of TC synapses they form, it is uncertain if particular neuronal types consistently receive specific proportions of their synaptic input directly from TC afferents. Here we report current efforts to answer this question by ascertaining the amounts of TC and other synapses in which different neuronal types are involved. Lesion induced degeneration is used to label TC axon terminals because previous work suggests this method reliably demonstrates all TC afferents in layer IV of mouse PMBSF cortex. Elements postsynaptic to TC axons are labeled by the Golgi/gold-toning method or by the retrograde transport of horseradish peroxidase (HRP). In a third approach, some non-spiny neurons have been identified by their appearance in serial thin sections and in 3-dimensional reconstructions. Analyses of synapses onto a gold-toned layer IV spiny stellate cell show its dendrites to receive 9% of their synapses from the thalamus, whereas dendrites of a layer IV non-spiny stellate cell in the same preparation receive 16% of their synapses from the thalamus. Dendrites of a gold-toned layer V non-spiny stellate cell receive 2.9% of their synapses from the thalamus, whereas in the same PMBSF barrel, neighboring dendrites of a layer IV non-spiny bitufted cell receive 17.3% of their synapses from TC afferents. These results indicate that neurons occurring even within the same thalamic terminal projection field may receive markedly different amounts of their synaptic input from the thalamus. Furthermore, counts show all dendrites examined in these studies pass through neuropil where 18% of the synapses are made by TC afferents and so the observed differences would seem not to be due to differences in the concentration of TC synapses in the fields through which the dendrites pass. Analyses of the synapses of neurons labeled by the retrograde transport of HRP injected into the vibrissal region of ipsilateral MsI cortex show dendrites of layer III and IV pyramidal cells consistently receive only few TC synapses. Further investigation should disclose if superficial pyramids projecting elsewhere receive different amounts of TC synapses.
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259.6 CURRENT SOURCE DENSITY ANALYSIS OF THE EVOKED POTENTIAL IN THE SOMATOSENSORY CORTEX OF THE CAT. P.B. Hoeltzell* and R.W. Dykes (SPON: G. Kostopoulos). Microsurgical Laboratory, Departments of Surgery and Physiology, McGill University, Montreal,PQ, Canada.

The sequence of potential changes following a brief punctate tactile stimulus was studied in primary somatosensory cortex. Each cat was anesthetized with sodium penobarbital. Tracheal and venous cannulae were inserted, a pneumothorax was performed and the animal was mounted in a stereotaxic device. Expired CO_2 was kept near 3% and body temperature near 37°C. After a craniotomy a low-impedance glass microelectrode was inserted in the forearm region of SI cortex and a biological transducer was positioned over the center of the receptive field which activated that cortical site. Fixed-amplitude, o.1s stimuli were delivered to the arm every 2s. These stimuli produced action potentials at the cortical focus in the depths from 500 to 1200 μm. The evoked potentials were amplified with conventional recording equipment, filtered to exclude DC and action potentials and digitized with a small laboratory computer.

Preliminary experiments were done to demonstrate stationarity of the potential under these experimental conditions and to calculate the maximum lattice spacing which could be used for sampling without spatial aliasing.

In subsequent experiments the cortical focus was identified and then the microelectrode was withdrawn and used to make a series of penetrations in rostrocaudal or mediolateral rows passing through or parallel to this site. In each penetration evoked potentials were recorded every 50 to 100 μm through the cortical depth. The penetrations were spaced either 100 or 200 μm apart. These potentials were then processed to compute current sources and current sinks in that cortical volume.

The results illustrate that the surface evoked potential is a reflection of neural activity in the upper cortical layers and that the surface maximum is not situated immediately over the site where the maximum thalamic afferent volley arrives, but some distance away where it reflects other, related neural events.

The spatial organization of the evoked potential appears to involve several populations of neurons at more than one cortical depth and more than a single cortical site. Interpretation of this data requires a detailed knowledge of the synaptic relationships among neurons in the somatosensory cortex as well as knowledge of the temporal sequence of activity in the afferent terminals ending in different cortical layers.
(Supported by the Medical Research Council of Canada and the National Institute of Health, USA.)

259.7 SELECTIVE NEURO-DEGENERATIVE CHANGES IN THE RAT SOMATOSENSORY CORTEX FOLLOWING TREATMENT WITH METHYLAMPHETAMINE, P-CHLORO-AMPHETAMINE BUT NOT 6-HYDROXYDOPAMINE. G. Ricaurte*, R.W. Guillery, L.S. Seiden, C.R. Schuster*. Dept. of Pharmacol. and Physiol. Sciences, Univ. of Chicago, Chicago, IL. 60637.

In a previous Fink-Heimer study (Ricaurte et al., 1980, submitted for publication) signs of neuronal degeneration were found in the somatosensory cortex of rats treated four days previously with high dose regimens (12.5 or 50.0 mg/kg x 3, 8 hours apart, s.c.) of methylamphetamine, a potent psychomotor stimulant drug. The affected neurons were located primarily in laminae III and IV of somatosensory cortical area 2, as defined by Krieg (J. Comp. Neur. 84: 221, 1946); this includes the region of the cortical "barrels" (Woolsey et al., J. Comp. Neur. 164: 79, 1975). These neurons were commonly pyramidal, had darkly-staining shrunken perikarya and argyrophilic dendrites which were frequently fragmented; their nucleus (when discernable) was pyknotic, argyrophilic and often eccentrically displaced. Further, silver sections counter-stained with cresyl violet revealed glial cells closely surrounding (as if phagocytizing) many of these argyrophilic neurons.

We now report that similar neuro-degenerative changes occur in the somatosensory cortex of rats treated four days previously with the serotonergic neurotoxin p-chloroamphetamine (PCA) (10 mg/kg, i.p.). In contrast to this, rats treated with the catecholaminergic neurotoxin 6-hydroxydopamine (6-HDA) (100 ug., i. vent.) do not display comparable neuropathological changes either when 6-HDA is given alone (Hedreen and Chalmers, Brain Res. 47: 1, 1972), or when it is given 45 minutes after a desmethylimipramine (25.0 mg/kg, i.p.) and pargyline (50.0 mg/kg, i.p.) pretreatment, as it was in this study.

Since 6-HDA generally destroys catecholamine-containing cells, the above observations suggest that the somatosensory cortical neurons affected by the amphetamines are not dopaminergic or noradrenergic. This is consistent with the fact that catecholamine producing cell bodies have not been identified in the cerebral cortex. Although the transmitter substance produced by the damaged somatosensory neurons remains to be determined, the fact that they are sensitive to the toxic actions of methylamphetamine and PCA, drugs with known serotonergic neurotoxic properties (Sanders-Bush et al., J. Pharmacol. Exp. Ther. 192: 33, 1975; Ricaurte et al., Brain Res. in press, 1980), suggests that they may be serotonergic or closely related.

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260.1 EVIDENCE FOR THE RELEASE OF ENKEPHALINS BY TYROSINE-ARGININE (KYOTORPHIN). Anita Rackham, Paul L. Wood, Roger L. Hudgin*. Merck Frosst Labs., Dept. of Pharmacology, P.O. Box 1005, Pointe Claire-Dorval, Quebec, H9R 4P8, Canada.

The endogenous dipeptide tyrosine-arginine (kyotorphin) has been reported to have naloxone reversible analgesic effects. A proposed mechanism for the analgesia seen after intracerebroventricular administration of this and related peptides is release of enkephalins rather than direct activation of opiate receptors. Unlike enkephalins and standard opiate agents, kyotorphin and related peptides do not inhibit the binding of either [^{3}H]naloxone, [^{3}H](D-ala^2-D-leu^5)enkephalin, or [^{3}H]ethylketocyclazocine at concentrations up to 1000 nM. Using neurochemical (GC-MS) techniques to monitor changes in dopamine (DA) and its metabolites in the rat striatum, the present study further supports the enkephalin-release hypothesis for the action of kyotorphin. At analgesic doses, 50 to 200 µg intracisternally in the rat and mouse tail flick and hot plate assays, the effect of kyotorphin and various dipeptides on DA metabolites was found to be similar to that of morphine and opioid peptides. Significant increases were seen in striatal levels of dihydroxyphenylacetic acid (DOPAC) (90% increase) and homovanillic acid (HVA) (60% increase) while no effect was seen on DA or 3-methoxytyramine (3-MT) levels. Also the kyotorphin-related peptides and standard opiates were found to have similar attenuating effects on cortical and hippocampal acetylcholine turnover (TR_{ACh}), with decreases of 50 to 75% in TR_{ACh} in both the cortex and hippocampus. These data show that the action of kyotorphin and related dipeptides on DA and TR_{ACh} parallels that of standard opiate receptor agonists, but the lack of direct effect on opiate receptors suggests stimulation of enkephalin release may be a possible mechanism of analgesic action.

260.2 EFFECTS OF MORPHINE AND OPIOID PEPTIDES ON THE ELECTRICALLY EVOKED RELEASE OF ENTERIC SUBSTANCE P. Alan R. Gintzler* (Spon: C. Noback), Dept. of Anatomy, Columbia Univ., P&S, New York, 10032.

Previous studies have demonstrated that naloxone-precpitated enteric withdrawal is mediated via 2 neuronal pathways. One involves the release of acetylcholine (ACh) and the activation of smooth muscle muscarinic receptors. The other involves the release of enteric serotonin which then acts through the enteric nervous system to release substance P (Gintzler, A.R., Brain Res., 182:224, 1980). The involvement of substance P in precipitated gut withdrawal suggests that acute administration of narcotics might also be effective in modulating release of substance P in naive preparations. Stimulation of naive ilea (20 Hz) produces an atropine-resistant contracture that can be blocked by pretreatment with tetrodotoxin (TTX; 10^{-6}g/ml) or by desensitization to substance P. This desensitization is highly specific; contractures in response to histamine, ACh, KCl, or 0.1 Hz electrical stimulation are not affected. The magnitude of the atropine-resistant contracture evoked by 20 Hz stimulation therefore, can be used as an indication of the release of substance P. Morphine (5×10^{-6}M) as well as the opioid peptides FK33-824 (9×10^{-7}) or D met^{2}pro^{5}enkephalin (3×10^{-7}M) can significantly reduce the magnitude of the atropine-resistant 20 Hz contracture without altering responses to exogenous substance P. This inhibition is stereospecific and is blocked by naloxone (10^{-6}M). Pretreatment with the nicotinic cholinergic receptor blocker, hexamethonium reduces by about 30% responses to 20 Hz stimulation but has no effect on the inhibition produced by morphine. Thus, although a portion of the electrically evoked release of enteric substance P is dependent on the release of ganglionic ACh, the inhibition of release produced by morphine does not involve a cholinergic mechanism. Furthermore, pretreatment of a naive preparation with naloxone significantly enhances the magnitude of responses to 20 Hz stimulation suggesting that endogenous opioid peptides can also modulate release of enteric substance P. (Supported by NIMH Grant #DA01772).

260.3 ß-ENDORPHIN-INDUCED INCREASE IN CENTRAL SYMPATHETIC OUTFLOW: INHIBITION OF ADRENOMEDULLARY RESPONSE BY INTRACEREBRAL SOMATOSTATIN. Nathan M. Appel and Glen R. Van Loon. Departments of Medicine and Physiology, University of Toronto, Toronto Canada M5S-1A8.

Endorphins have been characterized as peptides with opiate-like biological activities present in brain and pituitary gland. A variety of effects including sedation, catalepsy, analgesia, hypothermia and regulation of pituitary hormone secretion has been attributed to central nervous system actions of endorphins. Evidence for aminergic neuronal modulation of some of these endorphin effects has been provided. The present study was undertaken to examine the possibility that endorphins might alter central sympathetic outflow. That is, we examined the possibility that brain endorphins might alter peripheral release of catecholamines, thus altering plasma catecholamine concentrations. We examined the effects of intracisternal (ic) synthetic human ß-endorphin on plasma concentrations of dopamine, norepinephrine and epinephrine in unanesthetized, freely moving rats bearing a chronic intraarterial cannula. ß-endorphin produced major, prolonged increases in plasma concentrations of all three catecholamines when compared with the responses to ic saline. The increase in plasma epinephrine was much greater than the increases in plasma dopamine or norepinephrine, supporting greater stimulation by ß-endorphin of sympathetic outflow to adrenal medulla than to sympathetic nerve endings. Prior intraarterial administration of naloxone blocked these ß-endorphin-induced effects, supporting mediation by opioid receptors. Systemic naloxone did not alter plasma catecholamine responses to ic saline. Acute systemic administration of guanethidine blunted the plasma norepinephrine response to ic ß-endorphin, suggesting that a considerable portion of the ß-endorphin-induced increase in plasma norepinephrine is derived from sympathetic nerve endings. A variety of putative neurotransmitters may interact with endorphins in modulating central sympathetic outflow. Somatostatin has been implicated as a neurotransmitter in the central nervous system. Simultaneous ic administration of somatostatin together with ß-endorphin markedly inhibited the plasma epinephrine response to ß-endorphin while decreasing the dopamine response to a much lesser degree and decreasing the norepinephrine response nonsignificantly. In conclusion, endorphins increase central sympathetic outflow to both adrenal medulla and to sympathetic nerve endings. In addition, somatostatin neurons appear to interact with endorphin neurons to selectively block endorphin-mediated stimulation of sympathetic outflow to adrenal medulla, without affecting outflow to sympathetic nerve endings.

260.4 CALCIUM SENSITIVITITY OF ENKEPHALIN RESPONSE IN THE HIPPOCAMPAL SLICE. S. M. Bawin*, W. R. Adey, S. Lin-Liu* and M. D. Mahoney*. Research Service, VA Hospital, Loma Linda, CA 92357

Electrophysiological responses such as long-term hyperpolarization, long-term potentiation, and epileptogenic activity in the hippocampal slice, are critically dependent on the calcium concentration in the bathing medium, and are impaired at calcium levels lower than 1mM. Enkephalins have recently been shown to increase spontaneous activity and amplitude of population spike response to Schaffer collateral stimulation in CA_1 pyramidal cells. Therefore, the effects of low (1mM) and high (4mM) calcium concentrations on these excitatory effects were studied, using rat hippocampal slices.

The slices (450 µm) were continuously perfused with warm (33-35C) oxygenated physiological solutions at a rate of 2 ml/min. A double valve system allowed perfusion with either control or treatment solutions without interrupting the flow. Bipolar stimulating electrodes and 4M NaCl micropipettes were positioned in the Schaffer collaterals and the CA_1 pyramidal cells, respectively. Twenty minutes perfusion with 1mM calcium solution induced a progressive decrease in the evoked response, while 4mM calcium solution induced large increases in population spike amplitude. Perfusion with control (2mM Ca) solution containing 200nM (D-Ala^2, Met)-enkephalin resulted in large (50-70 percent), long lasting but reversible increases in spontaneous and evoked cell activity, as described by others.

The enkephalin response was weaker (20-40 percent increase) during perfusion with either high or low calcium concentrations. Furthermore, the induced activity was not maintained beyond the first 10 min of perfusion with enkephalin. Calcium has been shown to antagonize opioid effects in the brain, and could similarly antagonize enkephalin excitation in the CA_1 cells during perfusion with 4mM calcium solution. The decreasing response in low calcium medium could reflect loss of synaptic responsiveness. (Funding: DOE DE-AI101-79ET29078, FDA RO1-FD00963-03, and Southern California Edison Company.)

260.5 ROLE OF β-ENDORPHIN ON CALCIUM FLUXES IN SYNAPTOSOMES. S. Lin-Liu*, S. M. Bawin* and W. R. Adey (SPON: M. A. Baker). Research Service, VA Hospital, Loma Linda, CA 92357

It has been suggested that β-endorphin regulates release of neurotransmitters by inhibition of Ca^{2+} influx into the nerve terminals but does not affect Ca^{2+} release. The present study was aimed at studying the effect of β-endorphin on Ca^{2+} uptake during excitation by K^+ stimulation and further to determine whether Ca^{2+} efflux per se is affected.

Synaptosomes prepared from cerebri of rats according to Cotman and Matthews were suspended in 0.32M sucrose with 5mM Hepes buffer (pH 7.5) and preincubated at 32°C for 5 min. An equal volume of a 32°C. incubation solution I_1 (low KCl) or I_2 (high KCl) containing $^{45}Ca^{2+}$ 0.1 μCi/ml was added and incubation continued for 10 min. $^{45}Ca^{2+}$ uptake was ended by rapid filtration of synaptosomes through millipore filters (0.45 μm pore size). For uptake study, the filters were washed four times with 2.5 ml of stopping solution S_1 (normal NaCl) or S_2 (no NaCl). Radioactivity remaining on filters was assayed by liquid scintillation counting. Results between incubation with and without β-endorphin and between high and low K^+ were compared. The difference between normal- and no-Na^+ washing was interpreted as Na^+-sensitive intracellular Ca^{2+}.

In efflux studies, the filters were perfused continuously with Kreb's saline at 32°C at 2 ml/min. β-endorphin (10 μM) was injected into the perfusion line at 20 μl/min at 1 to 5 min intervals during perfusion. Results were compared with injection of deionized water. In low KCl incubation, β-endorphin decreased calcium uptake 15-20 percent, confirming the findings of others. In the absence of β-endorphin, high KCl stimulated calcium uptake by 30 percent, but with β-endorphin the stimulation was about 17 percent. This 10 percent inhibition by β-endorphin may arise from not only a reduction of K^+-stimulated Ca^{2+} influx, but also from increased Ca^{2+} release from bound pools for the following reasons. First, in both situations, the percentage of Na^+-sensitive Ca^{2+} are the same, suggesting a possible reduction in all Ca^{2+} pools. Second, with low K^+ incubation, while β-endorphin decreased total Ca^{2+} uptake, it increased the percentage of Na^+-sensitive Ca^{2+}. Third, when perfusing with a calcium-free solution, β-endorphin increased $^{45}Ca^{2+}$ in the perfusate. These results demonstrated a regulator role of β-endorphin during neural excitation and suggest that β-endorphin inhibits Ca^{2+} influx and enhances Ca^{2+} efflux at the same time. (Funding: DOE DE-AI101-79ET29078, FDA RO1-FD00963-03, and Southern California Edison Company.)

260.6 BOVINE CHROMAFFIN CELLS IN CULTURE ARE A MODEL FOR THE SYNTHESIS AND RELEASE OF THE ENKEPHALINS AND PUTATIVE PRECURSORS. J. Rossier, B. Livett†, J. Trifaro‡, D. Dean†, R. Lee‡, R. Lewis°°, S. Udenfriend°° Physiologie Nerveuse, CNRS, Gif-sur-Yvette, France, °Dept. of Pharmacol.,Mc Gill Univ.,Montreal, †Dept. of Neurology, Montreal General Hospital, °°Roche Inst.,Nutley,New Jersey.

10^6 Chromaffin cells freshly isolated from the adrenals contain per cells: .9 ng of oxidized Met-enkephalin, .6 ng of Met-enkephalin-Arg^6, 2.2 ng of Met-enkephalin, .8 ng of Leu-enkephalin, 2.5 ng of Met-enkephalin-Arg^6-Phe^7 and more than 100 ng of various putative enkephalin precursors. These putative precursors have M_r ranging from 3000 to 22000. Cells in culture maintain similar levels of enkephalins and putative precursors for more than a week. Biosynthesis: After 8 days in culture, cells were incubated with ^{35}S-Methionine for 2 hrs. An acid extract of the 105,000 g x 1 hr supernatant was separated by gel filtration on a Sephadex G-75 column. Fractions containing the 22,000 M_r enkephalin precursor were incubated with trypsin. The trypsin digest was separated by HPLC and the fractions corresponding to Met-enkephalin-Arg^6 and Met-enkephalin-Lys^6 were immunoprecipitated with a serum specific for the N-terminus of the enkephalin molecules. No radioactivity was precipitated in the tubes corresponding to Met-enkephalin. The 22,000 M_r precursor incorporated into the sequences Met-enkephalin -Arg^6 and -Lys^6 at least .04 % of the radioactivity found in the 105,000 g supernatant. Fractions containing M_r compounds below 1000 were analysed by HPLC. After the 2 hrs pulse, no radioactivity was found in the HPLC fractions corresponding to Met-enkephalin and Met-enkephalin-Arg^6-Phe^7. Six hrs after the pulse, these two HPLC fractions did contain .05 % of the radioactivity found in the 105,000 g extract. By immunoprecipitation it was confirmed that all the radioactivity associated with the two fractions containing Met-enkephalin and Met-enkephalin-Arg^6-Phe^7 respectively corresponded to these molecules. The specific radioactivity of Met-enkephalin was three times greater than that of Met-enkephalin-Arg^6-Phe^7 indicating a slower turn-over of this latter molecule. Release: Studies were carried out with chromaffin cells in culture for 6 days. Nicotine ($5x10^{-6}$ M, 5 min) stimulated the release of catecholamines along with enkephalins and their putative precursors. A single stimulus released 21 % of the total cellular content of noradrenaline, 10 % of adrenalin, 8 % of Leu-enkephalin and 5 % of the putative enkephalin precursors. Various concentrations of nicotine were tested. Dose response curves for the catecholamines and the enkephalins were completely parallel with a maximum for a nicotine concentration of $1x10^{-5}$ M. These results and other cytological evidences indicate that the catecholamines and the enkephalins are stored in, and released from the same vesicles. J.R. is Chargé de Recherche INSERM.

260.7 CHARACTERIZATION OF ENKEPHALIN DEGRADING ENZYMES. Charles Gorenstein and Solomon H. Snyder. Dept. of Pharmacol. Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205

Degradation of enkephalins can occur by a variety of peptidases. In an effort to determine which if any are concerned with the physiological breakdown of enkephalins, we have characterized several membrane bound brain peptidases. DEAE chromatography of Triton solubilized membranes resolves several enzymatic activities. Two enzymes, dipeptidylcarboxy peptidases A_1 and A_2 were separated. Identification as dipeptidylcarboxy peptidases was based on formation of tyr-gly-gly by thin-layer chromatography. Both enzymes are metallo-peptidases have an isoelectric point of 4.8 and are clearly resolved from angiotensin converting enzyme. Enkephalinase A_1 has been purified 1500 fold to apparent homogeneity.

A novel membrane bound dipeptidyl aminopeptidase designated enkephalinase B generates tyr-gly and can also cleave a series of basic dipeptides beta-Naphthylamide derivatives (lys-ala β-NA, arg-arg-β-NA). This enzyme has been purified to apparent homogeneity.

Of these enzymes, enkephalinase A_1 shows a regional distribution closely paralleling that of opiate receptors.

260.8 IN VIVO AND IN VITRO ENZYMATIC DEGRADATION OF DYNORPHIN-(1-13) AND STRUCTURAL ANALOGUES. F.M. Leslie* and A. Goldstein (SPONS: B.M. Cox) Addiction Research Foundation, 701 Welch Road, Palo Alto, CA 94304.

Dynorphin-(1-13), a recently synthesized opioid peptide, has been shown to be rapidly degraded by rat brain enzymes both in vivo and in vitro (Goldstein et al., Proc. Nat. Acad. Sci. 76: 6666 (1979)). In order to more fully characterize the pharmacology of dynorphin-like peptides, it is important to synthesize structural analogues which are completely resistant to enzymatic attack under the conditions in which they are studied. The present study investigates in vitro and in vivo enzymatic cleavage of ^{125}I-dynorphin-(1-13) and compares the relative stabilities of several ^{125}I-labelled structural analogues. Degradation was assessed by gel permeation, thin layer and high pressure liquid chromatography, using a combination of systems in which all potential radiolabelled dynorphin-(1-13) metabolites could be identified.

When incubated in vitro with well-washed rat brain membranes (50mM Tris-HCl buffer, pH 7.4), ^{125}I-dynorphin-(1-13) (20-100nM) was degraded by a combination of aminopeptidase and carboxypeptidase activities. At 22°C, in the absence of added ions, carboxypeptidase removal of the terminal lysine residue was the most prominent degradative process. At 37°C, or in the presence of Krebs ions, aminopeptidase activity was greatly increased. High concentrations (1mM) of enzyme inhibitors such as phenanthroline and bacitracin, or incubation at 0°C, attenuated but did not completely inhibit this degradation. Addition of a methyl ester group at the carboxy terminus protected the peptide to some extent from both carboxypeptidase and aminopeptidase attack. Substitution of a D-ala group at position 2 and amidation of the carboxy terminus further increased dynorphin-(1-13) stability in this system.

In vivo degradation was assessed 10 min after lateral intraventricular injection of radiolabelled peptide. Dynorphin-(1-13) (10 and 100 nmoles) was rapidly broken down in intact rat brain by aminopeptidase activity. An analogue, D-Ala^2-dynorphin-(1-11), was considerably more resistant to in vivo enzymatic attack, although there was still significant breakdown at the 10 nmole dose.

260.9 A NOVEL AMINOPEPTIDASE FOR ENKEPHALIN DEGRADATION.

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Enkephalins, the endogenous opioid peptides, are inactivated primarily by aminopeptidases, as demonstrated by the release of Tyr with brain homogenate and cultured human endothelial cells. Four peptidases were identified as being responsible for enkephalin degradation, three particulate enzymes, 1) aminopeptidase, 2) enkephalinase A, and 3) enkephalinase B, and 4) a soluble aminopeptidase. Enzymes 1 and 4 split the Tyr-Gly (1-2) bond; 2, the Gly-Gly (2-3)bond; 3, the Gly-Phe (3-4)bond. In the brain, the degradation of enkephalin from the amino terminus is 3-4 times higher than the activity of the other enkephalinases. We purified a soluble aminopeptidase from rat brain using enkephalin as substrate. The post-mitochondrial fraction was precipitated with $(NH_4)_2SO_4$ between 40 and 70% saturation and submitted to permeating chromatography, ion-exchange chromatography, and adsorption chromatography. The enzyme activity was assayed measuring the degradation of enkephalin by the disappearance of UV absorption at 210nm after the separation by high-pressure liquid chromatography equipped with a μBondapak C_{18} column. The enzyme degraded enkephalin only by release of the tyrosine; the disappearance of enkephalin and the appearance of des-Tyr-enkephalin was in 1:1 ratio; it did not split the des-Tyr-enkephalin. Compared to other known aminopeptidases, the enzyme was less active against aminoacyl β-NA: Tyr β-NA, Arg β-NA, Pro β-NA, or γ-endorphin. Disc gel electrophoresis revealed that the enzyme preparation contains 5 protein bands; only one of these was active when gel slices were incubated with enkephalin, and the R_f of the active band was different from that of other aminopeptidases. We observed differences between the breakdown of Met and Leu enkephalins. Bestatin, a potent aminopeptidase inhibitor, inhibited both Met and Leu enkephalin breakdown. SQ14225 had no effect on Met-enkephalin; however, it promoted Leu-enkephalin breakdown. Mn^{++} activated Met-enkephalin degradation but had no effect on Leu-enkephalin degradation. The aminopeptidase is a sulfhydryl enzyme. The effect of metals, inhibitors, the substrate specificity, and other characteristics demonstrate that this enzyme is different from leucine aminopeptidase, arylamidase, amino-peptidase, aminopeptidase A, aminopeptidase B, and aminopeptidase M. The enzyme was stable at 4°C for months but not after freezing and thawing. Its MW is 100K; the pH optimum is 7-7.5. Our results indicate that there exists in the brain a novel aminopeptidase specific for enkephalin, which has hitherto not been reported in the literature.

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261.1 THE EFFECTS OF ANESTHESIA ON THE METABOLIC RESPONSE TO CORTICAL SPREADING DEPRESSION IN THE GERBIL. A. Mayevsky, N. Zarchin* and U. Rosenshein*, Life Sciences Dept., Bar-Ilan Univ., Ramat-Gan, ISRAEL.

The present study was aimed at describing the effects of deep anesthesia on the metabolic responses to a large energy demand in the brain of gerbils. Metabolic responses were evaluated by monitoring the oxidation-reduction state of NADH by surface fluorometry in the awake or anesthetized gerbil. A large energy demand was induced by exposing the cortex to complete depolarization-spreading depression (epidural application of 0.3-0.5M KCl solution). The gerbil Meriones unguiculatus (50-60 gr) was anesthetized by Equi-Thesin (a mixture of Pentobarbital, Chloral hydrate, Magnesium sulfate, Propylene glycol and Alcohol) for the duration of the operation (0.3 ml/100 gr). The skull was exposed and drilled for the location of a light-guide holder, push-pull cannula (KCl application) and for ECoG electrodes. After cementation, the animal was connected to a DC fluorometer/reflectometer for the continuous monitoring of NADH redox state. The animal was exposed to 1 min N_2 breathing as a standard test for the state of the brain (Mayevsky, A., Brain Res. 140, 217, 1978). The response to spreading depression (SD) was measured at three stages: 1) 20-30 min after the end of the operation, while the gerbil was still anesthetized; 2) in the fully awake gerbil (2-3 hr after operation); and 3) after reanesthetizing the gerbil with Equi-Thesin or Pentobarbital. The results show a different response to SD in the anesthetized brain as compared to the awake state. The response to SD in the awake gerbil was a state 4 to 3 transition, which appeared as an "oxidation cycle", as was described for the rat brain model. Under deep anesthesia, the response to SD was a "reduction cycle", as was also described in the ischemic rat or gerbil brain (Mayevsky, A., Brain Res. 140, 217, 1978; Mayevsky et al., Neurol. Res. 1, 213, 1980). The effects of anesthetics were found only in the gerbil, but not in the rat, even when very deeply anesthetized. From our study we concluded that deep anesthesia in the gerbil decreases the ability of the cerebral circulation to compensate for the extra oxygen usage during SD, so that a reduction of NADH was recorded. In other studies (Freidli, Mayevsky and Mela, unpublished results) we found that under deep anesthesia induction of SD led to the re-uptake of K^+ from the extracellular space concomitant with the "reduction cycle".

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261.2 DECREASED INFLUX OF β-HYDROXYBUTYRATE INTO VARIOUS BRAIN STRUCTURES AFTER PORTACAVAL ANASTOMOSIS. A.M.Mans*, J.F.Biebuyck* and R.A.Hawkins, Depts. of Anes. and Physiol., The Penn State Univ., Coll.of Med., The M.S.Hershey Med.Ctr., Hershey, PA 17033

During hepatic failure in the rat, there are alterations in the blood-brain barrier transport mechanisms for neutral amino acids, leading to increased influx throughout the brain (1). The transport of substrates by other carriers may be differently affected, e.g. the brain uptake index of some monocarboxylic acids was decreased three weeks after a portacaval shunt (pcs) operation (2), while that of glucose was unchanged (3). The location of these changes to brain structures is important for a better understanding of the physiology of the blood-brain barrier in both health and disease. Therefore we have studied the influx of β-hydroxybutyrate, which is transported by the monocarboxylic acid carrier, into the brain of rats 7 weeks after pcs or sham-operation (control). The method used was modified from an autoradiographic technique previously used to study amino acid transport, and which enables measurement of influx into virtually any brain region in vivo. We found a decrease in influx in all 32 brain areas studied ranging from 40 to 50%. The mean values (± SEM) for some regions are shown in the table.

	Influx (nmol.min^{-1}g^{-1})		
	Control(6)	Pcs(6)	% decrease*
Frontal cortex	6.07 ± 0.60	2.54 ± 0.56	58
Parietal cortex	5.88 ± 0.63	2.54 ± 0.52	57
Amygdala	4.54 ± 0.56	1.98 ± 0.52	56
Hippocampus	3.88 ± 0.59	2.10 ± 0.51	46
Hypothalamus	3.64 ± 0.49	1.46 ± 0.45	60
Anterior thalamic nuclei	5.24 ± 0.72	2.84 ± 0.60	46
Medial geniculate	4.81 ± 0.86	1.99 ± 0.56	59
Interpeduncular	5.11 ± 0.59	2.41 ± 0.33	53
Superior colliculus	6.06 ± 0.76	3.21 ± 0.73	47
Inferior colliculus	5.78 ± 0.94	2.39 ± 0.61	59
Cerebellar gray	3.92 ± 0.70	1.64 ± 0.49	58
Cerebellar vermis	5.36 ± 0.65	2.13 ± 0.62	60

*$p<0.05$

The concentration of β-hydroxybutyrate in the plasma was not altered (control:222 ± 16 nmol/ml; pcs:272 ± 51 nmol/ml). Since other plasma substrates for this carrier were also unchanged at 7 weeks after pcs, the decrease in influx indicated an alteration in the characteristics of the transport carrier which occurred throughout the entire brain. These findings, together with other studies, indicate that very specific changes in blood-brain barrier transport occur during hepatic failure.

1. Mans et al., Trans. Am. Soc. Neurochem. 11:222, 1979.
2. Sarna et al., Brain Res., 160:69, 1979.
3. James et al., Science 200:1395, 1978.

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261.3 AGE-ASSOCIATED DECREASE IN LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) IN THE BEAGLE. A.S. Garden*, M. Ohata*, S.I. Rapoport and E.D. London. Lab. of Neurosciences, Nat'l. Inst. on Aging, GRC, Balto. City Hosp., Baltimore, MD 21224.

The ^{14}C-2-deoxy-D-glucose (DG) method of Sokoloff et al. (J. Neurochem. 28:897,1977) allows measurement of local cerebral glucose utilization (LCGU) within individual brain regions. Using the DG method, we showed that LCGU in the brain of the Fischer 344 rat increases between the ages of 1 and 3 mo, decreases between 3 and 12 mo, and remains unchanged between 12 and 24 mo and 24 and 34 mo (London et al., J. Neurochem., in press). In the present investigation, we studied LCGU in the beagle at the ages of 1 yr and 10-11 yr. We chose to study the dog because it shows senescence-associated neuropathology similar to that seen in man (Wisniewsky et al., Lab. Invest. 23: 287, 1970); age-associated neuropathology in the rat is minimal.

DG (50 μCi/kg) was injected i.v. in conscious dogs that were adapted to a harness. Radioactivity in timed arterial plasma samples was measured by liquid scintillation spectroscopy. After 45 min, the brain was removed and dissected into 24 regions for which radioactivity was also measured by scintillation spectroscopy. LCGU was calculated with the kinetic constants for transport and metabolism of DG in the rat (Sokoloff et al., 1977) and with a lumped constant of 0.558 for the beagle (Duffy et al., Trans. Am. Soc. Neurochem. 10:171, 1979). Between the ages of 1 and 10-11 yr, LCGU decreased in every brain region assayed. Most striking decreases were in some white matter areas (corpus callosum excluding the genu, anterior commissure, internal capsule), where LCGU fell by 50-60%. Other white matter areas showed smaller decreases (genu of corpus callosum, 29%; cerebellar white matter, 11%). LCGU fell by approximately 40% in the geniculate nuclei and inferior colliculus, by 20-30% in the superior colliculus and cortex on either side of the calcarine fissure, and by 22% in the olfactory bulb. These decrements may be related to a loss of sensory function with age. Structures involved in limbic function, such as the hippocampus, cingulate cortex, temporal tip, and amygdala, showed decreases in LCGU of approximately 25-30%, but the decline in the mammilary body was 14% and that in the pyriform cortex was only 4%. In gray matter areas involved in motor function, such as the caudate nucleus and cerebellar flocculus, decrements were 25-35%. Other regions which showed decreases in LCGU are: the hypothamus (24%), thalamus less geniculate bodies (31%), midbrain basis and tegmentum (30%), pons (37%) and medulla (22%). Thus, although there is an overall decrease in LCGU with age in the beagle brain, this decrease is not uniform but has a regional distribution that probably is associated with regional deficits in brain function.

261.4 AGE-ASSOCIATED ALTERATIONS IN REGIONAL CEREBRAL BLOOD FLOW IN THE FISCHER-344 RAT. M.Ohata*, U.Sundaram*, W.R. Fredericks*, E.D. London and S.I. Rapoport. Lab. of Neurosciences, Nat'l. Inst. on Aging, GRC, Balto. City Hosp., Baltimore, MD 21224

Regional cerebral blood flow (rCBF) is related to cerebral metabolism and is a measure of neuronal functional activity. rCBF was measured in specific brain regions of the conscious unanesthetized Fischer-344 rat at the ages of 1 mo, 3 mo, 12 mo, 24 mo or 34 mo. ^{14}C-Iodoantipyrine was infused i.v., and radioactivity in timed arterial blood samples was measured by liquid scintillation spectroscopy. The brain was removed and dissected into 14 regions for which radioactivity was also measured by scintillation spectroscopy. rCBF was calculated by applying the Kety-Schmidt analysis to the data (Sakurada et al., Am. J. Physiol. 234, H59, 1978; Ohno et al., Stroke, 10, 62, 1979). In 1 mo old rats, the mean rCBF in 9 anterior brain regions was 92 ml·100g^{-1}·min^{-1}. It was 76 ml·100g^{-1}·min^{-1} in 4 posterior brain regions. rCBF increased significantly ($P < 0.05$) in the 9 anterior regions between 1 and 3 mo of age, and continued to increase after 3 mo to a mean of 132 ml·100g^{-1}·min^{-1} at 12 mo, but did not change significantly in the posterior brain regions between 1 and 12 mo. rCBF tended to decline between 12 and 24 mo, more consistently in the posterior than anterior brain regions. White matter rCBF did not change with age. The findings indicate that rCBF increases during development and maturation of the anterior portion of the rat brain, between 1 and 12 mo. The posterior part of the brain, which is more mature than the anterior brain by 1 mo, shows no significant changes in rCBF between 1 and 12 mo. The decline in rCBF in some brain regions after 12 mo of age may reflect some of the degenerative changes that occur within the brain from 12 mo to death at 36 mo.

rCBF does not follow the same temporal pattern during maturation and aging of the rat brain as does local cerebral glucose utilization (LCGU). LCGU increases as does rCBF between 1 and 3 mo, but then falls by 24% between 3 and 12 mo while simultaneously rCBF continues to rise (London et al., J. Neurochem., in press). The parallel courses of rCBF and LCGU between 1 and 3 mo probably are related to developmental changes in the rat brain, which is not fully mature until 4 mo of age. However, the simultaneous rise in rCBF and fall in LCGU between 3 and 12 mo are unexplained. They may be due to an altered coupling relation between rCBF and cerebral metabolic rate of oxygen consumption. The dissociation between the time courses of rCBF and LCGU during maturation and aging of the rat brain, which is reported here for the first time, suggests that rCBF and LCGU are not indices of the same functional parameters.

261.5 EFFECT OF OXOTREMORINE, A MUSCARINIC AGONIST, ON LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) IN THE RAT. D. Dow-Edwards, J.M. Peterson*, P. Mahone*, S.I. Rapoport and E.D. London. Lab of Neurosciences, Gerontology Research Center, National Institute on Aging, Baltimore City Hospitals, Baltimore, MD 21224.

The distribution of specific muscarinic receptor binding sites in the rat brain has been described with the use of radioligand binding and autoradiographic techniques (Yamamura and Snyder, P.N.A.S. 71:1725, 1974;Kuhar and Yamamura, Brain Res. 110:229, 1975; Rotter, A. et al., Brain Res. Rev. 1:141,1979). In order to assess the functional significance of these binding sites, we employed the autoradiographic ^{14}C-2-deoxy-D-glucose (DG) technique of Sokoloff et al. (J. Neurochem. 28:897, 1977) to study local cerebral glucose utilization (LCGU) in the conscious rat. Oxotremorine (OXO), a potent muscarinic agonist, was given i.p. (0.75 mg/kg) 2 min before i.v. DG (125 µCi/kg) and the rats were killed 50 min after DG administration. Although many regions showed no changes in LCGU following OXO, LCGU was increased significantly ($p < 0.05$) in the sensory-motor and parietal cortices, the hippocampus, globus pallidus, caudate nucleus, nucleus accumbens, medial and lateral septal nuclei, olfactory bulb, and cerebellar gray matter. Several diencephalic nuclei showed increased LCGU; these include the ventral and anteroventral thalamic nuclei, the lateral geniculate nucleus and the lateral habenula. In the mesencephalon, the red nucleus and superior colliculus showed 2-fold increases in LCGU, and a 50% increase was noted in the substantia nigra pars compacta. In every region where OXO caused an increase in LCGU, this increase was blocked by the antimuscarinic drug scopolamine (2.5 mg/kg, i.p., 10 min before OXO) but not by atropine methylbromide (1 mg/kg, s.c. 20 min before OXO), which does not readily penetrate the blood-brain barrier. Scopolamine (2.5 mg/kg i.p.) without oxotremorine had no significant effect on LCGU. These findings indicate that increased LCGU in response to oxotremorine requires stimulation of central muscarinic receptors and is not dependent upon peripheral muscarinic stimulation.

We note no simple correlation between the magnitude of increase in LCGU following OXO administration and the reported densities of high affinity muscarinic binding sites. Thus, LCGU following specific pharmacologic stimulation depends on the presence of relevant receptors for the administered drugs and on the neuronal circuitry of that brain region.

261.6 **NEURAL ACTIVITY DURING HIBERNATION: APPLICATION OF THE ^{14}C DEOXYGLUCOSE TECHNIQUE.** **T.S. Kilduff, F.R. Sharp and H.C. Heller*. Dept. Biol. Sci., Stanford University, Stanford, CA 94305 and Dept. Neurology, Univ. Calif. Med. Cntr., San Diego, CA 92103.**

Knowledge of neural activity during hibernation is derived from EEG recordings from a limited number of brain areas. We have utilized the ^{14}C deoxyglucose technique of labelling tissues according to their glucose utilization to provide a more global perspective of brain function during deep hibernation and arousal from hibernation. Golden-mantled ground squirrels (Citellus lateralis) were implanted with chronic jugular catheters and polyethylene re-entrant tubes; some animals were also prepared with thermodes straddling the hypothalamus. The re-entrant tubes enabled introduction of a thermocouple subcutaneously to measure body temperature during experiments. The ^{14}C deoxyglucose was injected through the catheter (15uCi/100g body wt) during deep hibernation, arousal from hiberation, euthermia, and with thermogenesis stimulated by cooling of the skin or hypothalamus. After an incubation time appropriate to the particular experiment, brains were removed, sectioned in a cryostat and exposed to X-ray film. The optical density of 83 neural structures represented in the resultant autoradiographs was measured and these values were compared to the density of a white matter structure such as the optic tract. Analysis of this relative measure of glucose utilization revealed decreases during deep hibernation compared to euthermic controls in all gray matter structures examined except the lateral septal area and the paraflocculus; the greatest decreases of relative glucose utilization were found in the inferior colliculus (63%), cerebellar nuclei (54%), and mammillary body (50%). The predominant band of activity thought to be lamina IV in the auditory, somatosensory and visual cortex in the euthermic animals was not evident in deep hibernation. Areas showing little or no decrement in relative activity during deep hibernation include the cochlear nuclei, superior colliculus, nucleus of the olfactory tract, suprachiasmatic nucleus and, as mentioned above, the lateral septal area. As animals aroused from hibernation, an increase in relative activity was noted in the medullary reticular formation and dorsal horn of the spinal cord. A distinct lamina IV was evident by the time body temperature had reached 15°C. In addition, structures such as the habenula and mammillary body showed an unusual pattern of glucose utilization, suggestive of discrete regions of activity.

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261.7 ADENINE NUCLEOTIDE PROTECTION AGAINST LETHAL HYPOXIC HYPOXIA IN RODENTS. B.J. Kraynack, L.L. Kraynack*, J. Hinshaw*, J. Gintautas. Anesthesiology Research Laboratories, Texas Tech University Health Sciences Center, 3601 4th Street, Lubbock, Texas 79430.

Adenosine Triphosphate (ATP) (200/mg/kg) intraperitoneally (IP) administered significantly increased survival time (ST) in mice exposed to an otherwise lethal hypoxic atmosphere. Survival time increased 794%. Here, we compared the effects of the adenine nucleotides, nucleoside, base and cyclic nucleotide on ST. Male Swiss white mice (25-30gm) were injected IP with a constant volume of 0.9% saline while treatment groups received an IP injection of ATP (50, 100, 200 or 400 mg/kg); or one of the following drugs (200 mg/kg): 1) adenosine diphosphate (ADP), 2) adenosine monophosphate (AMP), 3) adenosine, 4) adenine phosphate, 5) dibutyryl cyclic AMP, and 6) phosphocreatine. Thirty minutes later, mice were exposed to 5% oxygen at room temperature. The interval between the introduction of the hypoxic mixture to the chambers and the last respiratory effort was measured for each animal and defined as the ST. The hypoxic observation period was truncated at 30 minutes. The percentage increase of ST for AMP, ADP and ATP are shown in Table 1. The other agents were ineffective. ATP (200 mg/kg) increased ST by 775% and was the most effective dose (and agent) tested. In rats, centrally administered ATP, ADP and AMP (500 µg) significantly increased ST. Although the physiological mechanism by which the adenine nucleotides promote resistance to lethal hypoxia remains undefined, these findings suggest that protection may not be related to an energy transfer mechanism. Moreover, while we can not entirely exclude a peripheral mechanism, the data indicate that the nucleotides probably exert their effect directly on the central nervous system.

Drug	% Increase ST over Saline
Saline	---
AMP	439
ADP	491
ATP (50 mg/kg)	178
(100 mg/kg)	188
(200 mg/kg)	775
(400 mg/kg)	399

This work was supported by the American Society of Anesthesiologists and Parker B. Francis Foundation.

261.8 RESPONSES OF CEREBRAL OXYGEN WAVES TO CHANGES IN BLOOD PRESSURE IN RABBIT. Jack P. Douglas; George J. Niemirowski*; Scott Hall; Robert G. Grossman. Division of Neurosurgery, UTMB, Galveston, Texas 77550.

Cortical oxygen tension, measured continuously using polarized platinum electrodes, has previously been observed to oscillate rhythmically. These oscillations are thought to be produced by rhythmic contraction and dilation of the cerebral vasculature, and may play a role in control of blood flow (Folkhow, 1962). We propose that the cerebrovascular network contains intrinsically oscillating series and parallel vascular elements that rhythmically control the amount of blood flowing through a given volume of tissue. We have investigated the changes in reactivity and participation of the various elements along the vascular tree following changes in mean systemic blood pressure (MSBP) in awake and anesthetized rabbits. Two platinum disk electrodes were implanted over the cortical surface of each hemisphere in homologous zones to monitor oxygen availability (aO_2) and for measurement of cerebral blood flow (CBF) using H_2 clearance. At least two weeks were allowed to pass prior to experiments to allow CBF and aO_2 to stabilize. In the awake, unmedicated animal oxygen was found to fluctuate in a complex yet sinusoidal fashion, sometimes very regularly. Power spectral analysis (PSA) showed dominant frequency peaks at each location which, in the resting state, were stable over time. Characteristic peak frequencies ranged from 0.15 to 0.20 Hz. Wave amplitudes were less than 5% of resting oxygen levels. Increases in MSBP, produced by infusion of angiotension II, decreased amplitude, increased peak PSA frequency and desynchronized the signal. CBF autoregulated up to the highest blood pressure obtained (MSBP=145 mm Hg). Hypotension produced by blood removal, on the other hand, caused a significant slowing of the dominant wave frequency, often by 50% from normotensive conditions, and increased wave amplitude. Waves often became more regular at low arterial pressures with ipsilateral and then bilateral wave synchrony occurring. At MSBP near 45 mm Hg, waves abruptly disappeared, and reappeared when pressure slowly increased. If pressure remained low for longer than five minutes, waves remained inhibited for periods up to 30 minutes. These observations suggest multiple vascular pacemaker sites which participate in a differential response to changes in blood pressure. Enhanced bilateral synchrony suggests large basal vessels dominate in responding to lowered pressure, possibly because arterioles are already maximally dilated, while all elements participate at elevated pressures. In addition, waves abruptly collapse at pressures near or lower than those pressures where CBF autoregulation is maintained.

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261.9 MERCURY VAPOR TOXICITY IN THE CENTRAL NERVOUS SYSTEM OF RODENT. Yam S. Tong* and Chakwan Siew. Res. Inst., Amer. Dental Assn. Hlth. Fdn., Chicago, IL 60611.

Elemental mercury (Hg) toxicity is generally believed to be associated with the central nervous system (CNS). Ethanol, one of the most commonly used CNS affecting drugs, was employed to probe the effect of Hg vapor on mice. Male Swiss-Webster mice were exposed to mercury vapor (0.2 mg/m^3, 24 hr/day, 7 days) in specially designed chambers. Ethanol sleep time was determined prior to and post Hg exposure. The results indicate that chronic low-level Hg exposure prolongs significantly the ethanol sleep time (Hg exposed mice, 45.7±2.2 vs. control 24.7±2.2 min $p<0.05$). However, there was no signfiicant difference between the two groups in blood ethanol levels which were measured when the mouse awoke. The brains of the exposed mice were then homogenized in 5mM Tris-HCl, pH 8.6. After centrifugation at 100,000 x g for one hour, the majority of the Hg was found bound to the soluble protein(s). The supernatant was exhaustively dialyzed against the same buffer before being chromatographed on a Sephadex G-200 column. The gel filtration column yielded two Hg peaks. The major one was eluted right after the void volume and overlapped with a major protein peak. The second Hg peak came out at a much later volume. The profile obtained from Hg-exposed Sprague/Dawley rats is very similar. When an aliquot of the dialyzed supernatant was brought to 2% sodium dodecyl sulfate (SDS) and then dialyzed against 0.1% SDS, no significant decrease in the Hg content of the sample was detected. This equilibrium dialysis study indicates that the Hg is most likely covalently bound to protein. The biotransformation of metallic Hg to ionic Hg, a form most commonly involved in specific binding with proteins, may possibly depend on the action of the enzyme catalase, which has been suggested to be responsible for an oxidation-reduction cycle of Hg in mammalian tissues. The nature of the binding as well as the identity of the Hg-bound protein(s) are being investigated. (Supported in part by grants from BRSG, RR 05889 and American fund for Dental Health.)

261.10 COLCHICINE: UPTAKE INTO BRAIN AND LONG-TERM MEMORY. E. Bennett, M.H. Alberti*, and J.F. Flood*+. Lab.Chem.Biodynamics, Lawrence Berkeley Lab., Berkeley, CA 94720 and Dept. of Psychology and Psychiatry, UCLA, Los Angeles, CA 90024.

Numerous experiments have shown that inhibitors of protein synthesis including anisomycin (ANI) and cycloheximide impair long-term memory formation in a variety of species. It has been suggested that microtubules may be involved in the transport of essential protein from the cell body to synaptic and dendritic endings. These transported proteins then produce long-lasting changes in membrane structure or cell morphology which are presumed necessary to establish long-term memory. Under training conditions where neither inhibitors of protein synthesis nor inhibitors of axoplasmic flow alone produce amnesia, we have shown that the combined peripheral administration of ANI and Colchicine, vinblastine, or podophyllotoxin will impair long-term memory formation in mice [Amer. Soc. Neurochemistry, Trans., 10, 220 (1978)]. The initial objective of the biochemical experiments reported here was to estimate the amount of colchicine entering mouse brain under the conditions of the behavioral experiments. When [ring A-4-^{3}H]-colchicine (2 µg) was administered, approximately 5% of the radioactivity was found in liver, but less than 0.01% was found in brain. About 40-50% of the radioactivity in brain was precipitated by vinblastine. This uptake into brain was considerably less than was anticipated based upon a report concerning the uptake of methoxy-labelled colchicine into rat brain by Stewart and Rose [J. Neurochem., 30, 595 (1978)]. We therefore compared the effect of species and position of radioactive label upon brain uptake of colchicine. A large species influence on uptake into rat or mouse was not found, but a major effect of label position was observed. Much more radioactivity was found in brain after administration of [ring C-methoxy-^{3}H]-colchicine than after administration of [ring-A-4-^{3}H]-colchicine. Most of the radioactivity in brain after administration of methoxy-labelled colchicine was not precipitated by vinblastine and appeared to be metabolic water. Experiments have now shown when ANI is administered prior to training and as little as 30 pg of colchicine is injected bilaterally into caudate shortly after training, that amnesia for active avoidance training is obtained in a significant proportion of the mice. This (30) pg is approximately equivalent to the amount of colchicine entering the brain after subcutaneous administration of 2 µg of colchicine. Taken together, these experiments lend support to the above hypothesis that structural alterations in synapses brought about by recently synthesized protein transported down the axons of dendrites may be an essential process for long-term memory formation.
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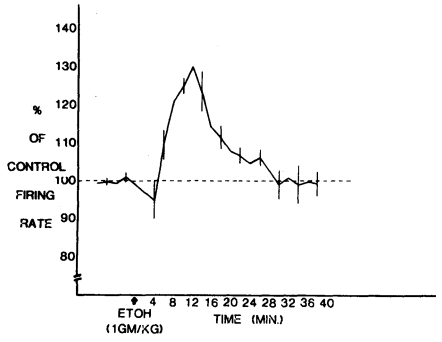
262.1 DIFFERENTIAL EFFECTS OF LOCAL AND SYSTEMIC ETHANOL ON RAT CEREBELLAR PURKINJE NEURONS: EVIDENCE FOR A LOCUS COERULEUS INVOLVEMENT. S.M. Sorensen, D. Carter*, J. Marwaha, R. Baker* and R. Freedman*. Alcohol Research Center, Univ. of Colorado Health Sciences Center, Denver, CO 80262, and Denver VA Hospital.

Ethanol (E), applied directly to Purkinje (P) cells using micropressure ejection, produces a dose-dependent inhibition of cell firing. When P cells were monitored continuously after an intraperitoneal injection of E (1 mg/Kg), a biphasic response was seen. An increase in firing rate to 130% of control within 12 minutes of injection is followed by a slow decline to control or below control firing rates at 30 min. (See figure below). The early excitation of the P cell following parenteral E was not seen in rats which had been pretreated with 6-hydroxydopamine to eliminate the noradrenergic (NA) afferents to the cerebellum. The excitation was also blocked by the β-adrenergic antagonist propranolol. In both these cases, after the NA input to the cerebellum is eliminated, only depression of the P cells was observed. These results suggest that the early excitation of P cells, seen after systemic E, may be due to a reduction of the tonic inhibitory input from the locus coeruleus, the source of the NA input to the cerebellum. The biphasic nature of the P cell response to parenteral E may therefore be due to an initial disinhibition followed by a direct depression of the P cell itself.



Supported by AA-03527 and DA-02429.

262.2 COMPARISON OF THE EFFECTS OF ETHANOL, TETRAHYDROISOQUINOLINES (TIQs) AND OPIATES ON NEURONAL ACTIVITY IN BRAIN: AN IONTOPHORETIC AND MICRO-PRESSURE STUDY. E.D. French*, T.W. Berger, G.R. Siggins, T.W. Shier*, and F.E. Bloom. Alcohol Research Center and A.V. Davis Center, Salk Institute, La Jolla, CA 92037.

Iontophoresis of opioid peptides predominantly depresses the discharge of neurons of most brain areas, whereas hippocampal pyramidal cells are usually excited (Nicoll et al. Proc. Natl. Acad. Sci. 74:2586, 1977). These actions are naloxone sensitive. This spectrum of actions was used as an initial comparative model to test two hypotheses: 1) that ethanol effects are mediated by an opiate-like mechanism, and; 2) that formation of acetaldehyde-catecholamine condensation products (TIQs) might contribute to some actions of ethanol. All drugs were applied to single neurons by electroosmosis or micro-pressure. Ethanol (1-3 M in the pipette) weakly inhibited most neurons of the cerebellum, caudate nucleus and parietal cortex, while 55% of presumed HPC pyramidal neurons (N=31) were markedly excited. Tetrahydropapaveroline (3 mM in the pipette) inhibited the spontaneous and glutamate- or acetylcholine (ACh)-induced firing of most (60-100%) neurons in all these regions. In contrast, salsolinol and 7-Me-salsolinol (3 mM in the pipette) excited 73 and 56%, respectively, of HPC pyramidal neurons, while depressing the activity of most parietal cortical neurons. Iontophoretic or systemic naloxone antagonized the excitatory actions of ethanol (75% of tested cells) and salsolinol (89% of tested cells) on HPC pyramidal cells, without change in the basal firing rate. The doses of naloxone used also blocked excitatory responses of HPC pyramidal cells to methionine-enkephalin; however, ACh-induced speeding was also antagonized in 1/3 of tested cells. Conversely, the antimuscarinic agent scopolamine antagonized the excitatory actions of salsolinol, but not those of met-enkephalin, in some HPC pyramidal cells.

These results show that acutely applied ethanol or salsolinol elicits a spectrum of neuronal effects in brain similar to that for opiates; namely inhibition of activity in several tested brain areas, but excitation in hippocampus. A fair percentage of these excitatory effects are antagonized by naloxone. However, the occasional non-specific effects of naloxone and the puzzling antagonism of the ethanol- or salsolinol-induced excitations by scopolamine cast doubt as to the opiate-like actions of these substances. Also, intracellular studies in the HPC slice (Pittman and Siggins, this volume) indicate that ethanol mechanisms are far more complex than for activation of opiate receptors. Supported by NIAAA (AA-03119 and AA 03504).

262.3 ETHANOL HAS MULTIPLE ACTIONS ON ELECTROPHYSIOLOGICAL PROPERTIES OF HIPPOCAMPAL (HPC) PYRAMIDAL NEURONS IN VITRO. Q.J. Pittman and G.R. Siggins. A.V. Davis Center and Alcohol Research Center, The Salk Institute, La Jolla, CA 92037.

The sequelae of alcohol ingestion indicate interactions with the CNS. This possibility is supported by reports of ethanol (EtOH)-induced alterations of EEG and single-unit activity in several brain regions, including HPC. However, dose-response relationships have not been forthcoming from in vivo work and field potential studies in HPC slices have not revealed significant direct EtOH actions at doses below those that would be fatal in vivo. To clarify this discrepancy we used intracellular recording to determine if directly-applied EtOH might selectively affect individual neurons in the HPC slice.

Hippocampi were quickly removed from 100 g rats and 400 μm transverse slices were cut and placed in a perfusion chamber where they were continuously perfused over both surfaces with warmed (35° C), oxygenated artificial CSF. The inflow system permitted introduction of drug-containing solutions without interrupting the flow of the perfusate.

Slices demonstrating large amplitude, stable field potentials in response to stratum radiatum (SR) stimulation were selected for further investigation. Stable intracellular recordings of 1-4 hrs duration were obtained from 20 CA1 neurons. In 10 of these, EtOH (10-400 mM) caused a slight but repeatable hyperpolarization (1-5 mV) whereas 4 other cells displayed weak depolarizations. Although changes in input resistance (measured by current injection) often accompanied these potential changes no obvious relationship was seen between these two properties. Also no clear relationship emerged between the concentration of ethanol and changes in membrane potential or resistance. The amplitudes of recurrent IPSPs evoked by SR stimulation were decreased in the presence of EtOH in 5 of 10 tested cells and increased in 2. SR-induced EPSPs increased in amplitude in 5 of 12 tested neurons; 3 of these increases were associated with increased input resistance and 2 with a decrease in resistance. Three of the 12 cells displayed decreased EPSP amplitude.

Many cells showed reproducible responses in membrane and synaptic properties to low EtOH concentrations (10-40 mM); nonetheless, many cells did not respond at much higher concentrations. These findings indicate that individual hippocampal neurons display marked differences in their sensitivity and responses to ethanol. This could account for the apparent insensitivity to EtOH shown by field potential studies of the HPC slice. Supported by grants from NIAAA (AA-03119 and AA-03504) and MRC (Canada).

262.4 EFFECT OF ETHANOL DEPENDENCE AND WITHDRAWAL ON GABA RECEPTOR BINDING IN RAT BRAIN. L. Volicer and T. M. Biagioni*. Edith Nourse Rogers Memorial Veterans Hospital, Bedford, MA 01730.

Altered binding of neurotransmitters to their receptors participates in mechanism of action of several psychoactive drugs. Since there is considerable evidence indicating that ethanol affects GABA-ergic system, we investigated the effect of chronic ethanol administration on GABA receptor binding.

Ethanol dependence was induced by administration of ethanol three times a day (6-12g/kg/day, per os) for 7 days. Control rats were intubated with water and the ethanol-treated rats were sacrificed 1 hour (dependent) or 16 hours (withdrawn) after the last ethanol dose. Synaptosomal membranes were prepared by differential centrifugation and were frozen and treated with Triton X-100 prior to assay. The membranes were suspended in a sodium free buffer, incubated with ^{3}H-GABA in the presence and absence of mM GABA, and separated on Whatman GF/B filters.

In the cerebral cortex specific GABA binding, determined at 13nM ^{3}H-GABA concentration, was lower in withdrawn rats than in control or dependent animals. This lowering was due to decreased receptor affinity (K_D = 22.41 ± 0.86 vs 12.65 ± 0.60 nM, p <0.001) and was prevented by repeated incubation of synaptosomal membranes with Triton X-100. It occurred also in some dependent rats which exhibited partial withdrawal syndrome between ethanol intubations. Low affinity binding was similar in control and withdrawn rats, but lower in dependent animals. This lowering was due to decreased number of low affinity receptors (0.695 ± 0.175 vs 1.565 ± 0.085 p moles/mg. of protein, p <0.005). In the cerebellum GABA binding, at 13nM ^{3}H-GABA concentration, was not significantly different in control, dependent or withdrawn rats. However, rats which were minimally affected by ethanol administration had higher binding than control or affected rats. In contrast to the ethanol intubation, feeding of rats for 7 or 20 days with a liquid diet containing 35% calories in the form of ethanol did not induce ethanol dependence and did not affect GABA receptor binding determined at 13nM ^{3}H-GABA concentration.

These results indicate that chronic ethanol administration affects both low and high affinity sodium-independent GABA binding. In the cerebral cortex the number of low affinity sites is decreased shortly after ethanol administration and returns to normal during withdrawal. Withdrawal decreases affinity of the high affinity sites possibly by increasing the amount or the binding of an endogenous inhibitor of GABA binding. Changes of GABA binding in the cerebellum indicate that compensatory changes of GABA binding might modify expression of ethanol withdrawal. (Supported by the Veterans Administration)

262.5 ALTERATION OF BRAIN BIOGENIC AMINE LEVELS AFTER CHRONIC ALCOHOL AND LITHIUM ADMINISTRATION. P.A. McGinley* and E.B. Truitt* (Spon: D.C. Riccio), Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272

Clinical studies have shown lithium to possibly be effective in reducing recidivism and depressive symptoms in alcoholism accompanied by depression (Kline et. al., Am. J. Med. Sci. 268: 15, 1974 and Merry et. al., Lancet 2:481, 1976). In an effort to find a neurochemical explanation for the observed lithium induced behavioral changes in rats maintained on chronic ethanol, brain area levels of norepinephrine (NE) and dopamine (DA) were measured in rats treated chronically with lithium chloride (1.0 and 1.5 mEq/kg) and ethanol (Lieber-DeCarli liquid diet). Following three weeks of daily injections of lithium chloride or sodium chloride (equimolar concentration; electrolyte control), the animals were maintained on the ethanol diet for seven weeks. Comparisons were made between the lithium-ethanol treated groups and those receiving lithium chloride only, sodium chloride plus ethanol and no treatment. Brains were removed and dissected according to the method of Glowinski and Iverson (Neurochem. 13: 655-69, 1966) into six major areas; cortex, corpus straitum, cerebellum, medulla, hippocampus and midbrain-hypothalmus. Each brain area was analyzed for NE and DA concentration using high pressure liquid chromatography with electrochemical detection (HPLC-EC). Significant changes in NE levels in the corpus straitum and midbrain-hypothalmus were found in the animals treated with lithium and ethanol when compared to all controls. These results corrleate with lithium-induced changes previously found in amphetamine or apomorphine-induced stereotypical behavior and reduction of audiogenic withdrawal seizures (Truitt et. al., Alc. Clin. Exp. Res. 4:231, 1980). These results suggest that lithium may alter the pattern of chronic ethanol-induced behavior and monoamine changes in the brain.

(Supported by USPHS Grant AA-03157)

262.6 HISTAMINE ANTAGONISM OF PENTOBARBITAL NARCOSIS AND HYPOTHERMIA. P.W. Kalivas*and A. Horita* (SPON: L.M. Halpern). Depts. of Pharmac. and Psychiat., Univ. of Wash., Sch. Med., Seattle, WA 98195

While a precise physiological function for central histamine (HA) is not defined, data has accumulated indicating that this biogenic amine has neurotransmitter-like qualities. Based upon EEG alterations and circadian fluctuations of central HA, a function in arousal mechanisms has been hypothesized. The pentobarbitalized rat was used to test this hypothesis. Prior to testing, male S.D. rats were bilaterally implanted with guide cannulae in the lateral ventricles. At least 7 days following surgery, animals received 45 mg/kg pentobarbital (PB), given i.p. Twenty min later animals received intraventricular (icv) injections of HA dissolved in buffered saline (pH 6.0-7.0) or buffered saline alone. Doses of 5-25 ug significantly shortened the duration of PB narcosis. Doses as low as 0.1 ug caused a significant attenuation of PB hypothermia. These effects of HA were not associated with changes in brain or plasma levels of C^{14}-PB or its metabolites. While neither H_1 nor H_2 antagonists blocked the analeptic response when given alone, 10^{-7}M cimetidine plus 10^{-7}M chlorpheniramine given icv 10 min prior to $3.4X10^{-8}$M HA (5 ug) were effective. In addition, a number of brain sites were examined for sensitivity to microinjection of HA. While the medial hypothalamic injections (5 ug/0.5 ul/60 sec) elicited the greatest analeptic response, a number of medial diencephalic and septal injection sites demonstrated sensitivity. Interestingly, the interpeduncular nucleus was very sensitive to HA antagonism of PB hypothermia, but insensitive to HA induced arousal.

262.7 INTERACTION OF BARBITURATES WITH BRAIN MEMBRANES. R. Adron Harris and F. Schroeder. Truman V.A. Hospital and Department of Pharmacology, University of Missouri School of Medicine, Columbia, Missouri 65212.

Effects of barbiturates on the physical properties of synaptic plasma membranes (SPM) and myelin prepared from mouse brain were studied after incorporation of fluorescent probes. Absorbance-corrected fluorescence output (CO), polarization of fluorescence (P) and membrane absorbance (AB) were determined (JBC 251:6747, 1976; BBA 511:356, 1978). Diphenylhexatriene (DPH) was used to monitor the hydrophobic membrane core while 1-aminopyrene (1-AP) was used to probe the membrane surface. In vitro addition of pentobarbital (0.1 to 1.0 mM) decreased the polarization (P) of DPH in SPM but did not affect P of DPH in myelin. Lipids were extracted from SPM and phospholipids were separated by silicic acid chromatography. Vesicles prepared from the total lipid extract of SPM were not affected by pentobarbital. However, the P of DPH was increased by pentobarbital in vesicles prepared from SPM phospholipids. Membrane rigidity, as determined by P of DPH, followed the order: intact SPM>total lipid>phospholipid. These results suggest that barbiturates fluidize the more rigid membranes (or membrane domains) yet increase the rigidity of very fluid membrane areas. This postulate was supported by results from experiments in which membrane fluidity and barbiturate effects were altered by changes in temperature. The fluorescence of 1-AP bound to SPM was not affected by pentobarbital, indicating that the predominant effect of the drug involves the hydrophobic core of the membrane. The effect of barbiturates on P of DPH in SPM was stereoselective as the R(+) isomers of pentobarbital and secobarbital were more potent than the S(-) isomers. P was decreased by R(+)-pentobarbital at concentrations as low as 45 μM. Barbiturates also decreased CO of DPH and membrane AB. These effects were not stereoselective and were found with both myelin and SPM. These effects probably reflect changes in membrane physical properties other than fluidity (e.g. polarity, refractive index, membrane thickness). In summary, barbiturates appear to fluidize the hydrophobic inner core of synaptic membranes. This effect is stereoselective and is not found with myelin membranes. Barbiturates also alter other membrane properties, but these effects are not selective for synaptic membranes or for the optical isomers. The fluidization of synaptic membranes by barbiturates, which is similar to that produced by ethanol (Fed. Proc. 39:745, 1980), may produce alterations in synaptic transmission which are ultimately expressed as barbiturate intoxication. (Supported in part by funds from the Medical Research Service of the Veterans Administration, the National Cancer Institute (CA24339), and the American Heart Assoc.(78-734)

262.8 THE EFFECT OF LESIONS IN THE DORSAL AND MEDIAN RAPHÉ NUCLEI ON THE DEVELOPMENT OF TOLERANCE TO ETHANOL-INDUCED HYPOTHERMIA. A.D. Lê*, J.M. Khanna, H. Kalant* and A.E. LeBlanc*. Dept. of Pharmacology, Univ. of Toronto, Toronto, Canada M5S 1A8, and Addiction Research Foundation of Ontario, Toronto, Canada M5S 2S1.

Previously we have reported that general manipulation of whole brain serotonin (5-HT) plays a role in the development of ethanol tolerance (Psychopharmacology, 67:143-146, 1980). In this study, we examined specific 5-HT pathways to further elucidate the role of 5-HT in ethanol tolerance. Electrolytic lesions were made in the median (n = 18), the dorsal (n = 18) and the dorsal plus median (n = 18) raphé nuclei of rats. A fourth group (n = 18) of sham-operated animals was also prepared. After one week of recovery from surgery, each group was tested with a standard test dose of ethanol (3 g/kg). Rectal temperatures were determined by means of a telethermometer fitted with a YSI recorder. The temperature was monitored at 0, 30, 60 and 90 min with a probe inserted 5 cm into the rectum. The maximum fall in temperature (ΔT max) was used to quantify the ethanol effect. The animals in each main treatment group were then divided into two subgroups matched on the basis of their maximum hypothermic response, and were designated to receive either daily treatment with ethanol (5 g/kg, p.o.) or calorically equivalent amount of sucrose. Tolerance to the ethanol-induced hypothermia was assessed at intervals of 5 days for 30 days.

Lesions of the dorsal raphé nucleus produced a negligible effect on the development of ethanol tolerance. Lesions of the median raphé nucleus delayed the development of tolerance. Combined lesions of the median plus dorsal raphé nuclei did not significantly increase the effect produced by the lesions of the median raphé nucleus alone. Biochemical analysis confirmed the differential depletion of 5-HT by the various lesions. These results indicate that the 5-HT pathway originating from the median raphé nucleus is important in the development of tolerance to ethanol.

262.9 DISCRIMINATIVE STIMULUS PROPERTIES OF ETHANOL: THE ROLE OF ACETALDEHYDE AND THP. Shippenberg, Toni S.*, Amirian, James H.*, Altshuler, H. L. Department of Neuropsychopharmacology, Texas Research Institute of Mental Sciences and Department of Pharmacology, Baylor College of Medicine, Texas Medical Center, 1300 Moursund, Houston, Texas 77030.

Ethanol (ALC) serves as a discriminative stimulus (DS) for rats in a double lever drug discrimination (DD) paradigm at doses of 0.6 gm/kg or greater. Our previous studies demonstrate that animals trained to discriminate intraperitoneally (IP) administered ALC from IP saline (SAL) are unable to perform that discrimination following central administration. This study evaluated the contribution of acetaldehyde and a tetrahydroisoquinoline (TIQ), tetrahydropapaveroline (THP) to the DS properties of ALC. 95 male Sprague Dawley rats ranging in weight from 300-450 gms. were subjects in these studies. The animals were shaped to perform ALC-SAL DD in a double lever discrimination paradigm on a DRL-10 reinforcement schedule following either intraperitoneal (IP) or central intraventricular (ICV) dosage. ICV testing of the DS properties of ALC confirmed our previous finding that ICV ALC (1.0 - 1500 μg) does not serve as a DS in rats trained to discriminate IP ALC from IP SAL. Further acetaldehyde (AcALD) administered either centrally (0.05 - 10.0 mg) or peripherally (1.0 - 50.0 mg) does not generalize to ALC in animals trained with IP ALC. A second group of rats were trained to discriminate centrally administered ALC from centrally administered SAL. Although 250 μg ALC is an effective DS, these experiments revealed that the period of training required to establish ALC as a central DS exceeds the usual functional life of the intraventricular cannula. The results of central training were highly variable. The stimulus properties of THP were assessed in IP ALC trained rats. THP was not found to be an effective DS in that group, although the results were highly variable. The results of these studies confirm our earlier studies that the DS cue associated with ALC does not appear to be mediated centrally. These data strongly suggest that the DS property of ALC is a peripheral phenomenon resulting from the irritating effects of ALC following IP dosage.

263.1 PURIFICATION OF CALMODULIN FROM MAMMALIAN NERVE. Z. Iqbal and S. Ochs. Dept. of Physiology and Medical Biophysics Indiana University School of Medicine, Indianapolis, IN 46223 USA

Calmodulin, a calcium-binding protein, has been shown to activate a wide range of enzymes with low levels of Ca^{2+} present. These include cyclic nucleotide metabolizing enzymes, protein kinases and Ca-Mg-ATPase. It participates in the dissociation of microtubules and could be involved directly in the mechanism underlying axoplasmic transport. It is present in mammalian nerve (Iqbal and Ochs, J. Neurobiol. 11:311-318, 1980) and it activates tubulin associated Ca-ATPase (Ochs and Iqbal, These Proceedings). In this communication we describe a simple procedure for the purification of calmodulin from mammalian nerve. Frozen dog nerves were boiled and homogenized in a 10 mM Tris HCl-buffer at pH 7.5 containing 0.1 mM each of 2-mercapto-ethanol and ethylene glycol-bis-(B-aminoethyl ether) N,N'-tetra acetic acid (EGTA) (buffer A). The heat stable proteins remaining in the supernatant after centrifugation at 50,000 x g for 30 min were subjected to 50% saturation with $(NH_4)_2SO_4$. The proteins soluble in 50% saturated $(NH_4)_2SO_4$ were separated by centrifugation and brought to a pH of 3.5 with acetic acid and the contents centrifuged to isolate precipitated proteins. The pellet was solubilized by dilution and by increasing pH to 7.5 and dialyzed extensively against buffer A. Final purification of the calmodulin was performed by affinity chromatography on Sepharose-4B columns using 2-chloro-10 (3-aminopropyl) phenothiazine hydrochloride (CAP) as a ligand employing the procedure of Jamieson and Vanaman (Biochem. Biophys. Res. Commun. 90:1048, 1979). Calmodulin binds to CAP in the presence of Ca^{2+} and it can be eluted from the column with EGTA containing buffers. The identification of the calmodulin in the various fractions eluting from the column was performed by the activation of calmodulin-dependent cyclic nucleotide phosphodiesterase (PDE) isolated either from cat brain or dog nerve. The yield of calmodulin obtained after affinity column chromatography was determined by protein estimation (Lowry's method) to be approximately 1 mg/100 g of frozen dog nerves. Purification of the calmodulin was shown by a single protein band on 12.5% acrylamide gels containing SDS and by thin layer isoelectrofocusing on acrylamide gels. Submicromolar concentrations of the purified calmodulin were able to activate PDE to half maximum level and the activation was prevented by trifluorperazine, also in submicromolar levels. Supported by NIH RO1 8706-11, NSF BNS 79-14029 and the MDA.

263.2 N-METHYLATION OF CALMODULIN IN BRAIN EXTRACTS. A. Sitaramayya*, T.J. Murtaugh*, L.S. Wright* and F.L. Siegel. Depts. of Pediatrics and Physiological Chemistry, University of Wisconsin, Madison, WI 53706.

Calmodulin (CaM) has considerable amino acid sequence homology with troponin C (TnC), but whereas TnC is not methylated CaM contains a simple residue of trimethyllysine (TML), and has also been reported to undergo carboxymethylation; the significance of calmodulin methylation is not known. Brain extracts incubated with S-adenosyl-L-(^{3}H-methyl)-methionine methylate several peptides, the major products having molecular weights of 17K and 35K daltons. Electrophoresis in SDS and native gels in the absence and presence of 2mM EGTA confirm the identity of the 17K peptide as calmodulin. Treatment of the in vitro methylation products with base prior to assay removes carboxymethylase groups, allowing one to estimate N-methylation. The enzyme responsible for the N-methylation of calmodulin has been partially purified and characterized; trimethyllysine has been shown to be the amino acid residue of CaM formed by the action of this enzyme, calmodulin lysine N-methyltransferase (CLNMT). CLNMT is present in brains of all mammals studied but is absent in both electroplax of E. electricus, a tissue particularly rich in CaM, and in other primitive animals and plants. Supported by NS 11652.

263.3 EVIDENCE THAT ACTIN-LIKE PROTEIN 'C' IS PHOSPHORYLATED PRESYNAPTICALLY. R. Hofstein* and M. Hershkowitz. Isotope Department, The Weizmann Institute of Science, Rehovot, Israel.

An actin-like protein named protein 'C', was identified in synaptosomal plasma membranes and found to be phosphorylated under control of calcium and magnesium (Hershkowitz, M., Biochim. Biophys. Acta, 542: 274, 1978; Hofstein R., et al. Biochim. Biophys. Acta, in press). It was proposed that the phosphorylation of protein 'C' is a step in neurotransmitter release. To test this possibility we investigated whether during development, there is correlation between the appearance of presynaptic terminals and the phosphorylation of protein 'C'. On the first day of life, protein 'C' is already present in synaptosomal plasma membranes but it does not undergo detectable phosphorylation until the third day of life. Its maximal phosphorylation is evident by the second week of life. 6-hydroxydopamine (6 OHDA) was used to test whether protein 'C' is localized presynaptically. Intraventicular injection of 6 OHDA into adult rats led to a selective reduction of the phosphorylation of protein 'C' by 30% and 20% in hippocampus and cortex, respectively.

Subcutaneous injections of 6 OHDA into neonatal rats, led to the following changes in the hippocampus: a) reduction by 90% of ^{3}H-noradrenaline (NA) uptake; b) enhancement by 90% in the binding of ^{3}H-dihydroalprenolol (DHA); and c) specific reduction of 60% in the phosphorylation of protein 'C'.

On the other hand, in the brain stem the following changes were observed: a) increase by 16% in the uptake of ^{3}H-NA; b) decrease by 20% of the binding of ^{3}H-DHA and c) increase in the phosphorylation of protein 'C' by 30%.

These results indicate that the phosphorylation of protein 'C' is correlated with the appearance of presynaptic terminals and with their integrity.

263.4 INCORPORATION OF ^{3}H-AMINO ACIDS INTO AXONAL PROTEIN OF DECENTRALIZED AND INTACT AXONAL FIELDS OF GOLDFISH RETINAL EXPLANTS. E. Koenig and P. Adams*. Div. Neurobiology, Dept. Physiology, SUNY/Buffalo, N.Y. 14214.

The goldfish retinal explant system, developed by Landreth and Agranoff (Brain Res., 118:299-303, 1976), is a favorable in vitro system to study the question of endogenous protein synthesizing activity of regenerating vertebrate axons. The explant produces retinal ganglion cell axon fasicles free of ensheathment and non neural cells that extend several mm out from the explant. Incorporation of ^{3}H-amino acids into axonal protein can be assessed in either isolated decentralized fields or in axons retaining their central connections. Protein content of individual axonal fields was determined by direct quantitative microanalysis, and tritium radioactivity was measured in a low background system (< 1 cpm) after combustion of microsamples. A mixture of ^{3}H-amino acids was used to assess endogenous axonal protein synthesis. ^{3}H-amino acids are incorporated into axonal protein of decentralized fields and the incorporation is inhibited 90% by cycloheximide (1 mM). The presence of unlabelled amino acids at the time axons are severed from the explant suppresses subsequent incorporation for 2-3 hrs. Ca^{++} present in the medium at the time of axotomy, diminishes the rate of protein synthesis progressively. Gel microelectrophoresis of axonal proteins shows evidence of proteolytic breakdown in axons 3 hrs after decentralization. Axons decentralized with Ca^{++} present exhibit much more degradation. On the basis of various agents that block axoplasmic transport, radioactive products from ganglion cells do not begin to appear in axonal fields for at least 2 hrs. Preliminary studies with the intact explant system show that Co^{++} (1.8 mM) blocks incorporation into retinal ganglion cells (90%), without affecting incorporation into axonal protein significantly. These studies indicate that optic nerve axons of the goldfish regenerating in culture are capable of endogenous protein synthesizing activity in both the intact and decentralized states.

Research was supported by grants NS 04656 and BNS 77-24886.

263.5 THE PREPARATION OF BIOLOGICALLY ACTIVE MESSENGER RNA FROM HUMAN POSTMORTEM BRAIN. J.M. Gilbert, B.A. Brown,* P. Strocchi,* E.D. Bird and C.A. Marotta. Laboratories for Psychiatric Research and The Lowell Laboratories, Mailman Research Center, Harvard Medical School, McLean Hospital, Belmont, MA 02178.

Messenger RNA (mRNA) was extracted from two specimens of human postmortem brain tissue by alkaline phenol extraction of polysomes followed by oligo (dT)-cellulose chromatography. The mRNA preparations stimulated protein synthesis in a cell-free system containing wheat germ homogenate. The products of protein synthesis were analyzed by one- and two-dimensional gel electrophoresis. These analyses indicated that numerous polypeptides were synthesized by the human mRNA including tubulin subunits and actin isomers. The molecular weight range of polypeptides synthesized by human mRNA fractions from two brains were identical; and analysis by two-dimensional gel electrophoresis indicated qualitatively similar products. The yield of mRNA extracted per gram of human tissue was less than the yield obtained with rat forebrains from animals sacrificed immediately before brain removal and mRNA purification. A decrease in the amount of polysomes isolated from human tissue relative to rat brain tissue was a major factor contributing to the low yield. The molecular weight distribution of polypeptides synthesized by human and rat brain mRNA fractions was similar; thus, there was no indication for selective breakdown or inactivation of high molecular weight mRNA species in the human tissue. Our studies indicate that it is possible to utilize postmortem tissue for molecular biological investigations of human brain mRNA.

263.6 CHANGES IN GENE EXPRESSION DURING CEREBELLAR DEVELOPMENT IN THE RAT. S.L.BERNSTEIN*,A.E.GIOIO*,and B.B.KAPLAN. Dept. Anatomy, Cornell University Medical College, New York, NY. 10021

The cerebellum (Cb) of the Norway rat, Rattus norviegicus, was selected as a model for development of the central nervous system. Alterations in gene expression during postnatal development were evaluated by RNA driven hybridization of radiolabeled single-copy DNA to (a) total cellular poly(A+)RNA, reflecting the complexity of poly(A+)hnRNA (b) poly(A+)mRNA, and (c) polysomal poly(A-)RNA. Total poly(A+)RNA from whole brain was used as an internal hybridization standard.

Total cellular RNA from adult whole brain and Cb at several ages was isolated by the guanidinium thiocyanate/CsCl method (Kaplan, et.al. Biochem. J. 183,184.1979). Polysomes were obtained by sedimentation through 2.0M sucrose, and residual hnRNA contamination subsequently removed by velocity sedimentation in sucrose density gradients. Polyadenylated RNA was obtained from all RNA fractions by affinity chromatography on oligo(dT)-cellulose.

Whole brain total poly(A+)RNA hybridized to 12.6±0.8% (±S.E.M.) of the single-copy DNA, a value consistent with that reported by Kaplan, et.al. (Biochemistry 17, 5516. 1978). Assuming asymmetric transcription, the sequence complexity of total poly(A+)RNA was $4.8X10^6$ nucleotides (nt), a value equivalent to 106,000 different hnRNA transcripts 4500 nt in length. The percentage single-copy DNA expressed as total poly(A+)RNA declined from 12.6±0.7% in the neonate Cb to 10.5±0.4% in the adult ($p<0.05$). Data from mixing experiments containing 3d and 60d total poly(A+)RNA reveal extensive homology (>90%) in the sequences present in these RNA populations. Hybridization values of poly(A+)mRNA from neonate and adult Cb declined from 4.8±0.2% to 4.1±0.2%, respectively ($p<0.01$), a loss equivalent to 15,000 different poly(A+)mRNAs 1800 nt in length. Polysomal poly(A-)RNA was hybridized to R_ot values $> 1.0X10^5$ M Sec. The percentage of single-copy DNA expressed by this RNA class reflects the sequence complexity of the nonadenylated mRNAs (Chikaraishi,D.M.,Biochemistry 18,3249.1979; Van Ness, et.al. Cell 18, 1341.1979). Values for neonate and adult poly(A-)mRNA were 3.4±0.2% vs 3.6±0.2%, respectively. Hybridization to R_ot values $>2.5X10^5$, generated by longer incubation times (>7d) or higher RNA concentrations (>28mg/ml) did not increase apparent saturation values.

The results of this study indicate that (1) the base sequence complexity of cerebellar poly(A+)hnRNA and poly(A+)mRNA decrease significantly during postnatal development, and (2) the age-related alterations appear restricted to specific RNA populations.

(This work supported by NIH grant HD11392.)

264.1 URINE INDUCES CHANGES IN CONCENTRATIONS OF LH-RH AND CATECHOLAMINES (CA) IN THE ACCESSORY OLFACTORY BULB OF FEMALE PRAIRIE VOLES. D. Dluzen, S. Carter-Porges, L. Getz and V.D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

We previously demonstrated that in rat and vole olfactory bulb, LH-RH is preferentially localized in the accessory (AOB) compared to the main (MOB) olfactory bulb. It was, therefore, of interest to measure concentrations (pg/mg) of LH-RH, dopamine (DA) and norepinephrine (NE) in these structures of female prairie voles (Microtus ochrogaster) following stimulation with conspecific male urine. Approximately 200 µl of urine from sexually experienced males or distilled water was applied onto the upper lip of females (N=7/group) who were decapitated at 1, 30, or 60 min following stimulation. Olfactory bulbs were removed, divided into main and accessory components and unilateral halves were either extracted in 0.1N HCl for LH-RH RIA or in 0.1N $HClO_4$ for CA radioenzymatic assay. In addition, blood was collected for LH RIA. In both urine and water treated females a significantly ($p<.001$) greater concentration of LH-RH was obtained in the AOB (16.4±1.5) vs MOB (5.9±0.4). A slight increase in LH-RH at 30 vs 1 min and a significant ($p<.05$) increase at 60 min limited to the AOB was obtained in urine stimulated females (13.1±1.3, 16.3±3.5, 25.3±5.6). No significant changes were obtained from MOB extracts of these females at 1, 30, or 60 min (6.7±1.3, 6.1±0.8, 8.1±1.5) nor did water treated females demonstrate time dependent changes in either the MOB (4.5±6.1, 4.2±0.3, 5.5±2.1) or AOB (16.9±4.5, 12.5±1.4, 14.3±2.9). One minute following urine or water stimulation NE concentrations in the AOB of both groups were higher than those of the MOB (170±16, 167±12 vs 116±32, 98±16, respectively). A significant ($p<.05$) time effect on NE concentration was observed in the urine stimulated group and limited to the AOB, with maximal depletion of 44% at 60 minutes. No difference between AOB and MOB DA concentrations were obtained, nor did DA in either structure vary as a function of time following stimulation. Using 50 µl samples, no detectable serum LH values were obtained at any interval for water treated females, while 43%, 29%, and 0% of the females treated with urine demonstrated detectable LH levels at the 1, 30, and 60 minute intervals, respectively. These results confirm that olfactory bulb LH-RH in the female vole is preferentially localized in the AOB and demonstrate a time dependent and stimulus specific response to conspecific male urine limited to the AOB. Similarly, a time dependent and stimulus specific response for NE, also limited to the AOB was observed, while DA fails to demonstrate this phenomenon. It would appear that olfactory/gustatory cues contained in male urine capable of producing changes in female serum LH levels may be mediated by direct stimulation of AOB LH-RH neurons possibly under NE control.

264.2 ANTAGONISTIC ANALOGS OF LHRH: SUPPRESSION OF LORDOSIS BEHAVIOR IN RATS. C.A. Dudley*, W. Vale, J. Rivier, R.L. Moss. Department of Physiology, Univ. of Texas Southwestern Med. Sch., Dallas, Tex. 75235 and Neuroendo. Dept., Salk Institute, 10010 N. Torrey Pines Rd. La Jolla, CA 92037.

Peptide analogs of luteinizing hormone-releasing hormone (LHRH) which have a variety of characteristics including extended duration of action, higher potency and antagonistic activity, may provide a tool for investigating the physiological role of LHRH as a modulator of extra-pituitary function. The present experiment was designed to test the effect of two antagonistic analogs as well as an antibody to LHRH on estrogen-progesterone induced mating behavior. A total of 60 female rats were ovariectomized and implanted with 23 guage stainless steel cannulae positioned in the third ventricle. The animals were primed with estrone (E; .20 mg) and progesterone (P; 2.5mg) and tested for lordosis behavior prior to entering the infusion stage of the experiment (mean lordosis-to-mount ratio (L/M)=.91±.08). Subsequently, the animals were randomly divided into two groups, primed with E at 0 hr, and then at 43 hr simultaneously primed with P and infused with either µl saline (S) or one of the following experimental drugs: 1)(D-pGlu; D-Phe^2, D-Trp^{3,6}) LHRH antagonist ($LHRH_1$) 50 ng in 1µL saline; 2) An antibody to LHRH (LHRH-Ab) 1µL diluted 1:10 in saline; 3) (Ac-dehydro-Pro^1, pCl-D-Phe^2, D-Trp^{3,6}) LHRH antagonist ($LHRH_2$) 100 ng in 1µL saline. Animals were tested for lordosis behavior at 48 hr. Ten days elapsed between infusion tests so a period of 60 days was required for the animals to receive S paired with each experimental agent in counterbalanced order. The L/M ratios of the S and drug-treated groups were compared by matched-pair t-tests.

LHRH-Ab had no effect on lordotic responding. This result may indicate that insufficient amounts of LHRH were bound up by the antibody, enabling the remaining LHRH to exert an effect. However, both of the antagonists suppressed lordotic behavior as compared to their S controls. $LHRH_1$ yielded a mean L/M of .45 ± .05 while the mean for the S controls was .60 ± .06 ($t=2.52$; $v=52$, $p<.02$). The decrement in mating behavior produced by $LHRH_2$, a long-acting antagonist, was even more dramatic. The mean L/M for this antagonist was .41±.05 while the S infused animals produced a mean of .88±.03 ($t=8.47$; $v=35$, $p<.01$). Thus, antagonistic analogs of LHRH, which act at the pituitary level to block gonadotropin release and ovulation, were also successful in blocking a CNS-mediated behavioral event, namely, lordosis responding. These results provide additional evidence for the role of endogenous LHRH as a physiological modulator of sexual receptivity.

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264.3 THE SUPPRESSION OF COPULATORY BEHAVIOR IN MALE RATS WITH ECTOPIC PITUITARY GRAFTS: ROLE OF PROLACTIN. P.C. Doherty*, A. Bartke* and M.S. Smith*. (SPON: V.F. Williams). Depts. of Anat. and Ob./Gyn., University of Texas Health Science Center, San Antonio, TX 78284, and Dept. of Physiol., University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

We have recently reported that hyperprolactinemia induced by grafting whole pituitaries under the kidney capsules suppresses copulatory behavior in male rats and mice (Svare et al., Biol. Reprod., 21:529, 1979). Whole rather than anterior pituitaries were used because it has been reported that they produce higher serum prolactin (PRL) levels (Aguado et al., J. Endocrinol., 72: 399, 1977). Numerous studies have indicated that ectopic pituitaries may produce significant amounts of hormones other than PRL and this may be especially true of whole pituitary grafts. Thus we decided to determine whether the suppressive effects of pituitary grafts on copulatory behavior are the result of high circulating levels of PRL. Adult sexually experienced male CD-F rats were given four whole pituitary grafts, four anterior pituitary grafts, or were sham-operated. Adult females of the same inbred strain served as pituitary donors. Tests of copulatory behavior were begun two and one half weeks after surgery. Males were placed in observation chambers with ovariectomized females made receptive by injection of estrogen and progesterone, and were scored for mount latency, number of mounts, intromission latency, number of intromissions, ejaculation latency, and number of ejaculations. Each test lasted thirty minutes. Both groups of grafted animals exhibited suppressed copulatory behavior patterns, but these effects were more striking in the animals which received only anterior pituitary grafts. Whole pituitary grafted animals had significantly longer latencies to mount ($p < 0.05$) and to intromit ($p < 0.01$) than the sham-operated controls, while the anterior pituitary grafted animals differed from the sham-operated controls in latencies to mount ($p < 0.05$) and to intromit ($p < 0.01$), as well as in the number of mounts ($p < 0.05$) and intromissions ($p < 0.05$). Treatment of sexually experienced male CD-F rats with ovine PRL (400 µg, 2X daily in a 50% polyvinylpyrrolidone-saline vehicle) also resulted in deficits in copulatory behavior. The oPRL treated animals showed a significant decrease in the number of intromissions ($p < 0.02$) as compared to the vehicle treated control animals. The intromission latency was also increased in the PRL-treated group to twice the value of the control group, however this did not prove to be significant ($0.10 > p > 0.05$). In summary, despite possible release of other behaviorally active compounds from the whole pituitary grafts, the behavioral deficits observed in grafted animals are probably the result of chronically high PRL levels. (Supported by HD-12671).

264.4 SEXUAL BEHAVIOR AND DECIDUALIZATION OF THE FEMALE ZUCKER RAT. A.M. Chelich* and E.S. Edmonds. Northwest Center for Medical Education, Indiana University School of Medicine, Gary, Indiana 46408.

The female genetically obese Zucker rat has been described as uniformly sterile (Zucker and Zucker, J. Hered., 52:275-278, 1961). It has been reported (Saiduddin et al., Endo., 93:1251-1256, 1973) that obese females become pseudopregnant following reserpine treatment but not following cervical stimulation, that uteri of pseudopregnant obese females do not decidualize in response to uterine trauma, and that obese females do not lordose. We have previously found that cervical stimulation does induce pseudopregnancy in obese females. To determine whether cervically stimulated pseudopregnant obese females decidualize, 0.1 ml of sesame oil was introduced into the lumen of one uterine horn on the fourth day of pseudopregnancy in five obese and six lean Zucker rats. Uteri were examined and weighed on the ninth day of pseudopregnancy. All obese and lean rats decidualized. The mean weight of the decidualized horns was 1639 ± 319(SEM) and 1449 ± 165 mg and the control horns weighed 232 ± 10 and 151 ± 16 mg for obese and lean rats respectively, except for the control horn of one obese rat which spontaneously decidualized and weighed 490 mg. Sexual behavior was tested twice in eight fat and nine lean females, 2 to 7 months of age. Proestrus obese females were placed with intact males and proestrus lean females with vasectomized males for 20 minutes or until ejaculation occurred. Solicitation (darting and hopping) occurred in all sessions with lean rats and in 12 out of 16 sessions with obese rats. Lean rats did not exhibit rejection (kicking or rolling over) but fat rats rejected in 2 out of 16 trials. In lean rats, lordosis occurred in all sessions with a lordosis quotient of 100%. Contrary to the literature, obese females also exhibited lordosis. All fat rats lordosed during at least one session. Lordosis was observed in 12 out of 16 sessions and occurred during 100% of the mounts except in one trial when it occurred with 68.7% of the mounts. Ejaculation occurred in all trials with lean females but in only 3 out of 16 trials with obese females even if the obese females were left overnight with the males. Of the three successful trials two resulted in pregnancy and one in at least pseudopregnancy. These results suggest than an inability to decidualize (prepare the uterus for implantation) is not a factor contributing to the reduced reproductive capacity of the obese female Zucker rat. Our data also indicate that sexual behavior is inadequate to insure ejaculation but that it is not inadequate because of an absence of solicitation or lordosis.
(Supported by funds provided by the Lake County Medical Center Development Agency)

264.5 EFFECTS OF STEROID ANTAGONISTS ON RAT SEXUAL BEHAVIOR. I.T. Landau. Dept. Psychology, Oakland Univ., Rochester, MI 48063

An initial series of experiments assessed whether the ability of the anti-estrogen, CI-628, to inhibit estrogen stimulated lordosis in ovariectomized adult rats depends upon its interference with the synergistic effects of estrogen with progesterone (P). Thus, the ability of CI-628 to inhibit lordosis was contrasted in ovariectomized rats brought into estrous by (a) a single injection of estradiol benzoate (EB) (2 µg) followed by a single injection of P (500 µg) 48 hours later vs. (b) a series of 4 daily EB injections (without any P). CI-628 (2 mg) substantially and equally effectively antagonized lordosis responding in both conditions, demonstrating an effect of CI-628 in the absence of exogenously administered P. Related studies suggested that CI-628's effectiveness was not dependent upon the subsequent daily EB injections acting as a progestin mimic or upon the influence of progesterone of adrenal origin. Implications of these results for attempts to inhibit androgen-stimulated male sexual behavior by CI-628, and the importance of androgen to estrogen metabolic conversions (aromatization), are discussed.

In a subsequent experiment, possible facilitatory effects of the aromatization inhibitor, 1,4,6-androstatriene-3,17-dione (ATD), on male sexual behavior were evaluated. ATD is typically proposed to antagonize the effect of testosterone propionate (TP) on sexual behavior, by inhibiting the behaviorally significant metabolic conversion of androgen to estrogen. In this study, castrated male rats were treated with daily injections of either ATD or the control vehicle, in combination with TP (50 µg), EB (25 µg), or sesame oil, for four weeks after castration, receiving weekly tests of sexual behavior with a receptive female. It was found that a high dose (10 mg) of ATD alone was sufficinet to maintain precastration levels of sexual behavior, equaling or exceeding scores of the TP controls. This dose of ATD also did not inhibit the stimulatory effect of TP on sexual behavior. This was true despite a lack of either a facilitatory or antagonistic effect of ATD on seminal vesicle weight. Results are discussed in terms of implications for the "aromatization hypothesis," possible central nervous system action of ATD, and possible qualitative differences in the mediation of androgenic effects in neural vs. peripheral target tissues. Related studies evaluated the interactive effects of CI-628 and the non-steroidal antiandrogen, flutamide, in the inhibition of androgen-stimulated male sexual behavior.

264.6 ECLOSION HORMONE: DEVELOPMENTAL TITERS AND ITS ROLE IN ECDYSIS. P. F. Copenhaver*, N. Tublitz*, P. M. Taghert*, and J. W. Truman (SPONS: T. Kennedy). Dept. of Zool, Univ. of Washington, Seattle, WA 98195.

Eclosion hormone (EH), an 8500 dalton peptide hormone which triggers adult emergence in Manduca sexta, has also been found to initiate pupal ecdysial behavior in this species (Truman, Taghert, and Reynolds (1980) J. Exp.Biol. in press). Recently we have been interested in the role of EH in the initiation of larval ecdysis behavior. EH injections into late fourth instar larvae successfully elicits premature ecdysial behavior in a dose and time dependent manner analogous to that seen under similar conditions in pupal ecdysis. An analysis of the blood of ecdysing larvae has demonstrated the presence of EH in significant levels shortly before the onset of ecdysial behavior, paralleling the time course of blood titres of EH in ecdysing pupae. In addition, measurements of the concentrations of EH in the brain and abdominal nerve cord (ANC) of pre- and post-ecdysis animals, using a biological assay, show a significant depletion of EH from the ANC while no similar change in brain concentrations is seen.

Since this evidence suggests that the release of EH is the critical trigger for both larval and pupal ecdyses as well as adult eclosion, we have measured the EH content of the brain and ANC throughout the life cycle of Manduca to determine its synthesis and release profile. Our results suggest that during development there appears to be a coordinated synthesis of these two pools of EH. However, release appears to be independently regulated. On the basis of gel filtration and isoelectric focusing, EH isolated from the brain is indistinguishable from that found in the ANC. Our data indicate that the strictly developmental events of larval and pupal ecdyses are triggered by the release of the peptide from the ANC, whereas adult eclosion, which is strongly gated by a circadian oscillator, is initiated by EH released from the endocrine center of the brain. Thus, there is an apparent correlation between the site of EH release and type of control over the ecdysial event.

264.7 NEUROENDOCRINE AND HISTOLOGICAL EVIDENCE FOR DIFFERENT ADRENAL GLAND RESPONSE PATTERNS IN DOMINANT AND SUBORDINATE JAPANESE QUAIL. D. L. Ely, F. S. Orcutt,* Jr., and F. Sadri.* Dept. of Biology, University of Akron, Akron, OH 44325.

In a previous study we have found that dominant colony mice have predominantly a sympathetic adrenomedullary response pattern to environmental stimuli, whereas subordinate mice respond with a pituitary adrenocortical pattern (Hor. and Behav., 10: 156-169, 1978). Using this model we replicated the study in Japanese quail (Corturnix corturnix japonica). Thirty male quail (4-8 months) were housed 5 nonsiblings to a group in 6 hardware cloth (1/2") cages (63 x 63 x 43 cm) in an environmental chamber (24-25°C, 14 hrs L, 10 hrs D). Behavior was observed 3x/week for each group for 30 min., and a ratio was calculated for pecks given/pecks received per bird for dominance determination. Subordinate animals showed significantly higher levels of plasma corticosterone during weeks 1 and 2 as compared to dominants (21 ug% vs. 14 ug%, $p<.01$, and 22 ug% vs. 11 ug%, $p<.05$). Dominant animals showed significantly higher ratios of adrenal chromaffin cells (CC) to steroidogenic cells (SC) ($p<.02$). Also the dominant males showed higher levels of seminiferous tubule activity ($p<.05$) and less myocardial fibrosis ($p<.05$) as compared to subordinate males. There were no differences in tissue weights or cloacal gland area between dominant and subordinate animals. Between colony comparisons showed that the more socially stable colonies had twice the adrenal weights of the unstable colonies. The stable colonies showed a peak corticosterone response the first week which declined until the 5th week whereas the unstable colonies showed a peak corticosterone response the 2nd which declined to the 6th week. There were no between colony histological differences with regards to CC/SC, heart fibrosis, or seminiferous tubule activity. The results suggest that dominant quail respond to social stimulation with a chromaffin cell proliferation similar to a sympathetic adrenomedullary response in mammals. Subordinate quail respond to social stimulation with an adrenal steroidogenic cell proliferation and increased plasma corticosterone similar to a pituitary adrenocortical response in mammals. (Supported in part by Akron University Faculty Research Grant #566).

264.8 STRESS, CORTISOL, TESTOSTERONE AND DOMINANCE RANK IN FREE-RANGING OLIVE BABOONS (PAPIO ANUBIS) R Sapolsky* (SPON: M Krieger) Rockefeller Univ. NY, NY 10021

Studies have shown that variability in baseline or stressed levels of glucocorticoids & androgens is associated with behavioral status. Generally, subordinate rodents & primates have elevated baseline corticosterone & enlarged adrenals while dominants have greater corticosterone responses to stress. Baseline testosterone, studies suggest, is highest in dominants; all subjects show suppression of testosterone by stress. Because behavioral nuances are lost in the laboratory, examination of these questions with feral animals would be fruitful.

Stress responses were studied in adult males of a troop of baboons living in the Mara Reserve, Kenya. Subjects have been observed for 5 years; rank is based on copulations & consortships with adult estrus females in the 6 months preceding the study. Animals were stressed by darting intramuscularly with an anesthetic syringe of Sernylan. Subjects were clearly stressed, showing agitation before becoming unconscious within 5 minutes. Blood samples were then taken at 5,10,15,30 & 60 mins. Darting was done over 2 weeks at 0800 each day; subjects were unaware they were to be darted. RIAs were conducted for cortisol & testosterone.

Cortisol: a stress response was shown, cortisol initially surging, declining by 10 or 15 minutes & then rising gradually thereafter. It is impossible to determine true maximal levels (since in some it occurs before the first sample at the 5 min. mark) or baseline in these stressed subjects; thus it is impossible to determine whether dominants have larger cortisol responses to stress than do subordinates. However, dominants have faster responses: in subordinates (ranks 5,8-12.5; $\bar{m}$= 8.9) cortisol is low at 5 min, surges at 10 & declines by 15. In dominants, (ranks 104,6,7; $\bar{m}$ = 3.8) levels are already their highest at 5 mins. & decline by 10. Baboons with these two different patterns are significantly different & non-overlapping in rank.

Testosterone: marked suppression of testosterone was shown 10 min. after stress; levels rose thereafter. There were several significant rank-related trends in the stress response. Dominance was correlated with high levels of testosterone (measured by the mean & maximum levels of the 5 sera samples taken from each individual). These measures must be considered the best approximations of baseline obtainable under these conditions. In addition, the relative degree of testosterone suppression following stress is significantly rank-related; dominants show the smallest suppression of testosterone by stress. Thus dominant individuals respond to stress with the most rapid secretions of cortisol. In addition, dominants, seemingly with higher baseline testosterone, show relatively less testosterone suppression by stress than do subordinates. (Supported by NIMH Grant MH-27934).

265.1 PERMEABILITY OF ACETYLCHOLINE-ACTIVATED POSTSYNAPTIC CHANNELS IN SYMPATHETIC NEURONS. Doju Yoshikami. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

The ionic conductance channels involved in the fast, nicotinic, postsynaptic potentials of principal neurons in sympathetic ganglia of frogs were examined. These acetylcholine (ACh)-activated channels are permeable to a number of different positively charged organic amines. Permeability was measured electrophysiologically and by uptake of ^{3}H-labeled amines. In electrophysiological tests, the reversal potentials (E_{rev}'s) of intracellular responses to iontophoretically applied ACh were measured when the Na^+ in the bathing Ringer's solution was replaced by various amines. An $E_{rev} > E_{K^+}$ was used as an indication that the amine could permeate the ACh-activated synaptic channel. In uptake studies, ganglia were exposed to low concentrations of the ^{3}H-amines either in the presence, or absence, of the ACh analog carbachol. A primary amino group in the labeled compound allows it to be histologically fixed in situ by glutaraldehyde so that uptake could be measured by autoradiography as well as by liquid scintillation counting of the ganglia.

These tests show that the ACh-activated channels which are permeable to Na^+ and K^+ are also permeable to various small primary amines including those having an alcohol, guanidinium, or amide moiety. Thus these neuronal postsynaptic channels appear to be relatively non-selective, cation-specific pores, and in this regard resemble their counterparts in skeletal muscle.

The use of radiolabeled amines in conjunction with autoradiography provides a novel means for the direct visual identification of individual neurons that have been been synaptically active. This provides a potentially powerful tool to probe the functional activity of other neuronal synapses.

265.2 CHANNEL LIFETIME AT INHIBITORY CHOLINERGIC SYNAPSES IS PROLONGED BY ESERINE. Roy L. White* and Daniel Gardner. Dept. of Physiology and Biophysics, Cornell Univ. Med. Coll., New York, N.Y. 10021.

At cholinergic synapses of Aplysia buccal ganglia, inhibitory postsynaptic currents (IPSC) show single exponential decay, implying that a single rate-limiting step (RLS) determines duration. Gardner and Stevens ((1980) J. Physiol. 304) showed that this RLS is unlikely to be either slow transmitter release or transmitter removal by diffusion or hydrolysis. They suggested instead that a conformational change, which closes open synaptic channels with exponentially-distributed lifetime τ, is the RLS determining IPSC decay. Acetylcholine fluctuation (ACh noise) spectra are consistent with this hypothesis. However, as in other cholinergic synapses, the anticholinesterase physostigmine (eserine) prolongs exponential decay of IPSCs, implying an additional action of eserine on the channel complex.

We now report that examination of IPSCs and ACh noise spectra with and without eserine is consistent with a direct eserine action on channel lifetime. IPSC decay follows single-exponential kinetics during and after eserine wash-in, showing that eserine alters an existing RLS, rather than slowing an additional, formerly fast process and thereby substituting a new RLS. Eserine 10^{-4} g/ml lengthened IPSC τ from 18±3 msec (n=7) to 38±5 msec (n=5). Without eserine, ACh was applied using pressure ejection, yielding double Lorentzian ($1/f^2$) spectra with corner frequencies f_1=8.1±0.6 Hz and f_2=60±5 Hz (n=16). If the 8.1 Hz noise process represents synaptic channel lifetime, and if eserine prolongs lifetime, then eserine should correspondingly lower f_1 to 4.2 Hz. In eserine, we recorded spectra with f_1=4.4±0.4 Hz (n=10), consistent with the hypothesis. In 4 cells, both IPSC and ACh noise were each recorded before and after eserine. Eserine lengthened IPSC τ from 16±2 to 37±5 msec; $1/(2\pi f_1)$ calculated from noise shifted correspondingly from 18±3 to 34±4 msec in eserine. The lack of any additional decay processes seen during eserine treatment, and the parallel effects on IPSC τ and noise f_1 provide further confirmation that τ indeed reflects the average channel lifetime, and imply that eserine directly modifies that lifetime.

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265.3 SINGLE ACETYLCHOLINE CHANNELS IN CULTURED HUMAN MUSCLE. M. B. Jackson,* H. Lecar,* V. Askanas and W. K. Engel (SPON: R. E. Taylor). N.I.H, Bethesda, Md. 20205.

Single channel ionic current fluctuations were recorded in cultured adult human skeletal muscle obtained by biopsy. A small open-ended extracellular patch electrode containing a dilute solution of the agonist suberyldicholine was used to record the current through a 1-2μ^2 patch of cell membrane. Cells were held at hyperpolarized membrane potentials of between -90 and -100 mV at temperatures of 22 to 26°C. Records of single channel jumps were made from muscle cells in cultures established by the technique of Askanas and Engel (Neurology, 25:58-67, 1975). The measured unit conductance was 47 pS and showed little variation for different cultures. That result is close to the value found with cultured embryonic rat muscle by the same technique (Jackson and Lecar, Nature 282: 863-864, 1979).

In contrast to the conductance measurements, the preliminary experiments did not give a single estimated value of the mean channel open-time. Duration distribution histograms show variability in the kinetics of channel closing. Analysis of these histograms shows that there are channels with mean open-times ranging from approximately 2 to 20 msec. Whether this reflection of varying rates of channel closing is a function of more than one population of channels and/or of developmental stages of the fibers, or of other factors, is not yet known. (Non-synchronous maturation is typical of adult human muscle in cultures). The present study establishes that this technique can be used to investigate such factors.

265.4 CHARACTERIZATION OF ENDPLATE CONDUCTANCE IN TRANSECTED FROG MUSCLE AND ITS MODIFICATION BY ATROPINE AND TUBOCURARINE. J.J. Lambert,* N.N. Durant,* L.S. Reynolds,* R.L. Volle and E.G. Henderson. Dept. of Pharmacol. Univ. of Conn. Health Ctr. Farmington, CT 06032.

Cutaneous pectoris muscles of Rana pipiens were transected distal to the innervated region. Within 10 mins, membrane potentials (E_m's) of -33±2.5mV and endplate potentials (e.p.p.s) (3-15mV) were recorded unaccompanied by muscle action potentials or twitch. The fall in E_m was associated with a net loss of $[K^+]_i$ and a net gain of $[Na^+]_i$. Although the input resistance fell by 50% and the space constant was slightly reduced in the transected muscle fibers, endplates could be adequately voltage-clamped with two microelectrodes. Endplate currents (e.p.c.s) with rise times of 350 to 700μsec were recorded as a function of holding potential (V_m). The current-voltage relationship of peak e.p.c.s over the range of -70 to +20mV was linear and the reversal potential (-6.6±2.2mV) was not different from that found for intact muscle fibers. The decay phase of e.p.c.s could be described as a single exponential at all V_ms and had a voltage (H=-140±14mV) and temperature (Q_{10}= 3.79±.20) dependence similar to that described for e.p.c.s of glycerol-treated muscles. Tubocurarine, ≥ 0.3μM caused a significant decrease in the time constant (τ) of e.p.c. decay and e.p.c. amplitude. The depression of e.p.c. amplitude by tubocurarine was partially reversed by 4-aminopyridine while the decrease of τ was not. Atropine (10^{-4}M) caused a monotonic shortening of e.p.c.s at a V_m of -90mV but e.p.c.s recorded at +50mV were biphasic(τ_{fast}= 0.44±0.02: τ_{slow}=4.03±0.46 msec). The transected muscle has utility for the study of conductance kinetics of the endplate uncomplicated by the presence of drugs or ions to eliminate contraction. (Supported by NS07540 and NS12563)

265.5 EFFECTS OF α-NEUROTOXINS FROM VARIOUS SNAKE VENOMS ON TRANSMISSION IN AUTONOMIC GANGLIA. V.A. Chiappinelli, J.B. Cohen and R.E. Zigmond. Dept. Pharmacol., Harvard Medical School, Boston, MA 02115

The pharmacology of nicotinic transmission in autonomic ganglia has been studied in vitro in intact ganglia by recording the compound action potential from postganglionic nerves following preganglionic nerve stimulation. Previously, we have reported that several, but not all, commercially available lots of α-bungarotoxin block transmission in ciliary and choroid neurons of both pigeon and chicken ciliary ganglia at a concentration of 10 μg/ml (1.2 μM), while having no effect on the rat superior cervical ganglion (Proc. Natl. Acad. Sci. USA 75: 2999 (1978)). This blockade is antagonized by preincubation with 100 μM d-tubocurarine. Two additional experiments were performed to test the postsynaptic nature of the blockade seen with this toxin. 1) Exposure to the toxin (1.2 μM) completely blocked the depolarization of ciliary ganglion cells produced by the cholinergic agonist carbachol (500 μM). 2) A solution of the toxin (1.2 μM) was incubated with membranes purified from Torpedo electric organ containing an excess (3 μM) of α-neurotoxin binding sites. After subsequent centrifugation to remove the Torpedo membranes, the solution was no longer capable of blocking transmission through the ciliary ganglion.

A high-affinity binding site for ^{125}I-α-bungarotoxin (K_D = 1 nM) was characterized in the chicken ciliary ganglion. However, since it is labelled equally well by lots of α-bungarotoxin which block transmission and those that do not, this site does not appear to be involved in the blockade of transmission.

Several α-neurotoxins from snakes other than Bungarus multicinctus were examined for ganglionic blocking activity. α-Cobratoxin (from Naja naja siamensis) and L.s. III (from Laticauda semifasciata) produced a partial blockade of transmission through the pigeon ciliary neurons, while having no effect on transmission through the chicken ciliary neurons or through choroid neurons in either species. Two other α-neurotoxins from Laticauda semifasciata, erabutoxin a and erabutoxin b, had no effect on these cell populations at similar concentrations (1.2 μM).

None of the α-neurotoxins tested had any effect on transmission in either the rat superior cervical ganglion or the rat pelvic ganglion at concentrations up to 100 μg/ml (12 μM). Collagenase treatment of these ganglia, in an attempt to increase access of the toxins to ganglion cells, did not alter these negative results.

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265.6 HISTRIONICOTOXIN BINDING TO TORPEDO NICOTINIC POST-SYNAPTIC MEMBRANES. Daniel C. Medynski* and Jonathan B. Cohen. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

The equilibrium binding of [^{3}H]-perhydrohistrionicotoxin([^{3}H]-H_{12}HTX) to nicotinic post-synaptic membranes isolated from Torpedo electric tissue has been studied by ultracentrifugation. The non-specific interaction of this ligand with the membranes is characterized by a partition coefficient, P=200 nmoles/g protein per nmole/ml. In the presence of 10μM carbamylcholine(carb) to occupy all cholinergic receptor binding sites, [^{3}H]-H_{12}HTX binds to a number of sites equal to one half(0.48±.09) the number of α-bungarotoxin sites(α-BgTx) with a K_{eq}=0.5μM. When d-tubocurarine or α-BgTx fully occupies the cholinergic sites, the K_{eq} of H_{12}HTX equals 0.8μM and 5μM respectively, while this alkaloid binds to the same number of sites but with a K_{eq}=5μM in the absence of cholinergic ligands. Thus the affinity of the HTX binding site is regulated by the ligand which occupies the acetylcholine(AcCh) binding site. A twenty-fold excess of non-radioactive H_{12}HTX displaces [^{3}H]-H_{12}HTX bound at equilibrium in the presence of carb on the hour time scale($t_{1/2}$=3 hrs.). There is no evidence that [^{3}H]-H_{12}HTX binds to the AcCh binding site, in contrast to the quaternary aromatic amine non-competitive antagonist, [^{14}C]-meproadifen, which interacts at the AcCh binding site directly in the absence of carb(Krodel et al., Molec. Pharm. 15, 294(1979)). Meproadifen displaces [^{3}H]-H_{12}HTX in a dose dependent manner in the absence(IC_{50}(-carb)=20μM) or presence(IC_{50}(+carb)=1.5μM) of 10μM carb, and non-radioactive H_{12}HTX reduces the binding of [^{14}C]-meproadifen in a manner compatible with competitive interaction at a common site.

The pharmacology of the HTX binding site is defined by the ligands that displace [^{3}H]-H_{12}HTX. The tertiary analogues of meproadifen, proadifen(IC_{50}(+carb)=0.6μM, IC_{50}(-carb)=4μM) and adiphenine(IC_{50}(+carb)=10μM), the aromatic amine, lidocaine(IC_{50}(+carb)=2.4mM, IC_{50}(-carb)=20mM), and the fluorescent ligands ethidium bromide(IC_{50}(+carb)=0.7μM, IC_{50}(-carb)=85μM) and quinacrine(IC_{50}(+carb)=50μM, IC_{50}(-carb)=400μM) all displace [^{3}H]-H_{12}HTX in a dose-dependent manner, exhibiting greater potency in the presence than in the absence of carb. Tetracaine displaces [^{3}H]-H_{12}HTX with greater potency in the absence of carb(IC_{50}(+carb)=100μM, IC_{50}(-carb)=0.35 μM).

This research was supported by USPHS Grant NS 12408 and predoctoral training grant GM 07306.

265.7 EFFECT OF BOTULINUM TOXIN ON END-PLATE CURRENTS IN THE RAT. L.C. Sellin* and S. Thesleff* (SPON: J. Nyquist) Department of Pharmacology, University of Lund, Lund, Sweden.

Local blockade of evoked transmitter release was produced by sublethal injection of botulinum toxin type A (BoTx) subcutaneously above the anterior tibialis muscle of adult male rats. At 7 days after injection, a time when extrajunctional receptors are present, the extensor digitorum longus nerve-muscle preparation was excised and end-plate currents (e.p.c.) were analysed by dual microelectrode voltage clamp. Control muscles were obtained from untreated rats. In order to facilitate the recording of e.p.c.s. without muscle contraction, the muscle fibers were crushed on both sides of the end-plate region leaving an intact area of about 1-1.5 cm. After this procedure, resting potentials ranged from -20 to -40 mV. In the BoTx-treated muscles and in some control muscles, transmitter release was enhanced by the addition of 2.5 μM 4-aminopyridine (4-AP) to the bathing medium. Data from e.p.c.s. were considered acceptable when the maximum membrane potential variation was less than 2% of the clamped voltage.

The relationship between current amplitude and voltage was linear and the reversal potential was about 0 mV in both the BoTx-treated and the control muscles. An analysis of the decay phase of the e.p.c. indicated that it could be characterized by a simple exponential function in both preparations. However, the decay phase of the e.p.c. was prolonged in the BoTx-treated muscles at 23°C, as indicated by an increase in the time constant (τ). This difference was more pronounced at hyperpolarized voltages than at depolarized voltages. When the rate constant ($\alpha, \alpha = 1/\tau$) of the decay phase of the e.p.c. was plotted logarithmically as a function of the clamped voltage, the BoTx-treated muscles showed an increased voltage sensitivity compared to normal muscles. Low concentrations (1 μM) of 4-AP increased the amplitude of e.p.c.s. without affecting the decay phase. At higher concentrations (5 μM), the time constant of the decay phase increased, but there was no effect on the voltage sensitivity.

These data suggest that alterations in the properties of the end-plate membrane occurs as a result of the paralysis produced by botulinum toxin. It is unlikely that BoTx had a direct effect, because similar changes were not observed at 2 days after toxin injection, although there was complete paralysis. (Supported by N.I.H. fellowship 3F32NS05935-02S1 and the Swedish Research Council).

265.8 **LOCAL ANESTHETIC BLOCK OF THE ENDPLATE CHANNEL IN HIGH EXTERNAL SODIUM.** **G. A. Redmann. Dept. of Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.**

The decay time constants and peak amplitudes of inward miniature endplate currents in varying concentrations of the quaternary lidocaine derivative QX-222 and external Na were examined in two microelectrode voltage clamped frog cutaneous pectoris muscle.

$[Na]_o$ (115 mM NaCl with added Na-glutamate) concentrations of 115, 230, and 345 mM gave peak currents of 3.1 ± 0.8 nA, 5.2 ± 0.6 nA and 5.6 ± 0.8 nA (±SD) respectively at -80 mV. Negative abscissal intercepts on double reciprocal plots of this data yielded a K_{Na} of approximately 400 mM at -80 mV, decreasing with hyperpolarization. The control decay rate constant decreased by about 60% in 345 mM Na. The slow to control exponential decay time constant ratios, $\tau s/\tau c$, plotted against varying (0-100 μM) concentrations of QX-222 showed a negative abscissal intercept that decreased with hyperpolarization with an e-fold change for about 60 mV and for a given voltage doubled in going from 115 to 345 mM $[Na]_o$, i.e., K_{QX} was about 40 μM at -80 mV in 115 mM Na, and about 90 μM in 345 mM Na at -80 mV. The amplitude ratios of the fast and slow exponential components of mepc decay, A_f/As, in the presence of 50 and 100 μM QX-222 were respectively 1.2 ± 0.5 (n = 6) and 2.0 ± 0.4 (n = 8) in 115 mM Na at -80, and 0.6 ± 0.2 (n = 4) and 0.9 ± 0.3 (n = 8) in 345 mM Na. A similar dependence of A_f/As on $[Na]_o$ was seen at hyperpolarized potentials. Reciprocal τ fast was a linear function of QX-222 concentrations. The slope of this relation decreased with increasing $[Na]_o$, being 2 x $10^7 M^{-1}s^{-1}$ in 115 $[Na]_o$, and 1 x $10^7 M^{-1}s^{-1}$ in 345 mM $[Na]_o$. The K_I for Na from competitive inhibition of the QX-222 channel block is in reasonable agreement with K_{Na} over the voltage range examined. Making the normal Ringer hypertonic with 460 mM sucrose reduced the mepc amplitude at -80 mV to 1.8 ± 0.9 nA and reduced α by about 30%, but did not change τ_s/τ_c as a function of QX-222 concentration.

The above data are consistent with the idea that both sodium and QX-222 compete for an intrachannel binding site. However, ionic strength effects on surface potential must also be considered. Supported by NS-14920 to P. Adams.

265.9 PROTECTION BY Mg^{++} OF ACETYLCHOLINE ACTIVATED IONIC CHANNEL BLOCKADE BY LIDOCAINE AND LOBELINE IN TRANSSECTED FROG MUSCLE.
E.G. Henderson, J.J. Lambert,* and L.S. Reynolds* (Sponsored by Linda Quenzer). Dept. of Pharmacol. Univ. of Conn. Health Ctr. Farmington, CT 06032.

We have previously shown that Mg^{++} (13mM) protected the Ach activated ionic channel from blockade by lobeline ($5X10^{-5}M$) in intact muscle fibers (Soc. for Neurosci. Abst. 1979:5,483.). In the voltage clamped transected cutaneous pectoris muscle lobeline ($1X10^{-5}M$) and lidocaine ($5X10^{-5}M$) caused a 32% and a 30%, respectively, decrease of the time constant of decay (τ) of e.p.c.s at a holding potential (V_m) of -90mV. After treatment of endplates with Mg^{++} (4-6 mM) lobeline and lidocaine, at the same concentrations, caused only a 16% and a 2%, respectively, decrease in τ. Mg^{++} by itself had no significant effect on τ, nor was the addition of Mg^{++} to the muscles treated with either lobeline or lidocaine able to reverse the block. Tubocurarine ($1.5X10^{-6}M$), atropine ($2X10^{-5}M$) and QX314 ($5X10^{-5}M$) caused a 25±2% decrease of τ which was unaffected by either pretreatment of the muscles with Mg^{++} (4-6mM) or the addition of Mg^{++} to the muscles already exposed to the blocking agent. In view of the possibility that the protection by Mg^{++} of the effect of lidocaine was associated with the degree of ionization at pH 7.4, an approximately equiactive concentration of QX314 ($2X10^{-5}M$) was also examined. As with the higher concentration, Mg^{++} did not protect. A quaternary derivative of lobeline is currently under investigation. These results suggest the possibility of multiple allosteric binding sites in the Ach activated receptor-channel complex. (Supported by NS 07540 and NS12563)

265.10 EFFECTS OF GUANINE NUCLEOTIDES ON THE BINDING OF CHOLINERGIC ANALOGUES TO THE MUSCARINIC RECEPTOR.
F.J. Ehlert*, W.R. Roeske* and H.I. Yamamura (SPON: T. Reisine). Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Guanine nucleotides have been shown to selectively reduce the binding of agonists to several neurotransmitter and hormonal receptors while producing only minimal effects on antagonist binding. Recent studies have demonstrated that guanine nucleotides also regulate muscarinic receptor binding in a manner which resembles the effects of guanine nucleotides on other neurotransmitter receptors. In this study, we have investigated the regulatory influence of guanine nucleotides on the binding of a series of cholinergic analogues and have found a correlation between the effects of guanine nucleotides and the efficacy of cholinergic drugs.

Muscarinic receptor binding assays were carried out on homogenates of the longitudinal muscle of the rat ileum using the specific antagonist affinity label, [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB). In the absence of guanine nucleotides, the carbachol /[^{3}H]QNB competition curve was much flatter than a simple Langmuir isotherm and could be rationalized in terms of two major binding sites (high and low affinity) and a third minor binding site (superhigh affinity). In the presence of the nonhydrolyzable GTP analogue, guanyl-5'-yl imidodiphosphate (Gpp(NH)p), the carbachol/[^{3}H]QNB competition curve shifted to the right and became steeper, demonstrating that guanine nucleotides convert a heterogenous receptor population into a more homogeneous one of lower overall affinity. The greatest effect of guanine nucleotides on ligand binding was observed with the highly efficacious agonists, carbachol and oxotremorine, whose IC_{50} values increased by a factor of 10 in the presence of Gpp(NH)p (100μM). Somewhat smaller increases (five fold) in the IC_{50} values of the partial agonists, pilocarpine and pentyltrimethylammonium, were observed. In contrast, Gpp(NH)p only produced a minimal increase in the IC_{50} value of the antagonist, atropine. Other cholinergic analogues were examined, and a correlation between the effect of Gpp(NH)p and the efficacy of the drug was observed.

The potency of Gpp(NH)p for reducing agonist binding was investigated using the agonist affinity label, [^{3}H]cis methyl dioxolane ([^{3}H]CD). Gpp(NH)p caused a dose dependent inhibition of [^{3}H]CD binding in the heart and ileum, with the IC_{50} for this effect being 1.5 and 25μM, respectively. In contrast, Gpp(NH)p caused a dose dependent enhancement of [^{3}H]QNB binding in the heart and ileum, with the IC_{50} for this effect being 0.2l and 1.0μM, respectively. These data demonstrated reciprocal effects of guanine nucleotides on muscarinic agonist and antagonist binding.

266.1 FORMATION OF TRANSIENT AND STABLE CONNECTIONS BETWEEN ADULT MOLLUSCAN NEURONS. Andrew G. M. Bulloch and Stanley B. Kater. Dept. Zoo., Univ. Iowa, Iowa City, IA 52242.

Traditionally the processes of neuritic growth and synapse formation have been associated with embryonic nervous systems. However, recent studies of adult nervous systems have demonstrated dynamic properties of mature neurons which can cause functional alterations of neuronal circuitry. Here we report the formation of both transient and stable electrical connections between identified buccal neurons of Helisoma trivolvis.

Previous studies of the dynamic capabilities of adult Helisoma neurons demonstrated that proximal nerve crush causes the generation of neurites both within the neuropil and at proximal axon stumps (Murphy and Kater, Brain Res. 186: 251-272). In the case of neurons L and R 5, which normally project only into ipsilateral nerve trunks, some centrally derived neurites enter foreign terrain by traversing the commissure between the paired buccal ganglia. Such sprouting underlies the formation of the novel, stable 5-5 electrical connection observed in the present study.

Isolated buccal ganglia with proximal crushes of Esophageal trunks were cultured in vivo for periods of 1-40 days as described previously. After 1 day of culture, 5-5 coupling was observed in 25% of preparations tested (n=12), whereas at 2 days all preparations (n=11) exhibited this novel connection. The synapse is apparently stable since it was found in >95% of ganglia after 3-40 days of culture (n=40). It is also thought to be highly specified, since neuron 5 was not found to be connected to any other neuron tested (n=32) in preparations 3-40 days of culture.

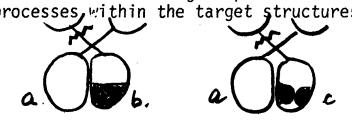
In contrast to the stable 5-5 connection, a transient electrical synapse forms between neuron 5 and neuron 4. Measurable coupling was detected between these neurons in 75% of preparations (n=8) after 1 day of culture. However, this connection is very weak at 2 days and no longer detectable by 7 days of culture (n= 6).

It is concluded that neuron 5 possesses a latent coding to form a stable connection with its homologue. The mechanism of 5-5 coupling may involve transient formation of a number of "inappropriate" connections with subsequent stabilization of the 5-5 synapse.

Supported by grant NS 15350.

266.2 EVIDENCE FOR THE ROLE OF TARGET-PRODUCED DIFFUSABLE FACTORS AND AXONAL REMODELING IN THE REINNERVATION OF A CAUDAL TECTAL ISLAND BY OPTIC AXONS IN GOLDFISH. M. A. Edwards, S. C. Sharma and M. Murray. Dept. Anatomy, The Medical College of Pennsylvania, Philadelphia, PA and NY Medical College. Introduced by J. Prendergast.

A regenerating optic nerve will establish a compressed retinotopic projection upon a rostral half tectum. If in addition a caudal tectal island is preserved, a compressed pattern will be established which spans both rostral half and caudal island (Murray and Sharma, 1977). The innervation of the island is achieved by the formation of a single prominent bundle which crosses the gap. This successful reinnervation could be accomplished by: (1) non-selective axonal outgrowth from the edge of the rostral tectum plus loss of axons unsuccessful in finding a tectal target; (2) non-selective outgrowth, stimulated by presence of a deafferented tectal island, followed by loss of grossly inappropriate axons; (3) selective outgrowth stimulated by tectal island. We examined the caudal border of the rostral half tectum in half tectal (b) and island (c) fish using EM and HRP. No organized bundles of axons emerge from the caudal border in the half-tectal fish. One or a few bundles of axons, usually closely associated with glial cells, were seen between the meninges and underlying cerebellum or tegmentum, in island preparations. The presence of the island then appears to stimulate this organized outgrowth from the rostral half-tectum. Normal density of boutons and normal appearing optic terminals, stained by orthograde HRP, are seen in the island. The location of retinal ganglion cells whose axons form the bundle projecting to the island was compared following HRP injection into the island (c) or into a comparable caudal site in control tecta (a). Labeled cells in control animals are confined to a small focus in nasal retina. Labeled cells in island fish, injected 1-2 mo. p.o., are distributed over ¼-½ nasal retina. At later stages (> 5 mo. p.o.) labeling is more restricted but still more extensive than normal. Axon outgrowth therefore appears to be stimulated by the presence of the island and presumably by diffusable factors emanating therefrom but outgrowth is somewhat non-selective. With time, the more inappropriate axons appear to be withdrawn. The extent to which aberrant synaptogenesis occurs remains unknown. The data are consistent with the view that precise topographical projections arise through a process of local remodeling of axonal processes within the target structures. (Supported by NS13768).



266.3 SIMULTANEOUS GENERATION OF MOTONEURONS AND INTERNUCLEAR NEURONS IN THE RABBIT ABDUCENS NUCLEUS: A HYPOTHESIS FOR NEURONAL DIFFERENTIATION. Marjorie D. Shaw* and Keith E. Alley (SPON: R. Spetzler). Dept. of Anat., Case Western Reserve University, Cleveland, Ohio 44106.

The rabbit abducens nucleus contains internuclear neurons that project to the oculomotor nucleus, as well as motoneurons that project to the lateral rectus muscle of the eye. These two cell types are fairly evenly mixed throughout the nucleus, and resemble each other so closely that only their axonal projections distinguish them.

By combining ^{3}H-thymidine autoradiography with retrograde transport of HRP, we determined the time of origin of each cell type. In one series of experiments, HRP was injected into the lateral rectus muscle of newborn rabbits which had been injected with ^{3}H-thymidine in utero. Motoneurons were defined as those cells containing HRP. Neurons without HRP were presumed to be internuclear neurons. In a complementary series of experiments, HRP was injected into the oculomotor nucleus of young adult rabbits which had been injected with ^{3}H-thymidine in utero. Internuclear neurons were then defined as those containing HRP granules, while motoneurons did not.

Both motoneurons and internuclear neurons originate over gestation days 9-12, with by far the greatest numbers arising on day 11. No differences could be detected in the generation times of these two populations.

We inferred from the anatomical arrangement of the two cell types and from direct histologic study of embryos, that both cell types originate in the same area of the germinal epithelium. Each has the potential to become either a motoneuron or an internuclear neuron. Not until the cell has migrated into the abducens nucleus primordium does the cell become determined as one type or the other. Within the nuclear primordium, local structural features, such as intercellular ependymal channels (Silver and Sidman, 1980), may guide the growing axons towards one target (oculomotor nucleus) or the other (lateral rectus muscle). By leading the axons to experience different environments, local features of the environment may determine the course of the cells' differentiation.

266.4 SEQUENTIAL GENERATION OF MOTONEURONAL POOLS IN THE OCULOMOTOR NUCLEUS OF THE RABBIT: A THEORY OF NUCLEAR CONSTRUCTION. Keith E. Alley and Marjorie D. Shaw* Dept. Anat., Case Western Reserve University, Cleveland, Ohio 44106.

Motoneurons innervating the same muscle congregate into distinct pools during development. In the oculomotor nucleus five populations of somatic motoneurons are present. These supply four of the eye muscles and the levator palpebrae. This study has analyzed the arrangement of the motor pools in the oculomotor nucleus of the newborn and adult rabbit by means of HRP transport. Their organization was then correlated with the birth dates of the oculomotor neurons as determined with ^{3}H-thymidine autoradiography.

Oculomotor neurons are generated on embryonic days 9 through 12. Plots of the position of labeled cells for each of these days revealed a spatiotemporal gradient of neuron production in the IIIrd nucleus. The sequence of cell formation advances from rostro-ventral to caudal-dorsal; thus the order of neuron origin correlates with its final position in the nucleus. Based on the HRP maps of pool location, this would indicate that the motoneurons innervating the inferior rectus, medial rectus, inferior oblique, and superior rectus muscles are produced in a sequential fashion.

At first, these observations might suggest that the order of neuron origin represents the critical step for forming distinct motor pools through changes in the developmental potential of the germinal epithelium. However, evidence from our investigation of the abducens nucleus would allow another interpretation. Perhaps the environment of the oculomotor primordium consists of a series of compartmentalized microenvironments. Each compartment would be sequentially filled with newly formed oculomotor neurons. Properties of the individual compartments would then specify the proper muscle target for each neuron.

In summary, we propose that motor nuclei are fabricated by the addition of younger neurons onto a base of older neurons. In this way a spatiotemporal gradient is formed. If the gradient covers a single uniform microenvironment, the differentiation of all neurons will be identical. If the gradient covers a series of environmental compartments, compact pools of neurons innervating different muscles will be laid down.

266.5 TRANSITION FROM A CONTINUOUS TO A DISCRETE DISTRIBUTION OF CORTICOSPINAL PROJECTION NEURONS IN THE RAT PARIETAL CORTEX.
Carolyn A. Bates* and Herbert P. Killackey. Dept. of Psychobio., Univ. of Calif., Irvine 92717

In the adult rat, corticospinal projection neurons are found in the medial portion of the parietal cortex. These projection neurons are large pyramidal cells which are located exclusively in layer Vb. Previously, it has been reported that callosal projection neurons which are discretely distributed in layers III and Va in the adult rat are more widely distributed in the neonatal rat (Brain Res., '79, 173:532). The present study was undertaken to see if the corticospinal projection neurons undergo a similar transformation in distribution during the course of maturation.

Forty rats, postnatal days 1 to 14 were injected at the thoracic level of the spinal cord with Sigma VI HRP. The animals were sacrificed 48 hours after injection and processed according to the technique of Mesulam (J. Hist. Cyt., '76).

From the earliest time sampled (PND 3) to PND 6, a continuous band of labeled cells is present along the entire mediolateral extent of parietal cortex (Fig. 1A). The labeled cells are immature pyramidal cells which are destined to reside in layer Vb. By PND 8, the most lateral cells are no longer labeled. The distribution becomes further restricted, so that by PND 11, only the most medial areas of parietal cortex contain labeled cells. At this point, the labeled pyramidal cells have almost reached their mature proportions, and their distribution is very close to that seen in the adult rat. By PND 16 (Fig. 1B), the pyramidal cells have matured and the adult distribution of corticospinal projection neurons is reached.

The present study suggests similarities in the way in which the cortical projection neurons of the different cortical layers develop and that a progressive restriction in the source of efferents from the cortex may be a general feature of cortical development. (Supported by NSF Grant #BNS74-00626.)

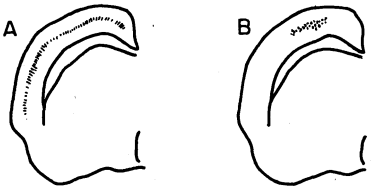


Fig. 1. Distribution of labeled corticospinal neurons at (A) PND 3 and (B) PND 16.

266.6 TRANSITION FROM A CONTINUOUS TO A DISCRETE DISTRIBUTION OF CORTICOTECTAL PROJECTION NEURONS IN THE RAT PARIETAL CORTEX.
G.O. Ivy and H.P. Killackey. Dept. of Psychobiology, Univ. of Calif., Irvine 92717.

We have previously demonstrated a developmental transition from a continuous to a discrete distribution of callosal projection neurons in the rat parietal cortex (Brain Res., '79, 173: 532). We decided to explore the generality of this developmental phenomenon by investigating the distribution of cortical neurons which project to the tectum.

Rat pups of various postnatal ages were injected in the tectum with a 50-60% solution of Sigma VI HRP via a glass micropipette attached to a 1 µl Hamilton syringe. Survival times varied from 6 to 30 hours. The tissue was processed according to the technique of Mesulam ('76).

We have found that in the adult rat, the corticotectal projection neurons are large pyramidal cells located in layer Vb in restricted portions of the parietal cortex (Fig. 1B). In contrast, from the day of birth (postnatal day 0, PND 0) until at least PND 4 (Fig. 1A) these neurons form a continuous band which extends throughout parietal cortex mediolaterally. This band is located in layer Vb, and individual neurons which have filled with the reaction product can often be identified as large pyramidal cells with apical dendrites extending to the pial surface. By PND 7, the band in layer Vb has lost some of its continuity; stretches of layer Vb are often devoid of labeled cells or may contain a few cells which are only lightly labeled. By PND 15, the discontinuous adult distribution pattern is established.

Thus the present findings, in conjunction with our previous findings on the callosal projection neurons and those of Bates and Killackey (this volume) on spinal projection neurons, indicate that the transition from a continuous to a discrete distribution of corticofugal neurons may be a general developmental phenomenon in the neocortex. (Supported by NSF #BNS74-00626).

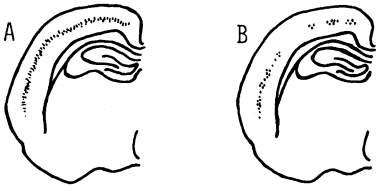


Fig. 1. Distribution of labeled corticotectal neurons at (A) PND 4 and (B) in the adult.

267.1 PERIPHERALLY ADMINISTERED PHLORIDZIN ALTERS SHORT-TERM FOOD INGESTION IN THE RABBIT. D. A. VanderWeele, P. J. Geiselman, and J. D. Sanderson*. Psychology Department, Occidental College, Los Angeles, CA 90041 and Psychology Dept. and Brain Research Inst., UCLA, Los Angeles, CA.

Intact, female New Zealand rabbits were infused with 10 cc of a 2.5 x 10^{-3}M solution of phloridzin or isotonic saline through cannulae entering the hepatic portal system or the upper duodenum and observed for feeding. Infusions were made in both the light and dark portions of the circadian cycle, but the results were virtually identical for both periods, and night results only will be presented. Duodenal administration of phloridzin significantly increased feeding during the first two hours following infusion. Hepatic portal infusion also significantly increased first half-hour feeding; over the first half-hour, food intake was 94% greater for duodenal phloridzin and 117% greater for portal phloridzin than following saline administration via the same routes, $t = 3.01$, $df = 17$, $p<.05$. Hepatic portal infusions, however, produced prolonged periods of anorexia following the immediate enhancement of feeding. The anorexia was not seen after duodenal infusion and lasted 2-3 hours. Duodenal administration produced hyperglycemia, presumably attributable to liver glycogenolysis, while portal infusions produced mild hypoglycemia, probably attributable to renal glucose transport blockade as glycosuria developed, followed by mild hyperglycemia. These results indicate again the lack of importance of absolute levels of glycemia in determining feeding, as both hyperglycemia and hypoglycemia were accompanied by strong feeding responses. We hypothesize that duodenal and portal phloridzin enhanced immediate food ingestion because the drug inhibited liver absorption of glucose and activated glycogenolysis. Phloridzin may, however, interfere with glucose transport into and out of the liver (Goresky & Nadeau, 1974) and, hence, allow liver stores to remain repleted after portal infusion promoting the anorexia observed. Duodenal infusion of phloridzin does not allow significant amounts into the venous system to affect liver or kidney function, as was observed in the present study, and therefore, no anorexia was seen. Alternatively, the blockage of renal reabsorption of glucose by hepatic portal phloridzin could activate sympathetic-mediated, compensatory muscle glycogenolysis which postpones further food intake. The latter hypothesis was suggested as preliminary results with hepatic-portal infusion of phloridzin in subdiaphragmatically vagotomized rabbits shows no immediate enhancement of food ingestion but these animals do show the prolonged anorexia following normal intakes over the first half-hour postinfusion.

Partially supported by grants AM 17259 and NS 7687

267.2 BRAIN REGIONAL SEROTONIN DEPLETION IN HYPOTHALAMIC OBESITY: LESION VS. OVEREATING AS CONTRIBUTORY FACTORS. D.V. Coscina, J.J. Warsh, and G.H. Anderson*. Sects. Biopsychol. and Biochem. Psychiat., Clarke Inst. Psychiat., and Dept. Nutrit. and Food Sci., Univ. Toronto, Toronto, CANADA.

Research into brain monoamine systems which mediate overeating and obesity suggests that impaired serotonin (5HT) metabolism may be one causative factor. Past work from our laboratories has supported this notion in that lesions of the medial hypothalamus (MH) in rats has consistently produced hyperphagia and obesity in association with depleted forebrain 5HT. However, other data from the Biochemical Nutrition literature suggests that overconsumption of protein can itself modify brain 5HT levels. In keeping with this possibility, we have recently obtained behavioral (1) and biochemical (2) evidence that brain 5HT depletion in obese MH-lesioned rats may be a consequence rather than a cause of overeating. To test this hypothesis further, the present study determined 5HT content in 6 discrete brain regions from MH-lesioned rats who either overate food or ate normal amounts of food. Subjects were 30 female rats weighing 230-250 g at surgery. Of these, 18 received bilateral radiofrequency heat lesions (55°C for 1 min per hemisphere) and 12 received sham surgery. All except 9 lesioned rats were allowed unrestricted access to food for 2 weeks. The hyperphagia of these 9 lesioned rats was curtailed by restricting intake to control levels during this time. Analyses of terminal plasma samples showed that hyperphagic rats had lower ratios of tryptophan to other neutral amino acids than sham-operated or restrained-lesioned rats, the latter two not differing from each other. Hypothalamic 5HT was depleted 13-16% ($p < .05$) in lesioned rats regardless of feeding condition. However, hippocampus showed less depletion after restricted feeding (-33%) vs. overeating (-41%; $p < .05$ difference). Thalamus and midbrain were not depleted unless rats overate (-22% and -30%, respectively, $ps < .05$). Neocortex and neostriatum were unaffected by lesion or feeding condition. These data show that overeating after MH injury substantially enhances the lesion-induced depletion of brain 5HT. As such, our findings suggest that overeating must be controlled in experiments designed to elucidate brain 5HT changes associated with this behavior as excessive intake confounds interpretation of other treatment effects.

(1) Anderson et al., Physiol. Behav., 1979, 23, 751.
(2) Coscina et al., Internat. J. Obes., 1978, 2, 362.

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267.3 MIDBRAIN KNIFE-CUTS WHICH SPARE FOREBRAIN SEROTONIN NEURONS DO NOT BLOCK HYPOTHALAMIC OBESITY. V.M. Dewan and D.V. Coscina. Sect. of Biopsychol., Clarke Inst. Psychiat., and Dept. of Psychol., University of Toronto, Toronto, CANADA.

Previous work from this laboratory (1) has shown that female rats with 70% depletion of forebrain serotonin (5HT) following dorsal and median raphe lesions show little overeating or bodyweight (BW) gain following medial hypothalamic (MH) lesions. This suggests a role for intact 5HT neurons in the expression of hypothalamic obesity. However, the possibility exists that this blockade is not due to 5HT depletion, but instead to disruption of fibers of passage along the midbrain axis. The present study tested this hypothesis by determining if coronal knife cuts (KCs) placed caudal to the raphe nuclei so as to sever such fibers, but leave 5HT afferents to the forebrain intact, would block overeating and obesity following subsequent MH lesions. Subjects were 20 adult female rats: 10 received 3 mm wide, 4 mm deep coronal KCs 0.55 mm behind the raphe nuclei; 5 received sham cuts wherein the knife guide was lowered into brain but the wire knife was not extruded; 5 served as normal controls. Following surgery, BW plus food and water intakes were measured daily for the first week, then weekly for 2 remaining weeks. Six weeks after surgery 5 of the KC rats plus the 5 shams were sacrificed for forebrain assays of 5HT and histologic assessment of KC loci. The remaining 5 KC rats plus 5 normals all received bilateral radiofrequency MH lesions. BW and intake measures were taken as before. One week after KC surgery, cut rats lost 22 g BW compared to 6 g for shams ($p < .05$). However, by the end of the second week, both groups had recovered and were comparable in BW to normal controls. KCs produced little (-14%) depletion of forebrain 5HT compared to sham surgery. BW change 3 weeks after MH surgery was equivalent for KC (+ 149 g) and normal (+ 165 g) rats, reflecting the equivalence of their hyperphagia (32 vs 39 g consumed/day, respectively). The results of this study suggest that the blockade of MH hyperphagia and obesity which follow severe 5HT depletion due to midbrain raphe lesions (1) is not due to transection of rostro-caudal fibers running through the midbrain axis at the level of these raphe nuclei. As such, these data continue to support the possibility that expression of MH hyperphagic requires intact forebrain 5HT systems.

(1) Coscina and Stancer, Science, 1977, 195, 416.

267.4 THE ROLE OF ENDORPHINS IN REGULATORY EATING AND DRINKING: NARCOTIC-INDUCED HYPERPHAGIA AND HYPERDIPSIA. A. Riley, M. Ortuno, K. Hoffman, M. Siemon, and M. Heft. Psychopharmacology Laboratory, The American University, Washington, D. C. 20016.

Although the endorphins have primarily been examined in relation to analgesia (Riley, Zellner, & Duncan, Neurosci. Biobeh. Rev., in press), these endogenous opiates have recently been implicated in the regulation of eating and drinking. This view is based on the suppression of food and water intake by narcotic antagonists (Holtzman, Life Sci., 24: 219-226, 1979) and on elevated endorphin levels in genetically-obese mice and rats (Margules, Moisset, Shibuya, & Pert, Science, 202: 988-991, 1978). If endorphins are mediating consumption, it would be expected that endorphin agonists, e.g., morphine, would produce hyperphagia and hyperdipsia.

Groups of animals were injected daily with distilled water, 20, 40, or 80 mg/kg morphine sulfate for 28 consecutive days. Food and water consumption, as well as body weight, were monitored 1, 3, 6, 12, and 24 hr post injection.

While morphine initially had no differential effects on consumption, with repeated injection (Days 8-14) a significant dose-related hyperphagia and hyperdipsia occurred 3-6 hr post morphine injection. With further injections (Days 15-28) morphine-induced eating and drinking continued to increase and occurred earlier following the morphine injection, i.e., 1-3 hr. That the morphine induced increases in consumption are delayed post injection and develop over injections suggest that morphine may produce several effects, e.g., catatonia and hyperphagia and hyperdipsia, and it is the interaction of these effects that determine the likelihood of consummatory changes following morphine administration. Initially, the morphine-induced catatonia limits changes in consumption. With the development of tolerance to the catatonic effects the morphine-induced changes in consumption can occur. Body weight increases paralleled the drug-induced changes in consumption.

These data parallel the aforementioned effects of narcotic antagonists on consumption and are consistent with the suggestion that endorphins may in part mediate regulatory eating and drinking.

267.5 AMPHETAMINE-INDUCED ANOREXIA IN GENETICALLY OBESE(obob) MICE: CORRESPONDENCE WITH HYPOTHALAMIC NOREPINEPHRINE DEPLETION.
R. Olsauskas and G.A. Oltmans. Dept. Pharmacol., Chicago Medical School, Chicago IL 60612

Genetically obese mice with the obob mutation have elevated hypothalamic(HT) levels of norepinephrine(NE)(Lorden et al., Brain Res., 1975; Feldman et al., Hormone Res., 1979). The relationship of this NE excess to the hyperphagia of the obob mouse is not known. In the current study the effects of amphetamine (AMPH) on food intake and HT NE content were studied in obob mice.

In the initial studies, female mice were adapted to a restricted 6 hour food access schedule and then treated chronically with 3 or 10 mg/kg of AMPH. Saline-treated obob mice ate significantly more and had significantly higher HT NE levels than saline-treated lean mice. AMPH-treated lean or obob mice demonstrated a dose dependent decrease in both food intake and HT NE levels during the initial 3 hrs following drug treatment. Furthermore, the food intake and HT NE levels were no longer different in lean and obob mice treated with the same dose of AMPH. Thus, when HT NE levels in obob mice were similar to those in lean mice, the food intake of the obob mice no longer differed from that of lean mice.

Preliminary studies indicated that the administration of AMPH on a mg/kg basis produced brain AMPH levels in obob mice which were approximately twice those in lean mice. Consequently, obob mice were administered 5 mg/kg AMPH and compared to a group of lean mice administered 10 mg/kg AMPH. The food intake of the 5 mg/kg group of obob mice was significantly decreased, but remained significantly higher than that of lean mice treated with the 10 mg/kg of AMPH. Furthermore, the HT NE levels of these obob mice were intermediate to those of the saline and AMPH-treated lean mice. Thus, equilibrating brain AMPH levels did not produce comparable food intake or HT NE levels in lean and obob mice.

In summary, the AMPH-induced decreases in food intake corresponded to the degree of depletion of HT NE. These results indicate that the decreases in food intake in the obob mice may be functionally related to the HT NE depletion. Thus, it appears that activated release of the NE stores can suppress feeding in this mutant. This may mean that the elevated HT NE levels reflect an abnormal release mechanism in the obob mouse which is related to the hyperphagia of this mutant.(Supported in part by a grant from NINCDS # RO 1 NS15600)

267.6 INCREASED CARBOHYDRATE PREFERENCE IN THE RAT AFTER INJECTION OF 2-DEOXY-D-GLUCOSE AND CLONIDINE. S.E.Fahrbach*, J.R.Tretter*, P.F. Aravich*, J.McCabe* and S.F.Leibowitz (SPON: N.Miller). The Rockefeller Univ. NY 10021 and Brooklyn Coll. NY 11210.

Feeding in the rat can be produced by stimulation of α-adrenergic receptors within the hypothalamic paraventricular nucleus (PVN) and by glucoprivation. There is evidence that hypothalamic catecholamine (CA) neurons may participate in the feeding response to glucoprivation. We further examined this hypothesis by testing the impact of 2-deoxy-D-glucose (2DG) and clonidine (CL) on preference for specific dietary constituents. The experiments reported use a self-selection feeding paradigm. Subjects were adult male rats. Diets varying in carbohydrate, protein, and fat content were compared, including chow-based sugar vs fat diets and dextrin vs casein. Insulin(7U/kg), 2DG(600mg/kg) and CL(50ug/kg) were injected intraperitoneally; in separate experiments CL(10μg) was injected into the PVN in brain-cannulated rats. Food intake was recorded 2,4,6, and 24 h post-injection.

In an attempt to show that chow diets (40% sucrose, 20% corn oil) could provide a sensitive measure of preference, intake after insulin injection, known to stimulate carbohydrate intake, was recorded. The ratio of sugar diet to total intake significantly increased from.59 after vehicle to .85 after insulin.Tests with 2DG showed a change from .57 to .76. CL, tested in the same paradigm, also produced an increase in food intake and a shift in preference to the sugar diet. To further elucidate the mechanism of action of CL, this drug was injected into the PVN, the most sensitive site for α-adrenergic induction of feeding. Intake of dextrin (non-sweet starch) and casein was compared in addition to intake of the chow-based diets. In both cases CL increased preference for the carbohydrate diet, indicating that sweet taste is not important. Thus, CL, presumably acting as a central α-adrenergic agonist, and 2DG, acting through intracellular block of glycolysis, produce similar behavior with respect to nutrient selection. These findings are consistent with the recent evidence of Tretter & Leibowitz (this meeting) that PVN injections of NE selectively enhance carbohydrate intake and the report of Leibowitz & Brown (Br.Res. 1980) that midbrain lesions of the dorsal NE bundle projecting to the PVN impair glucose intake and eating to glucoprivation. We suggest that these data corroborate a role for hypothalamic CA neurons in regulation of carbohydrate intake.
Research supported by MH-22879 and Whitehall Foundation Grant. SEF is an NSF Graduate Fellow.

267.7 HINDBRAIN CATECHOLAMINE PROJECTIONS TO THE PERIFORNICAL HYPOTHALAMUS: THEIR ROLE IN THE MEDIATION OF DRUG-INDUCED ANOREXIA AND HYPERPHAGIA. J. McCabe*and S. F. Leibowitz. (SPON: J. Orbach). The Rockefeller University, New York, New York.

Dopaminergic and adrenergic stimulation of the perifornical region of the hypothalamus (PFH) is known to suppress food consumption in hungry rats. When injected directly into the PFH, via chronically-implanted cannula, the neurotransmitters dopamine (DA) and epinephrine and the classic anorectic amphetamine (AMPH) each reduce food intake. The DA receptor blocker chlorpromazine (CPZ), however, when injected centrally or peripherally results in a hyperphagic food intake response. In the present study the role of various catecholamine (CA) fiber systems, previously defined in histochemical studies, were disrupted at several levels of the neuraxis using primarily the wire knife-cut (KC) technique. Food intake of deprived KC animals and sham-operated animals were compared after administration of DA, AMPH, or CPZ.

Results may be summarized as follows: 1). Electrolytic lesions of the proposed PFH receptor area, at the level of the ventromedial nucleus, resulted in a loss of the anorectic response seen after intracranial (IC) and intraperitoneal (IP) AMPH, and a diminished CPZ hyperphagic response. 2). A coronal KC immediately caudal to the proposed PFH receptor area, which severed the most medial fibers of the medial forebrain bundle, produced a loss of anorexia from IC and IP AMPH. The effect of IC injection of DA to the PFH, however, was actually potentiated by the KC, as was the hyperphagic response to CPZ, suggesting the development of denervation supersensitivity in PFH receptors. 3). Moving the cut further caudally into the midbrain tegmentum produced effects that depended on the dorso-ventral extent of the cut. When the KC was more ventrally placed, just dorsal to the medial lemniscus, an attenuated AMPH response was seen to both IC and IP injection. In contrast, if the KC was moved 2 mm dorsally, just lateral to the central grey, it generally failed to disrupt the IP AMPH anorexia, and actually potentiated IC AMPH anorexia. Both midbrain cuts attenuated CPZ hyperphagia. This evidence indicates the importance of the ventral CA fibers in the mediation of AMPH anorexia, while both dorsal and ventral fibers appear to mediate CPZ hyperphagia. Further experiments with KC at the level of the pons are currently in progress.

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267.8 PHARMACOLOGICAL AND LESION MANIPULATIONS OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN): EFFECTS ON EATING ELICITED BY ELECTRICAL BRAIN STIMULATION AND TAIL PINCH. Ronnie Halperin, Christine L. Gatchalian* and Sarah F. Leibowitz. The Rockefeller Univ., New York, NY 10021.

Eating elicited by central norepinephrine (NE) injection, electrical brain stimulation (EBS), and tail pinch (TP) show behavioral similarities and have been explained in terms of forebrain catecholamine mediating mechanisms. Through the use of pharmacological and brain lesion manipulations, the present studies investigate the possible interaction, at the hypothalamic level, of the systems underlying these three elicited eating responses.

Pharmacological Studies. Eleven rats were each chronically implanted with a drug-injection cannula aimed at the PVN and with a bipolar stimulating electrode aimed at the ipsilateral lateral hypothalamus. Rats were satiated and then centrally injected with NE (0.4 to 40 nmoles), the α-adrenergic receptor blocker phentolamine (PHT) (40 to 60 nmoles), or vehicle just prior to a 20-min test session in which eating in response to EBS, TP, or no stimulation (drug alone) was tested. Current thresholds for eating in response to EBS, and eating responsivity to TP were unaltered by a preceding PVN injection of NE. The effects of NE and EBS, and of NE and TP on food intake appeared to be additive, suggesting that these effects may be mediated by independent mechanisms. Furthermore, PHT, which blocked eating in response to NE injection, did not alter eating thresholds in response to EBS or eating in response to TP.

Lesion Studies. To further assess a possible role for the PVN in the mediation of TP-elicited eating, we tested this response in 6 rats before and after bilateral PVN or sham lesions. PVN lesions did not disrupt feeding elicited by TP and, in fact, TP-elicited eating appeared facilitated as measured by response latency and duration. These data indicate that the PVN is not a necessary component in the mediation of TP-elicited eating and that it may actually exert an inhibitory influence on TP-elicited eating.

At the level at which we are studying these responses, the hypothalamic system mediating eating in response to NE injection appears to be independent of the system(s) mediating eating in response to EBS and TP, responses which may be mediated via extrahypothalamic catecholamine systems. The apparent lack of interaction suggests that these systems may be involved in different aspects of the feeding process. (Supported by N.Y.State Health Research Council award #1424, and 1F32 MH 107656 and MH 22879 from USPHS)

267.9 EFFECTS OF CENTRAL CATECHOLAMINE DEPLETION ON THE FOOD INTAKE AND BODY COMPOSITION OF THE DIABETES MOUSE (db/db). Joan F. Lorden and Mary Ann Pelleymounter*. Dept. of Psychology, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

The diabetes mouse (C57BL/Ks-db) is genetically obese, hyperphagic, hyperglycemic and hyperinsulinemic. Central nervous system involvement in the syndrome is suggested by the observation that this mutant has elevated levels of hypothalamic norepinephrine (NE) in comparison with lean littermate controls. Furthermore, reduction of central NE has been shown to reduce body weight and blood glucose in the db/db (Lorden, J.F., J. Comp. Physiol. Psych. 1979, 93, 1085-96). Studies were undertaken, therefore, to assess the effects of central catecholamine depletion on both the size of the fat stores and also on food intake, activity and body temperature in an attempt to determine the means by which central lesions alter energy balance in this mutant.

Female db/db mice and their lean controls received 80 nmol infusions of the neurotoxin 6-hydroxydopamine (6-OHDA) dissolved in a .9% saline-.02% ascorbic acid vehicle. The 6-OHDA was delivered in a 2 µl volume through a 30 ga cannula stereotaxically aimed at the third ventricle. Additional groups of lean and diabetes mice received injections of the vehicle solution alone.

At sacrifice 100 days after surgery, large decreases in both hypothalamic and telencephalic NE and dopamine were noted in all 6-OHDA treated groups. Body temperature measured at 23°C was decreased in all 6-OHDA treated groups. Activity levels were not significantly altered by the lesions except in the homozygous lean group. These mice decreased their activity in comparison with vehicle treated animals of the same genotype. The 6-OHDA lesions reduced food consumption by 33% in the db/db mice but did not alter food intake in the lean controls. Blood glucose levels were also reduced in diabetes mice treated with 6-OHDA.

Food deprivation was sufficient to reduce both the body weight and blood glucose of vehicle treated db/db mice to the levels of 6-OHDA treated db/db mice. However, in the vehicle treated mice it was necessary to decrease food intake by over 60% to achieve this effect. Body fat was also decreased in both 6-OHDA and food restricted db/db mice in comparison with free-feeding vehicle treated mice. Food restriction and 6-OHDA treatment differed, however, in that food restricted mice also had a reduced lean body mass in comparison with the 6-OHDA treated group. No effects of the lesions were noted on the body composition of the lean control mice. Thus, in the diabetes mouse central catecholamine depletion produces decreases in body fat which are attributable only in part to a reduction in food intake. Since there was no evidence of increased energy expenditure through thermogenesis or activity, the lesion may also alter some as yet unspecified metabolic factor. (Supported by NINCDS grant 14755-02)

268.1 NEURAL ORGANIZATION PREDICTS STIMULUS SPECIFICITY FOR A RETAINED ASSOCIATIVE BEHAVIORAL CHANGE. Joseph Farley*, Dept. Psychology, Princeton Univ., Princeton, NJ 08544 and Daniel L. Alkon, NINCDS, NIH, MBL, Woods Hole, MA 02543.

Complementary behavioral and neurophysiological studies examined stimulus specificity for an associative behavioral change exhibited by the nudibranch Hermissenda. Paired, but not random, presentations of a light step (30 sec duration, intensity 4.6 x 10^3 ergs.cm^{-2}.sec^{-1}) with rotation-produced stimulation of caudal hair cells (94 rpm; 2.24 g) produced a significant increase in response latency for the animals' movement towards a test light. This associative behavioral change did not occur when cephalic, rather than caudal, hair cells were those stimulated by rotation. Indeed, response latencies were faster for animals receiving paired vs. random presentations of light and cephalic hair cell stimulation, and, in some instances faster than original baseline latencies. Intracellular recordings from type B photoreceptors in the isolated nervous system indicated the enhancement of a long-lasting membrane depolarization (LLD, Alkon and Grossman, 1978; Alkon 1979) under stimulus presentation regimens paralleling those involved in training of intact animals. A single pairing of light and rotation-produced excitation of caudal hair cells was sufficient to produce an average 3-4 mV depolarization of type B cells, detectable some 60 sec after the offset of light. Depolarization increased with a second pairing to a cumulative level of 7 mV. Unpaired presentations of light and rotation, as well as light-alone presentations, did not result in an enhanced LLD or a cumulative depolarization. Similarly, paired presentations of light and cephalic hair cell stimulation did not produce an enhanced LLD. Indeed, a slight hyperpolarization (1 mV) was obtained. This sensitivity of cumulative photoreceptor depolarization to the quality of hair cell stimulation derives from the differences in synaptic effects of hair cells upon type B photoreceptors for caudal vs. cephalic hair cell stimulation (Alkon, 1979). The magnitude and duration of type B depolarization following light paired with rotation-produced stimulation of caudal hair cells is enhanced by two sources: increased synaptic excitatory input from the ipsilateral optic ganglion and facilitated disinhibition (from caudal hair cells) of the type B photoreceptor. Turning the circumesophageal nervous system 180° with respect to the center of rotation results in the failure of B cells to receive this synaptic excitation and disinhibition and hence enhancement of the light-induced LLD. This cumulative depolarization specific to stimulus pairing can be expected to result in persistent depolarization ($\geq$3 days) which has been observed for these animals which undergo persistent associative behavioral changes (Crow & Alkon, 1978, 1979).

268.2 IN VITRO CLASSICAL CONDITIONING OF THE GILL WITHDRAWAL REFLEX IN APPLYSIA CALIFORNICA. Ken Lukowiak* and Chris Sahley, Division of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada and Department of Biology, Princeton University, Princeton, New Jersey. (SPON: L. Boyarsky)

The neural mechanisms mediating 2 types of non-associative learning (habituation and sensitization) of the gill withdrawal response (GWR) have been extensively studied in the siphon, mantle, gill and abdominal ganglion preparation (SMGA) of Aplysia californica. However, associative learning in this preparation has not been demonstrated. We now present evidence which shows that the gill withdrawal reflex (GWR) in this in vitro preparation can be classically conditioned. Previously, Lukowiak and Jacklet (1972, 1974) demonstrated that light (photic) stimulation to the siphon evoked a siphon withdrawal response. In contrast, the photic stimulus does not result in a gill withdrawal reflex. In our experiments, we pair photic stimulation of the siphon (conditioned stimulus, CS) with strong tactile stimulation of the gill (unconditioned stimulus, US). Tactile stimulation of the gill always evokes a GWR. Following 60-80 paired CS-US presentations, a GWR was observed on a CS alone test trial in 6 out of 9 preparations. A GWR to the CS was never observed in separate control preparations which received either CS alone presentations, US-CS presentations, or random CS, US presentations. The GWR to the CS, then, depended upon the CS-US pairing and, thus, we conclude is the result of associative learning.

Extinction of the learned response is observed following 3 CS alone trials. No systematic study has yet determined the duration of the learned response. Further experiments are being undertaken to determine the neural pathways and changes in neural activity or connectivity which underlie associative learning in this in vitro preparation.

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268.3 DOPAMINE MODULATION OF APLYSIA GILL REFLEX BEHAVIORS ACTS PRIMARILY VIA PERIPHERAL TERMINATIONS OF L_7. P. Ruben* and K. Lukowiak* (SPON. C. Beiswanger). Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

The gill withdrawal reflex (GWR) and its habituation, evoked by tactile stimulation of the siphon, are mediated by the integrated activities of the central and peripheral nervous systems. The GWRs are at least 35% of the amplitude of a spontaneous gill contraction. The CNS is capable of modulating (suppressing or facilitating) the reflex according to the central state of the animal. The facilitated state results in reflexive contractions well in excess of the amplitude of spontaneous contractions, and an absence of habituation or a retardation of its rate, despite normal synaptic decrement to central gill motor neurons. No environmental factors or whole animal behaviors have been correlated with the facilitated GWR. However, we have found two manipulations which can produce physiological mimicry of the facilitated state. First, stimulation of the gill motor neuron L_9 facilitates the GWR and prevents its habituation. Second, these same effects are achieved by the perfusion of dopamine (DA; 10^{-7}M - 5×10^{-7}M) through the gill. DA perfusion prevents habituation despite the decrement of evoked activity in central gill motor neurons which normally accompanies habituation. This demonstrates that DA induced facilitation is being exerted in the periphery. DA could be acting upon elements of the PNS and/or at the terminations of central efferent neurons. It appears that DA modulation of the GWR involves the peripheral terminations of central efferents, as its effects are ablated when the CNS is removed. Furthermore, motor neurons with their axons in the ctenidial nerve are most affected by DA perfusion through the gill. DA fails to exert a facilitatory influence when the ctenidial nerve is cut. DA acts primarily upon the endings of L_7. Hyperpolarization of L_7 to inhibit its contribution to the GWR prevents the facilitation due to DA perfusion. Hyperpolarization of other gill motor neurons does not reduce the facilitation. Repetitive firing of L_7 at 10-12 spikes per second once every 30 seconds produces a decrement of the evoked movement. In addition to its effects on the GWR, DA also facilitates the L_7 evoked movement and prevents its habituation. Preliminary experiments suggest that the ctenidial nerve radiations of L_7 are involved. These data show that dopamine, which may be the neurotransmitter responsible for the naturally occurring state of facilitation, acts to presynaptically or postsynaptically enhance the efficacy of L_7's neuromuscular transmission. These data lead us to favor the presynaptic hypothesis. (Supported by the MRC.)

268.4 PUNCTATE TACTILE GILL STIMULI EVOKE ACTIVITY IN CENTRAL GILL AND SIPHON MOTOR NEURONS IN APLYSIA. J. Goldberg* and K. Lukowiak* (SPON: L. Vaughn). Div. Med. Physiol., Fac. of Med., Univ. of Calgary, Calgary, Alberta T2N 1N4

The gill withdrawal reflex (GWR) can be evoked by tactile stimulation of the siphon or gill. The peripheral nervous system (PNS) mediates the GWR, while the central nervous system (CNS) exerts modulation over reflex behaviors. It has been suggested that tactile gill stimulation of moderate intensity does not send afferent input into the CNS. Brief punctate stimuli did not evoke an excitatory response in central gill motor neuron L_7, while strong shearing stimuli evoked only sub-threshold excitation (Kupfermann et al., 1971; 1974). It was therefore concluded that the PNS within the gill is functionally independent of the CNS. The subsequent demonstration that habituation of the GWR evoked by repetitive gill stimulation transfers its suppression to the GWR evoked by siphon stimulation, and vice versa, contradicts this conclusion since the transfer is mediated by both the CNS and PNS (Goldberg and Lukowiak, 1979). In these present experiments, the question of whether gill stimulation sends afferent input to the central motor neurons is re-evaluated. Brief punctate stimuli of varying intensity (200 mg to 2000 mg) were presented to the siphon and gill, with the CNS intact and removed. With the CNS intact, measurements of reflex latency and latency of excitation recorded in central gill and siphon motor neurons were taken. With the CNS removed, only measurements of GWR latency were taken. The results show that tactile gill stimulation at intensities as low as 200 mg evokes a short latency depolarizing excitatory response in both central gill and siphon motor neurons. The magnitude of excitation is usually slightly less than that evoked by siphon stimulation. The latency of excitation, for both siphon and gill stimulation, decreases sharply as the stimulus intensity is increased from 200 mg to 1000 mg. Upon further increases in stimulus intensity, there is little change in the latency of excitation. Behaviorally, the latency of the GWR evoked by siphon stimulation also decreases as the stimulus intensity is increased from 200 mg to 1000 mg, after which there is little change. The latency of the GWR evoked by gill stimulation is shorter than that evoked by siphon stimulation and decreases more gradually as the stimulus intensity is increased. For siphon and gill stimulation with the CNS removed, the GWR latencies are also short, and decrease only gradually as the stimulus intensity is increased. These data show that: 1) Brief punctate gill stimuli evoke excitation in central gill motor neurons; 2) The CNS controls the latency of the GWR when evoked by siphon stimulation, but not gill stimulation.

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268.5 DENTATE SINGLE UNIT AND FIELD POTENTIAL ACTIVITY DURING NM CONDITIONING IN RABBIT. D. J. WEISZ*#, G. A. CLARK*#, B. Y. YANG*§, P. R. SOLOMON*¶, T. W. BERGER° and R. F. THOMPSON#, #Dept. Psychobiol., Univ. California, Irvine, CA 92717, §Dept. Biol., Fudan Univ., Shanghai, P.R.C., ¶Dept. Psychol., Williams Coll., Williamstown, MA 01267, °Dept. Psychol., Univ. Pittsburgh, Pittsburgh, PA 15260.

Previous research has demonstrated an early, substantial, and persistent conditioned increase in hippocampal cell activity during classical conditioning of the rabbit nictitating membrane (NM) response. A much smaller conditioned increase in cell activity was seen in the part of entorhinal cortex (layers II and III) which contains cells afferent to the hippocampus and dentate gyrus. In the present two studies we examined the possibility that the conditioned increase in entorhinal cortex was amplified at the perforant path-granule cell synapse and/or relayed through dentate to the rest of hippocampus.

In one study we analyzed the responses of single cells from dentate gyrus recorded during classical conditioning of the NM response. Cells that demonstrated short latency activation following perforant path stimulation were identified as granule cells. The response patterns of identified granule cells during paired CS-US presentations were much different from the patterns of entorhinal and hippocampal CA1 and CA3 cells. During paired conditioning, granule cells exhibited short latency responses to the tone. Unlike patterns of entorhinal, CA1, and CA3 cells, those of granule cells did not form a temporal model of the conditioned NM behavior. Rather, many granule cells responded to the tonal CS with a rhythmical bursting pattern (burst frequency was approximately 8 Hz). During unpaired CS and US presentations, the dominant response of granule cells was a decrease of firing rate. While responses of entorhinal, CA1, and CA3 cells have been typically time-locked to the conditioned NM behavior, granule cell responses were temporally linked to the stimuli.

In a second study, monosynaptic field potentials from dentate gyrus were elicited by perforant path stimulation during NM conditioning. Granule cell excitability during the CS period was higher in the paired conditioning group than in the unpaired control group. A decrease in excitability was seen following tone presentations in the unpaired group.

These findings strongly suggest that the previously observed learning-dependent increase in entorhinal unit activity is not amplified at the perforant path-granule cell synapse nor relayed through dentate granule cells during acquisition of the conditioned NM response. The data do suggest a role in processing of stimuli for the dentate granule cells.

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268.6 EXCITABILITY INCREASES IN FACIAL MOTONEURONES OF THE CAT AFTER SERIAL PRESENTATION OF GLABELLA TAP. M.Matsumura* and C.D.Woody. Depts. of Anatomy and Psychiatry, Mental Retardation Research Center, UCLA Medical Center, Los Angeles, CA 90024.

Neuronal plasticity of facial motoneurones was investigated in awake cats after serial delivery of taps to the bridge of the nose (glabella). The effects of these stimuli which were used as unconditioned stimuli (US) in the production of conditioned blinking were assessed by intracellular recording and stimulation. Cats were classified into three groups: 1) "Conditioned" group: the cats were classically conditioned to eye blink by repeatedly presenting a click as conditioned stimulus (CS) followed by glabella tap as US; 2) "US-only" group: only glabella tap was presented; (This group did not exhibit an overt conditioned response when click was delivered). 3)"Naive" group: they were not given presentations of the CS or US.

Intracellular recordings were obtained using glass microelectrodes filled with 3M KCl (resistances 15 to 30MΩ), and inserted through a previously implanted guide tube oriented stereotaxically toward the facial nucleus. Facial motoneurones were identified by their response to stimulating the zygomatic branch of the facial nerve with single or double shocks every 500 msec. as well as by collision test. Of 47 neurons tested, 27 responded antidromically to facial nerve stimulation and 20 responded orthodromically. Resting potentials averaged -56 mV, while spike amplitudes averaged 30 mV. There were no significant differences in resting potential or spike amplitude among the three groups of cats or between antidromic cells and orthodromic cells.

Intracellular depolarizing current pulses were applied in each neuron to induce spike generation. The minimum current which repeatedly produced spike activity was taken as the threshold current for excitation. The threshold currents were distributed widely and evenly up to 7 nA in the "Naive" group. However, the distribution was shifted toward the lower values in the "Conditioned" and "US-only" groups. The mean threshold currents of neurons in the "Conditioned" group were 1.2 ± 1.2 nA; those in the "US-only" group were 1.0 ± 1.0 nA; and those in the "Naive" group were 3.5 ± 2.2 nA. The differences between "Conditioned" and "Naive" and between "US-only" and "Naive" groups were significant ($p < 0.01$). Besides the changes in threshold currents, membrane resistances of neurons from "Conditioned" and "US-only" groups were higher than those from the "Naive" group.

The results indicate that excitability changes may be produced in motoneurones by a US regardless of whether or not a CS is associatively paired. These changes could support the latent facilitation of motor behavior that occurs subsequent to presentation of US alone and has been identified previously by behavioral studies (supported by USPHS HD05959).

268.7 PLASTICITY OF A STARTLE-LIKE RESPONSE ELICITED ELECTRICALLY FROM THE ACOUSTIC STARTLE CIRCUIT IN THE RAT. Thomas Parisi and Michael Davis. Dept. Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06508.

The acoustic startle response has proven to be an excellent model system with which to study phenomenon such as habituation, sensitization, and reflex modification caused by antecedant stimulation (pre-pulse modification). Recent evidence suggests that the primary acoustic startle circuit in the rat consists of the auditory nerve, ventral cochlear nucleus, nucleus of the lateral lemniscus, nucleus reticularis pontis caudalis, spinal cord, and muscle (Gendelman & Davis, Neurosci. Abst., 5, 1964, 1979). Startle-like responses can be elicited from various points along this circuit by brief, single-pulse electrical stimuli. The purpose of this study was to evaluate how variables that are known to alter acoustic startle would alter 'startle' electrically elicited from selected structures.

Rats were implanted with chronic bilateral electrodes in either the ventral cochlear nucleus (VCN), or ventral sites in the nucleus reticularis pontis caudalis (NRPC). During testing, startle was elicited electrically by bilateral, single, cathodal pulses (1 msec, .05-.2 mA) to each site.

Similar to acoustic startle, electrically elicited 'startle' amplitude at both sites was directly related to stimulus intensity and inversely related to interpulse interval over a range from 0.5 to 32 sec (temporal recovery process). In a pre-pulse modification paradigm, a weak noise burst (70 db, 20 msec) with a lead time of 10 msec produced marked facilitation of 'startle' amplitude elicited from the VCN but only weak and non-significant facilitation of 'startle' amplitude when elicited from the NRPC. On the other hand, at 80 or 120 msec lead times, the pre-pulse depressed startle amplitude elicited from either site by about the same amount. These results suggest that pre-pulse facilitation, reported for acoustic startle, may be mediated primarily by an action on structures in the acoustic startle circuit prior to the NRPC, whereas pre-pulse inhibition may be mediated by an action on the NRPC or structures beyond.

Like acoustic startle, repetitive elicitation of electrical 'startle' from the VCN using interpulse intervals of 30 sec resulted in an initial increase in 'startle' amplitude (sensitization) followed by a gradual decrease in amplitude (habituation). In contrast, these same procedures resulted in a marked increase in amplitude across the session (sensitization) when 'startle' was elicited from the NRPC. These results suggest that under these conditions, habituation may be mediated by effects on structures prior to the NRPC whereas sensitization may be mediated by actions on the NRPC or structures beyond.

268.8 THE EFFECTS OF SENSORY DEPRIVATION ON SPATIAL RESPONSES OF HIPPOCAMPAL NEURONAL ACTIVITY IN RAT. P. J. Best and A. J. Hill. Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22901.

In rat, the activity of hippocampal cells is related to the animal's position in space. Maximum rates occur only within certain "spatial fields", specific for each hippocampal cell. In normal animals, if the field of a cell is determined in an open radial maze, and the maze is rotated, the field persists relative to the fixed surroundings and does not shift with the maze. Entorhinal lesions disrupt this spatial persistence and impair performance of a spatial task on the maze.

Neuroanatomical studies have indicated that entorhinal cortex is the primary source of sensory afferents to hippocampus. The present study investigates the effects of sensory deprivation on spatial firing in hippocampus. Rats were made deaf by chronic injection of neomycin sulfate and blindfolded with an opaque rubber mask. Fields of place cells were determined as rats ran on an elevated 6-arm radial maze for sucrose solution. The maze was rotated and place fields again determined. In 10 out of 13 cases, spatial fields did not persist, but followed the maze. In the 3 cases in which spatial firing persisted, rats were spun rapidly at the same time the maze was rotated again. The procedure disrupted the persistence of these cells as well. The same animals were tested in a spatial task on a 17-arm radial maze. Although these animals did not perform quite as well as controls, their scores did not differ significantly from those of controls.

Although loss of auditory and visual cues effects hippocampal cellular activity in a manner similar to entorhinal lesions, the cue deficit has less of a debilitating effect on behavior.

268.9 POST-REINFORCEMENT SYNCHRONIZATION IN THE EEG IS A CORRELATE OF LEARNING ABILITY IN CATS, Cynthia M. Harris*, Glenn T. Livezey*, and Thaddeus J. Marczynski, Univ. Ill. Med. Ctr, Chicago, ILL., 60612.

Pavlov postulated that learning requires an internal inhibition of maladaptive and nonreinforced responses and that the inhibition is coupled with phasic suppression of arousal. Clemente, Sterman and Wyrwicka (1964) hypothesized that phasic bursts of alpha-like EEG activity during conditioned behavior reflects the strength of the internal inhibitory process.In agreement with their hypothesis we have postulated that this phenomena will correlate with learning ability in cats.

To test our hypothesis we recorded EEG in cats during and after behavioral training. We inspected the records for occurence and magnitude of alpha-wave like segments of slow high amplitude synchronized EEG activity following rewarded behavior. The correlation between this Post-Reinforcement Synchronization (PRS) and learning ability was subjected to statistical analysis.

We implanted 27 cats with epicortical stainless steel recording electrodes over the posterior marginal and ectosylvian gyri. Each cat was placed in a training chamber where 1 cc. aliquots of milk were presented via remote control by the experimenter observing the animal's exploratory behavior. Exploratory tendencies that led stepwise toward association of the milk reward with lever pressing were partially rewarded by the experimenter to direct the animal's choice of exploratory behaviors. Thus the training proceedure required flexibility of behavior by the animal to suppress actions that were only partially rewarded in favor of those that were more fully rewarded until the animal learned to consistently depress the lever with the paw. Band pass frequency filters and a Grass integrator allowed quantification of the 7-14 c/s EEG frequency characteristic of PRS. PRS magnitude was scored as a ratio of the deflection of the integrator trace during the PRS activity and the deflection during a comparable time period before or after this PRS segment. Learning rate was scored in terms of length of training time required for each cat.

Across the 25 cats which learned to lever-press a significant correlation was found between the mean magnitude of PRS and the time required to learn to lever press ($r=.77$, $P<.001$). Cats with small PRS responses showed a tendency to perseverate in non-effective behaviors and required 3-6 fold greater training periods compared with cats with large PRS responses.

A neural model to account for this correlation between learning ability and PRS magnitude is presented in a companion abstract (Ladien et al.). The model incorporates the notion of PRS involvement in response evaluation and learning consolidation. The model also incorporates behavioral pharmacology data supporting a role for opiate and cholinergic receptors in PRS generation. As both expectation of reward and stimulus reinforcement value are required for PRS manifestation, a neural substrate for a match/mismatch process is discussed.

268.10 AN EXPANDED PARSIMONIOUS MODEL OF THE BRAIN. K. Ladien, T.J. Marczynski, and C.M. Harris, Dept of Pharmacology, Univ. of Illinois Med Ctr., Chicago, IL 60612

Numerous studies have shown that cats develop an alpha-like post-reinforcement synchronization (PRS) centered over their parieto-occipital area during consumption of milk in a bar pressing paradigm. We report in a companion abstract (Harris et al) that slow learning cats after acquisition of response have poorly developed PRS as compared to fast learners. Also, PRS in well-trained cats is blocked in a dose-dependent fashion by naloxone and is augmented by low doses of morphine.*Such observations support the notion that PRS activity and an accompanying Reward Contingent Positive Variation (RCPV) slow cortical potential are dependent both upon expectations and the stimulus reinforcement value. Similarly to Vanderwolf, Lindsley and others, we have observed hippocampal theta rhythms during approach and irregular activity during consumption which may be a necessary, but not sufficient condition for the appearance of PRS. We discuss the role of hippocampal match/mismatch feedback processes within the context of phasic reticular inhibition upon task completion. A circuit diagram accounting for the neuroanatomical substrates of the expectation, reinforcement, and matching processes is presented. It is proposed that task-specific channels are set up between stimulus- and response-sets during learning. Operant behaviors may be mediated by hippocampal spatial and cognitive maps reflected in the convergence of processed sensory inputs via the entorhinal perforant path with reticular and reinforcement information through medial septal-fascia dentate cholinergic tracts. Channels reflect the temporally coherent ensemble activity of multiple areas leading from the selective attention to and categorization of a stimulus to the matching of this stimulus with a particular response. For PRS, hippocampal maps may help couple parieto-occipital, reticular, and endogenous reinforcement pathways which are cooperatively activated depending upon learned associations and expectations. These areas remain coupled until a response is completed. The PRS-RCPV is seen as a cortical correlate of stimulus-response evaluation and may be important both in channel consolidation as well as in preparing the animal to shift attention. Such evaluation effects may also correlate with the P300 activity observed in human evoked potentials during sensory and cognitive discriminations. This work represents an update and elaboration upon a previously presented model of PRS-RCPV and other electrophysiological data (Marczynski, Multidisciplinary Perspectives in Event-Related Brain Potential Research, D. Otto, ed, EPA, 1978).

* Enkephalinergic projections between and within the amygdalar nuclei and the bed nucleus of stria terminalis may partially underlie these morphine-related reinforcement effects.

269.1 MONOCULAR DEPRIVATION AFFECTS CELL MORPHOLOGY IN LAMINAE C AND C_1 IN CAT LATERAL GENICULATE NUCLEUS. D. M. Murakami* and P. D. Wilson (SPON: J. Myerson).

Monocular deprivation leads to reduced cell growth in the binocular segment of the deprived A-laminae of the cat lateral geniculate nucleus (LGN). However, in order to measure the effect of monocular deprivation on cells in the C-laminae, laminae C and C-2 receiving contralateral input must be discriminable from lamina C-1 receiving ipsilateral input, and this is not possible with only Nissl stained material.

In order to study the C-laminae, kittens monocularly deprived 20-38 weeks received a 25 µl injection of 20% horseradish peroxidase (HRP) in the posterior chamber of either the deprived or experienced eye. After 4 days survival, the animal was perfused with Karnovsky's solution, and 40 µm brain sections were reacted for HRP with TMB and counterstained with neutral red. The presence and absence of HRP reaction product in the optic tract terminals clearly defined all laminae within the LGN.

Soma sizes were measured at 1000x for over 100 cells in each lamina, with care taken to measure only cells clearly within the boundaries of a lamina and only those profiles containing a clear nucleolus and Nissl substance. A significant reduction in cell size was found in laminae C and C-1 as well as in the A-laminae. Cells in the deprived laminae relative to those in the non-deprived laminae were reduced 27% for lamina A, 35% for A-1, 25% for C, 28% for C-1, and 3% for C-2, with a significant difference for all laminae except C-2.

Previous reports concerning cells in the A-laminae have shown that Y-type relay cells are more affected by monocular deprivation than X-type relay cells. Since W-type relay cells are found in laminae C (the ventral portion), C-1 and C-2 of the LGN, our results suggest that the soma size of W-cells may also be affected by monocular deprivation (Supported by Biomedical Research Grant, UCR)

269.2 EFFECTS OF MONOCULAR EYELID SUTURE ON THE STRUCTURE OF PHYSIOLOGICALLY IDENTIFIED NEURONS IN THE DORSAL LATERAL GENICULATE NUCLEUS. Michael J. Friedlander, L.R. Stanford* and S. Murray Sherman. Dept. of Neurobiology and Behavior, State Univ. of New York, Stony Brook, NY 11794.

The relationship of neuronal structure to physiological type was investigated in the A laminae of the dorsal lateral geniculate nucleus of cats raised to adulthood with monocular eyelid suture. This was done by intracellularly injecting physiologically described cells with horseradish peroxidase (HRP) for subsequent morphological analysis. Intracellular recording, iontophoresis of HRP, and tissue processing was as previously described in this laboratory[1,2].

Twenty neurons from deprived animals (10 cells each from deprived and nondeprived layers), have been recovered, to date. The morphological properties of these cells have been compared to those of identified X- and Y-cells from normal animals[1,2]. The following similarities to and differences from normal X- and Y-cells have been seen: 1) Nondeprived Y-cells appear physiologically and morphologically normal. 2) Nondeprived X-cells appear physiologically normal. The nondeprived X-cells all have soma sizes at the upper end of the normal X-cell size distribution, but their morphology is otherwise unremarkable. 3) Deprived X-cells appear normal in soma size, but have some morphological dendritic properties usually associated with Y-cells. 4) Finally, deprived Y-cells, some with normal and some with abnormal physiological properties have been recovered. All of these deprived Y-cells have somata smaller than any normal Y-cell previously seen. The physiologically normal, surviving deprived Y-cells are small but otherwise morphologically like normal Y-cells. Some of the deprived Y-cells with unusual physiological properties show dendritic structure not associated with normal Y-cells.

1. Friedlander, M.J., C.-S. Lin, and S.M. Sherman, (1979) Science, 204, 1114-1117.
2. Friedlander, M.J., C.-S. Lin, and S.M. Sherman, Soc. Neurosci. Abstr., 5, 785, 1979.

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269.3 RETURN OF Y-CELLS IN THE LATERAL GENICULATE NUCLEUS OF MONOCULARLY DEPRIVED CATS. E.E. Geisert, P.D. Spear and A. Langsetmo.* Dept. of Psychology and Neuroscience Training Program, Univ. of Wisconsin, Madison.

Previous studies have shown that removing inputs from the experienced eye of monocularly deprived cats increases the ability of the deprived eye to drive striate cortex neurons. The present experiment investigated whether the loss of Y-cells that occurs in deprived laminae of the lateral geniculate nucleus of monocularly deprived cats also is reversed following removal of the experienced eye. Single cell recordings were made in the lateral geniculate of four groups of animals: normal adult cats; 7 mo. old monocularly deprived cats (MD); 7 mo. old monocularly deprived cats that had the experienced eye removed at 4 mo. of age (MD-DE); and 7 mo. old monocularly deprived cats that had the experienced eye removed and the deprived eye opened at 4 mo. of age (MD-DE-O). From 5-7 cats and 126-155 neurons were studied in each group.

In normal cats, 41.2% of the cells were Y-cells. In agreement with previous studies, monocular deprivation produced a reduction in the proportion of Y-cells encountered: 18.2% were Y-cells in deprived laminae of MD cats, and this is significantly lower than in normal cats (p=0.013, Mann-Whitney test). Removing the experienced eye did not produce an increase in the proportion of Y-cells if the deprived eye remained closed: 17.0% were Y-cells in MD-DE cats. This is significantly lower than in normal cats (p=0.001) but indistinguishable from MD cats. However, removing the experienced eye and opening the deprived eye did produce an increase in the proportion of Y-cells: 40.2% were Y-cells in MD-DE-O cats. This is significantly higher than in both MD (p=0.009) and MD-DE (p=.001) cats, but indistinguishable from normal cats.

The results of this study demonstrate several interesting dissociations. In striate cortex of monocularly deprived cats, the percentage of cells driven by the deprived eye increases when the experienced eye is removed (MD-DE), and this is not altered by opening the deprived eye (MD-DE-O). Therefore, striate cortex shows an increase in the percentage of neurons that can be driven by the deprived eye when there is no change in the percentage of Y-cells in the lateral geniculate nucleus (MD-DE cats), and the return of Y-cells in the lateral geniculate does not have a noticeable effect on the response properties of striate cortex neurons (MD-DE-O cats). Furthermore, in the lateral geniculate nucleus of MD-DE cats, the cells return to nearly normal size, while the percentage of Y-cells remains the same as in MD cats.

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269.4 BINOCULARLY MEDIATED INTERACTIONS APPEAR TO MODIFY THE PHYSIOLOGY OF THE ADULT LATERAL GENICULATE NUCLEUS AFTER MONOCULAR PARALYSIS M.G. MacAvoy* and W.L. Salinger. Dept. Psych., University of North Carolina at Greensboro, Greensboro, NC 27412.

Contrary to the traditional view, it has been established that the adult visual system is altered by sensory modifications. For instance, in the lateral geniculate nucleus (LGN) of the adult cat, the relative encounter rate for X-cells (X/Y ratio) is reduced by such perturbations of visual stimulation as chronic monocular paralysis (CHMP;<14 days), acute and chronic binocular paralysis, chronic monocular tenotomy, and reverse monocular deprivation.

To analyze mechanisms responsible for adult neural plasticity we have used the chronic monocular paralysis preparation. As previously reported, the X-cell encounter rate was reduced by CHMP in the three major laminae in the LGN contralateral to the paralyzed eye, even in the lamina which is innervated by the unoperated eye. This reduction of encounter rate for X-cells in the lamina innervated by the unoperated eye led Salinger et al. (1977) to suggest a binocular inhibitory interaction as a mechanism underlying the shift in the X/Y ratio with CHMP. Central to this hypothesis was the assumption that there is a parallel effect of CHMP in the LGN ipsilateral to the paralyzed eye. However, this assumption remained untested for technical reasons. The present experiment provided a test of this assumption by examining the effects of CHMP on the previously unstudied (ipsilateral) LGN.

The change in X/Y ratios in the ipsilateral LGN after monocular paralysis was found to be parallel to that previously reported for the contralateral LGN. Thus reductions were found in all major laminae of the LGN's of both hemispheres, whether the laminae were innervated by the mobile or the paralyzed eye. These findings of parallel effects validate the assumption central to the hypothesis that a powerful binocular inhibitory interaction is involved in the suppression of X-cells during chronic monocular paralysis. These results were particularly striking in light of the traditional picture of the LGN as an entity composed of monocular cells which are subject only to weak, subtle binocular interactions.

269.5 CELL SHRINKAGE IN GENICULATE NEURONS FOLLOWING BRIEF UNILATERAL BLOCKADE OF RETINAL GANGLION CELL ACTIVITY. Baruch Kuppermann* and Takuji Kasamatsu (SPON: D. Van Essen). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Tonic background activity in visual afferents is indispensable for maintenance of synapses in the adult brain (Lund and Lund, Science, 171, 1971). By brief reversible blockade of tonic retinal discharges, the functional connectivity of geniculo-cortical synapses can be modified in binocular cortical cells of kittens (Kasamatsu, Exp. Brain Res., 26, 1976).

Currently we have studied morphological changes in the dorsal lateral geniculate nucleus (LGN) and physiological changes in the primary visual cortex of kittens induced by monocular blockade of tonic retinal activity. We injected tetrodotoxin (TTX) intraocularly, 10 μg per dose given twice, 3 days apart, to 6-week old kittens. TTX reversibly blocks ganglion cell firing in the retina. Five kittens received unilateral intraocular injections of TTX and were kept either in the light or in a dark room for a week-long period of TTX-induced monocular deprivation. After one week, histological examination of the celloidin-embedded LGN showed cell shrinkage in the deprived laminae in both the animals kept in the light and in the dark. The magnitude of cell shrinkage in deprived laminae of the dark-animals was remarkable despite the brief, one week blockade of tonic retinal discharges. Cell shrinkage in the deprived monocular segment was also observed, as expected with a pure deprivation effect (Hickey et al, J. Comp. Neurol., 172, 1977).

Single unit recordings were made on five kittens subjected to unilateral intraocular TTX (10 μg per dose in two injections 3 days apart) and kept in the light or in a dark room until the recording session. The duration of effects of TTX on ganglion cell firing was determined by physiological recording in the cortex. The end of the effect coincided with reappearance of the pupillary reflex of the injected eye. The period of suppression was found to last from 11 to 13 days. In all recordings a striking loss of binocular cells was observed, with a larger number of cells driven exclusively through the contralateral eye than through the ipsilateral eye.

In summary, we found that total blockade of tonic retinal discharges could induce striking morphological changes in the LGN after only one week of treatment and also resulted in a decrease of the number of binocular cells in the cortex. These changes were found in animals which were kept in the dark as well as in the light throughout the TTX treatment.

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269.6 THE EFFECT OF BINOCULAR ENUCLEATION ON LAMINAR DEVELOPMENT IN THE DORSAL LATERAL GENICULATE NUCLEUS. J. K. Brunso-Bechtold and V. A. Casagrande, Depts. of Anatomy and Psychology, Vanderbilt University, Nashville, TN 37232.

In the tree shrew, the dorsal lateral geniculate nucleus (LGN) appears cytoarchitecturally homogeneous at birth. During the first 10 postnatal days, the six layers typical of the adult nucleus gradually become distinct (Brunso-Bechtold and Casagrande, 1980). The object of the present study was to determine the effects of retinal input on the development of LGN layers by removing both eyes of tree shrews on the first postnatal day. The animals were allowed to survive for several intervals up to maturity (3 mo.) and then sacrificed, the brains embedded in celloidin, cut,and stained using standard procedures.

Our main results can be summarized as follows: First, in the adult bilateral enucleate the presence of layers can be distinguished on the basis of cellular morphology and packing density, although such distinctions are not as obvious as in the normal adult nucleus. This suggests that LGN cells can undergo maturation without retinal input. However, comparisons between bilaterally enucleated and normal animals sacrificed on the 8th postnatal day, indicate that the maturation of LGN neurons is slowed by the absence of retinal input. Second, our results show no evidence of clear interlaminar spaces in the absence of retinal input. Since it is unlikely that removal of afferents could prevent cell death, this result would seem to argue that interlaminar spaces form as a result of migration or cell movement rather than cell death. Furthermore, although the interlaminar spaces receive heavy input from superior colliculus and cortex (Casagrande, 1974), their presence in cases with bilateral enucleation demonstrates that these afferents are not sufficient to induce the formation of interlaminar spaces. Moreover, the lack of clear interlaminar spaces after 1, 2, and 3 weeks indicates that the absence of interlaminar spaces at 3 months is not a secondary loss. Finally, bilateral enucleation at birth appears to have a much less profound effect on LGN organization than does unilateral enucleation (unpublished observations). In cases with unilateral enucleation, the deafferented layers can scarcely be distinguished, and the nucleus appears dominated by the layers still receiving retinal input. This suggests that additional factors such as binocular interactions may play a major role in the development of LGN.

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269.7 LOSS OF RETINAL X-CELLS IN CATS WITH NEONATAL VISUAL CORTEX REMOVAL. P.D. Spear, L. Tong, R.E. Kalil, and E.C. Callahan*. Depts. of Psychology and Ophthalmology, and Neuroscience Training Program, Univ. of Wisconsin, Madison, WI 53706.

Previous anatomical experiments indicate that transneuronal retrograde degeneration of retinal ganglion cells occurs following neonatal removal of visual cortex in both cats and monkeys. In addition, size measurements and the central projections of the remaining ganglion cells have led to the suggestion that the degeneration selectively affects the X-cell functional class. We tested this possibility directly by recording from retinal ganglion cells in cats that had visual cortex removed neonatally.

Kittens received unilateral lesions of areas 17, 18, and 19 within 24 hours of birth, and they were studied when they were 13-14.5 months old. Recordings were made from single ganglion cell somas using intraocular microelectrodes. Cells were sampled from the nasal retina of the eye contralateral to the lesion; i.e., from the portion of the retina that normally projects exclusively to the hemisphere with the visual cortex lesion. An area of retina spanning 5°-15° from the area centralis and 8° above to 8° below the zero horizontal meridian was sampled systematically. Recordings also were made in normal adult cats using identical sampling procedures. Ganglion cells were classified as X, Y, or W on the basis of a large battery of tests, including the presence of linear or non-linear spatial summation, cut-off velocity, receptive field size, and response latency to optic chiasm stimulation.

Thus far, recordings have been made from four normal cats and three cats with neonatal visual cortex lesions. In normal cats, cell body recordings were encountered on about 40% of the retinal penetrations. Among 85 ganglion cells that have been studied, 50% were X-cells, 33% were Y-cells, and 17% were W-cells. In cats with neonatal visual cortex removal, cell body recordings were encountered on only about 25% of the retinal penetrations. Among 60 ganglion cells that have been studied, only one (1.5%) was an X-cell while 62% were Y-cells and 36.5% were W-cells. It is interesting to note that the decrease in ganglion cell sampling density (% of penetrations in which a cell body recording was encountered) can be accounted for entirely by the decrease in X-cells encountered. In addition, the ratio of remaining Y-cells to W-cells encountered in cats with neonatal lesions is about the same (1.7:1) as that in normal cats (2:1). Therefore, the results indicate that the neonatal visual cortex lesions produce a severe loss of retinal ganglion cells in the X-cell functional class, and that retinal Y-cells and W-cells are unaffected by the lesions.

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269.8 RETROGRADE DEGENERATION OF RETINAL GANGLION CELLS FOLLOWING REMOVAL OF VISUAL CORTEX IN THE NEWBORN KITTEN. R.E. Kalil, Dept. of Ophthalmology, Univ. of Wisconsin, Madison, WI 53706.

It is well known that ablation of visual cortex in the adult cat produces retrograde cell degeneration in the dorsal lateral geniculate nucleus. Removal of visual cortex in the newborn kitten also causes retrograde cell changes in the primary visual pathway and these changes appear to be more extensive than those which follow adult lesions. Thus damage to visual cortex in neonatal animals leads not only to cell death in the lateral geniculate nucleus, but also, as the present findings demonstrate, to transsynaptic retrograde degeneration of ganglion cells in the retina.

The degeneration of retinal ganglion cells has been studied quantitatively in cats from which visual cortex had been removed on the day of birth. As adults, the animals were perfused with phosphate buffered glutaraldehyde and a whole mount of the retina from each eye was prepared, and then stained with cresyl violet. The cross-sectional, perikaryal areas of ganglion cells were measured at two locations in each retina. Both locations were approximately 2.5 mm above the area centralis, and either 1.0 mm nasal or temporal to it. In addition, all large ganglion cells (cross-sectional area 800 μ^2 or greater) were counted in a 45 mm^2 area of the upper quadrants of the nasal and temporal hemiretinae of each eye.

Cell size frequency distributions were constructed from the cross-sectional area measurements. The distributions from the temporal and nasal retinae ipsilateral and contralateral, respectively, to the early cortical lesion show a 60% to 70% reduction in the number of medium sized ganglion cells (cross-sectional areas 300 μ^2 to 800 μ^2), but little or no change in the number of small ganglion cells (cross-sectional area less than 300 μ^2). Large ganglion cells appear to be unaffected by early removal of visual cortex insofar as their density and cross-sectional area are not altered.

These results indicate that damage to visual cortex in the newborn kitten leads to specific retrograde transneuronal changes in the ganglion cells of the retina: medium sized cells are reduced in number dramatically, whereas small and large ganglion cells are unaffected. Functionally, these results predict a deficit in X type ganglion cells but not in W- or Y-cells.

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270.1 MECHANISM OF ACTION OF PHENCYCLIDINE (PCP) IN THE CNS: AN IONTOPHORETIC STUDY. S.N. Raja*, and P.G. Guyenet. Univ. of Virginia, Dept. Pharmacology, Charlottesville, VA 22908.

The following electrophysiological experiments were undertaken to determine the effects of PCP on noradrenergic (NE) and dopaminergic (DA) neurons and to examine whether the drug alters the postsynaptic action of ACh, NE, DA, opioid peptides, Glu, GABA and substance P. Single unit recordings were made in chloral hydrate anesthetized rats using single or multi-barrel glass micropipettes. NE neurons were recorded in the locus coeruleus (LC), DA neurons in the substantia nigra pars compacta (SN). The rest of the recordings were obtained for CA1 hippocampal pyramidal cells (CA1 cells) or cerebellar purkinje cells (P cells).

PCP (0.75-1.8 mg/kg i.v.) produced a 55-65% decrease in the spontaneous firing rate (SFR) of all 6 NE neurons tested, lasting for 15-20 min. Iontophoretic PCP (0.2M, pH 4.0, 10-20nA) also decreased the SFR of these cells by 50 to 100%. In the LC, PCP (3-7nA) inhibited the action of three excitatory neurotransmitters (ACh, Glu, subs P) to approximately the same degree while the action of inhibitory substances was unchanged (met-enkephalin, GABA and clonidine). The antagonism by PCP of excitatory transmitters was observed only at doses which also decreased the SFR of LC cells; in contrast, the local anesthetic procaine antagonized the excitation caused by ACh and Glu without altering the SFR.

PCP (1.0-1.8 mg/kg i.v.) increased the SFR of a fraction of DA cells in the SN characterized by a slow (1-3/sec) firing rate (5 cells) and did not alter the SFR of the others (7 cells). PCP pretreatment (5.2-10 mg/kg i.v., 10 min) produced a 2.3 fold increase in the average dose of i.v. amphetamine necessary to inhibit the SFR of DA cells. Iontophoretic PCP (20-40nA) slightly inhibited the SFR of DA neurons and did not alter the inhibitory response to iontophoretic DA. These results demonstrate that PCP is neither a dopaminergic antagonist nor an amphetamine-like drug and suggest that the action of PCP on DA neurons must be indirect, possibly via a decrease in the activity of a striatonigral feedback.

From these and additional experiments conducted with CA1 and P cells the following generalizations could be made: 1) iontophoretic PCP depressed the SFR of all cells recorded 2) PCP antagonized the effects of all excitatory neurotransmitters tested and didnot alter the action of inhibitory transmitters (GABA, opiates, NE at α or β receptors) but its effects were qualitatively different from that of procaine 3) all effects of PCP (i.v. or local) could be mimicked with doses of the related anesthetic ketamine 3 to 10 times larger. Supported by NIDA grant # 1R01DA 02310-01.

270.2 EFFECT OF PHENCYCLIDINE (PCP) ON ELECTROSHOCK-INDUCED SEIZURES AND ON GLUTAMIC ACID DECARBOXYLASE ACTIVITY OF THE MOUSE BRAIN. Marie T. Spoerlein, Christina VanderWende and William Pritchard*. Rutgers University, Coll. Pharmacy, Box 789, Piscataway, N.J. 08854.

Male, Swiss Webster mice were administered various doses of PCP intraperitoneally. The animals were sacrificed by decapitation 35 min. after the injection; the brains were removed and dissected over a bed of crushed ice to isolate the cerebellum, striatum and hippocampus. Glutamic acid decarboxylase (GAD) was assayed by a modification of the method of Lowe, Robins and Eyerman (J. Neurochem. 3: 8-18, 1958) to scale it up to a more macro procedure. Each area was homogenized in 3.0 ml cold distilled water and centrifuged at 1,000 g for 20 min. One half of 1.0 ml was added to 0.5 ml of buffered substrate mixture containing pyridoxal phosphate and sodium glutamate to give final concentrations of 0.5 mM and 25 mM, respectively in 0.1 M phosphate buffer, pH 6.4.

PCP caused a 15 % increase of GAD at 5 mg/Kg and a 27% increase at 10 mg/Kg in the hippocampus, but at higher doses where the animals exhibited increased excitability and seizures, there was no difference from the control. There was no effect of PCP on GAD in the cerebellum or striatum at 10 mg/Kg.

PCP was examined for anticonvulsant activity at doses that caused no seizure activity in themselves. Animals were protected against maximal electroshock-induced seizures at 35 min. after injections in a dose related manner. At 10 mg/Kg, 100% of the animals tested were protected whereas at 5 mg/Kg and 2.5 mg/Kg, 60% and 50% of the animals were protected, respectively.

Although it cannot be definitely concluded from these data, the increase of GAD activity in the hippocampus may relate to the ability of PCP to prevent seizures.

270.3 BUSPIRONE: A MODEL FOR ANXIOSELECTIVE DRUG ACTION. Duncan P. Taylor, Deborah K. Hyslop* and L. A. Riblet. Biologic Research, Mead Johnson Pharmaceutical Division, Evansville, IN 47721.

Buspirone has been shown to be an effective antianxiety agent at the same dose as diazepam in a double-blind placebo-controlled study of psychoneurotic outpatients with a primary diagnosis of anxiety neurosis (Am. J. Psychiatry 136: 1184). Behavioral studies in animals as well as clinical observations have revealed that buspirone possesses an anxioselective pharmacological profile. Specifically, while buspirone shares diazepam's antianxiety properties, buspirone does not elicit sedative-hypnotic, anticonvulsant, or muscle relaxant effects. Further, buspirone exhibits minimal interaction with CNS depressants and does not display any potential for physical dependence or abuse in animal models. In in vitro binding experiments buspirone exhibits minimal inhibition of [^{3}H]-ligand binding at α_1, α_2, β, ACh, glutamate, glycine, H_1, H_2, opiate, 5-HT_1, and 5-HT_2 receptors. Notably, buspirone neither inhibits nor stimulates [^{3}H]-benzodiazepine binding, is without effect on the stimulation of binding by GABA or halide anions, and does not interfere with GABA binding or uptake. Buspirone interacts with reasonable potency only at the dopamine receptor. Buspirone appears to be an antagonist (100 µM GTP produces no shift in K_i) of lower potency (K_i=90 nM) at [^{3}H]-spiperone binding sites (postsynaptic) and as an agonist (a GTP-induced loss of inhibitory potency is present) of higher potency (K_i=18 nM) at [^{3}H]-N-propylnorapomorphine binding sites (presynaptic). Preliminary experiments indicate that the concentration of buspirone in the brain is of the order of 3 nM after therapeutic dose levels. This is consistent with the hypothesis that buspirone interacts at low concentrations at a receptor responsible for the relief of anxiety, and at higher concentrations at receptors responsible for other dopamine-mediated responses. However, unlike spiperone or other postsynaptic dopamine receptor antagonists, buspirone does not cause catalepsy. If presynaptic inhibitory dopamine receptors are located on dopaminergic neurons, stimulation of these receptors should inhibit dopaminergic transmission. If DA receptors are located on synaptic endings of other neurons (NE, ACh, peptides), stimulation of these receptors would alter nondopaminergic forms of neurotransmission. For instance, disinhibition of central noradrenergic transmission might be responsible for the electrocortical activation observed in animals treated with buspirone (Fed. Proc. 39: 752). A dose-dependent biphasic response to buspirone has been observed in vivo which provides pharmacologically revelant support for this hypothesis.

270.4 TOLERANCE FOLLOWING CHRONIC BENZODIAZEPINE TREATMENT: CHANGES IN BEHAVIOR AND IN NUMBER OF RECEPTORS. Howard C. Rosenberg and Ted H. Chiu*. Dept. of Pharmacology, Medical College of Ohio, Toledo, OH. 43699.

Previous reports from this laboratory have shown that intensive treatment of rats with the benzodiazepine flurazepam (FZP) caused an apparent reduction in the number of benzodiazepine receptors (Life Sci. 23:1153, 1978). This is a specific effect since similar treatment with barbital caused no significant change in benzodiazepine binding (Life Sci. 24:803, 1979). The present report describes further research into the time course of change in receptors, and the possible role of receptor modification in tolerance.

FZP was dissolved in a .02% saccharin solution and rats were allowed free access to this as their only liquid supply. Control rats were given saccharin solution to drink. Based on each rat's weight and volume of fluid consumed, FZP was added so that each rat would consume a dose of 100 mg/kg daily for the first week, then 150 mg/kg daily for the remainder of the treatment. Specific ^{3}H-flunitrazepam (^{3}H-FNP) binding to cerebral cortical membranes was studied following different durations of treatment. After 28 or 56 days of treatment, there was a highly significant decrease of 18-22% in the maximal binding capacity (B_{max}). Treatment for 7 or 14 days had no effect on B_{max}. Another series of rats received 28 days of treatment, then undrugged saccharin solution was given for various times before performing ^{3}H-FNP binding assays. B_{max} was reduced up to 12 hrs after the end of chronic treatment, but returned to control value by 24 hrs. There was no significant change in the dissociation constant (K_D) in any group.

The ataxia produced by acute i.p. FZP (20 to 200 mg/kg) was measured in control rats and in rats that had been treated for 28 days with FZP. Ataxia was rated on a scale of 0 (normal gait) to 3 (unable to walk at all). Dose-response analysis showed significant tolerance in rats tested 12 hrs after terminating chronic FZP consumption. However, in rats tested 24 hrs after the end of chronic treatment, tolerance had been lost. Thus, chronic FZP caused adaptation that was seen both as a reduction in the number of benzodiazepine receptors, and as tolerance to FZP-induced ataxia. Both measures returned to control between 12 and 24 hrs. after stopping chronic treatment. This suggests that the ataxia produced by FZP is mediated by specific benzodiazepine receptors, and the observed tolerance is a result of reduction in the number of benzodiazepine receptors.

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270.5 A GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC IDENTIFICATION OF FLURAZEPAM AND ITS METABOLITES IN PLASMA AND CEREBROSPINAL FLUID OF THE CAT. C. C. Chiueh, M. Ohata*, S. I. Rapoport, W. Mendelson and D. Greenblatt*. Lab. of Neurosciences, NIA, NIH, Baltimore City Hospitals, Baltimore, MD 21224; Lab of Clinical Psychopharmacology, NIMH, Bethesda, MD 20205; Clinical Pharmacology, Tufts-New England Medical Center Hospital, Boston, MA 02111.

The metabolic fate of flurazepam has been studied in animals and humans by using radioactive tracer techniques, spectrophotofluorometry and gas chromatographic techniques. Most of these studies described the pharmacokinetics of flurazepam in plasma or urine samples and indicate that N_1-desalkyl flurazepam is slowly biotransformed and accumulated during chronic therapy. Because the N_1-unsubstituted metabolite of this drug is pharmacologically active, it may be responsible for the unwanted toxic effects of flurazepam in the elderly following a 30 mg per night dose regime. The present experiments were designed to study the accumulation of lipophilic metabolites in the central nervous system of the cat after i.v. administration of 30 mg/kg of flurazepam. A gas chromatographic-mass spectrometric procedure is used in order to identify flurazepam and its metabolites in cerebrospinal fluid and plasma samples. A positive identification of benzodiazepines by mass spectrometry requires at least 50 to 100 ng per injection of sample into the Hewlett-Packard-5992 gas chromatographic-mass spectrometric system. Results confirm that the major metabolite of flurazepam in the blood was the N_1-desalkyl analogue, which accumulated significantly up to 24 hr after administration of a single dose of flurazepam. A positive identification of unchanged and N_1-desalkyl flurazepam in the cerebrospinal fluid was obtained 60 min after i.v. administration of the drug. There was an accumulation of this N_1-unsubstituted metabolite in the cerebrospinal fluid 24 hr after a single dose of flurazepam. The half life of flurazepam in plasma and cerebrospinal fluid appeared to be less than 2 hr. In summary, after a single i.v. administration of flurazepam, the unchanged analogue disappears quickly in plasma and cerebrospinal fluid while the N_1-desalkyl metabolite is slowly accumulated in blood and cerebrospinal fluid.

270.6 EFFECTS OF PHENYTOIN ON [^{3}H]DIAZEPAM BINDING IN DISSOCIATED PRIMARY CORTICAL CELL CULTURE. D. W. Gallager, P. Mallorga,* K. Swaiman* and P. Nelson. Biological Psychiatry Branch, NIMH and Lab. of Developmental Neurobiology, NIH, Bethesda, MD 20205

Primary cell cultures of fetal mouse cortex were grown in the presence of various concentrations of the anticonvulsant drug phenytoin (DPH). In these cultures, total [^{3}H]diazepam binding successively decreased in a dose-dependent manner, decreasing to 39% of control when maintained for 7 days in media containing 50 µg/ml DPH. Pharmacological characterization of [^{3}H]diazepam binding was accomplished by measuring the ability of a centrally active benzodiazepine, clonazepam (CLON), and a centrally inactive benzodiazepine, RO5-4864 to displace [^{3}H]diazepam binding (see abstract this volume, Mallorga et al.). In DPH-treated cultures, a dose-dependent decrease in CLON displaceable [^{3}H]-diazepam binding was observed decreasing to 6% of control culture binding at the 50 µg/ml dose of DPH. Scatchard analysis of membranes from DPH and control cultures showed that the decrease in CLON-displaceable [^{3}H]diazepam binding was due to a decrease in the total number of binding sites without a significant change in [^{3}H]diazepam binding site affinity. For example, membranes from cells grown in culture with media containing 25 µg/ml DPH had a K_D = 9.6 nM and B_{max} = 478 fmol/mg protein as compared to binding in control cultures with a K_D = 7.2 nM and B_{max} = 645.4 fmol/mg protein. The ratio of 10^{-7} M CLON/10^{-7} M RO5-4864 displaceable binding progressively decreased with increasing doses of DPH from 6.5 in non-DPH treated cultures to 0.15 in cultures treated with 50 µg/ml DPH. These changes can be correlated with DPH dose-dependent decreases in neuronal cell number as documented in a parallel cell culture study (see abstract this volume, E. A. Neale et al.). Decreases in the total number of [^{3}H]diazepam binding sites in fetal cortical cells maintained in culture with DPH are also consistent with decreases in [^{3}H]diazepam binding site number observed in cortical membranes prepared from pups exposed in utero to DPH (Gallager and Mallorga, Science, 208:64, 1980). These data are consistent with the possibility that DPH causes a dose-dependent reduction in developing cortical neurons containing [^{3}H]diazepam binding sites.

270.7 PHENYTOIN INCREASES THE SPONTANEOUS RELEASE OF ^{3}H-GABA IN CRUDE SYNAPTOSOMAL FRACTIONS OF RAT BRAIN. A. D. Vanker and S. J. Prevost*. Information Science Group, Univ. of Missouri, Columbia, MO 65211 and Dept. of Biology, Georgia State Univ., Atlanta, GA 30303.

Sprague-Dawley rats were given daily intraperitoneal injections of phenytoin at 10 mg/kg for 0-28 days. The animals were sacrificed by decapitation and the cerebral cortex divided into hemispheres. Each hemisphere was minced with a razor blade into prisms with sides of approximately 1 mm. One mince was used immediately to obtain a crude synaptosomal fraction for uptake studies using standard methods. The other was placed in a physiological buffer containing ^{3}H-gamma-aminobutyric acid (^{3}H-GABA) at 37°C and incubated under oxygen for 10 min, 'preloading' the various elements of the mince with ^{3}H-GABA. This mince was then used to obtain a crude synaptosomal fraction. Approximately 40 min elapsed between the end of the incubation and the actual release experiments.

Low-affinity and high-affinity uptake of ^{3}H-GABA were not affected in any experimental group; neither was elevated K^+-induced release of ^{3}H-GABA from the 'preloaded' fractions. Spontaneous release of ^{3}H-GABA, however, was significantly increased in all experimental groups except day 0. No significant inter-group differences were observed. It is apparent from our data that phenytoin affects a GABA pool not directly involved in the uptake or depolarization-induced release of GABA. This pool could be synaptosomal, glial, or perhaps even mitochondrial in origin. If it occurs into the synaptic region, the increased spontaneous release of this inhibitory neurotransmitter may be part of the anticonvulsant mechanism of action of phenytoin.

This research was supported by a grant from the Epilepsy Foundation of America.

270.8 EFFECTS OF γ-VINYL GABA ON CHEMICALLY INDUCED SEIZURES IN MICE: CORRELATION WITH BRAIN GABA CONTENT. D.A. Kendall,* D. Fox and S.J. Enna. Depts. Pharmacol. and Neurobiol. and Anat., Univ. Texas Medical School, Houston, Texas 77025

A number of inhibitors of GABA transaminase (GABA-T), the enzyme responsible for the metabolism of this neurotransmitter, have been reported to be effective in attenuating audiogenic or chemically induced convulsions. Curiously, however, while these drugs presumably act by enhancing GABAergic transmission, they tend to be substantially less effective against seizures induced by bicuculline, a GABA receptor antagonist, than against other types of convulsants. In the present investigation, the neurochemical and anticonvulsant effects of γ-vinyl GABA (GVG), an irreversible inhibitor of this enzyme, were investigated in an attempt to better understand the mechanism of action of this agent.

Male mice (Timco) were injected with 1500 mg/kg GVG and the protection against bicuculline (3 mg/kg s.c.), picrotoxin (10 mg/kg s.c.), isoniazid (250 mg/kg s.c.) and strychnine (2 mg/kg i.p induced seizures was determined at 4 and 24 hr after administration of GVG. The characteristics of the seizures induced by these agents differed with regard to severity and latency to clonus and tonus, presumably because of their differences in potency and sites of action. The parameters measured were time to first clonus, loss of righting reflex, onset of tonus and death. At the 4 hr time, significant protection was noted against all convulsants except bicuculline. Surprisingly, at 24 hr, while the degree of protection against picrotoxin, isoniazid and strychnine had substantially diminished, bicuculline-induced seizures were now significantly inhibited. Neurochemical analysis revealed that brain GABA content was elevated 15-fold at 4 hrs, but had declined to 7-fold at the 24 hr time point. In order to determine whether the time after injection or the brain GABA levels correlated best with the apparent selectivity of the anticonvulsant effect, animals were treated with a dose of GVG (1000 mg/kg) which elevated brain GABA 7-fold at the 4 hr time. In this case, bicuculline seizures were inhibited in a manner similar to that seen at 24 hr after the larger dose of GVG.

The results suggest that brain GABA content must be substantially elevated to inhibit chemically-induced seizures. Furthermore, the anticonvulsant effect appears to be somewhat selective in that protection against bicuculline-induced seizures occurs only within a narrow dose range of GVG. Thus, clinically, the antiepileptic dose of GVG may vary, not only with severity, but also with the type of seizure being treated. (Supported in part by USPHS grants NS-13803 and NS-00335).

270.9 THE ACTIONS OF VALPROIC ACID ON DORSAL ROOT FIBERS IN THE FROG SPINAL CORD. John C. Hackman, Robert A. Davidoff and Victoria Grayson.* Neurophysiology Laboratory, Neurology Service, V.A. Hospital and Department of Neurology, University of Miami School of Medicine, Miami, Florida 33101.

Valproate (n-dipropylacetate, DPA), an effective anticonvulsant, is thought to act by increasing CNS GABA levels. But recently it has been shown to augment GABA-induced inhibition of neurons. Our present experiments were designed to evaluate the effect of DPA on GABAergic synapses located on primary afferent fibers.

We used the hemisected frog spinal cord continuously superfused with HCO_3^--buffered Ringer's solution maintained at 15°C. Synaptic and amino acid-induced responses were recorded from dorsal roots (DR) using sucrose gap techniques.

DPA, added to the superfusate in concentrations of 10^{-5}M or greater, usually produced a small depolarization of primary afferent terminals (∿ 0.5mV). This potential change did not appear to result from activation of GABA receptors or Cl^- ionophores since it was not blocked by picrotoxin, bicuculline, piretanide or low $[Cl^-]_o$. Nor did it result from release of transmitter from GABAergic synapses, since DPA did not affect the spontaneous efflux of preloaded [^{3}H]GABA from cord slices. Futhermore, there was no "cross-desensitization" between GABA and DPA.

DPA (10^{-5}M) enhanced the amplitude (130%) and duration of DR depolarizations produced by applications of GABA [and depressed those caused by K^+ (10mM), glutamate and aspartate] in both normal and Mn^{++} (1.5mM)-Ringers. Two processes contributed to the effect: (1) The fading ("desensitization") of GABA-depolarizations seen with prolonged applications of the amino acid was suppressed by DPA; (2) DPA significantly reduced the high-affinity uptake of [^{3}H]GABA by cord minislices (1% of control uptake). The postsynaptic potentiating effect of DPA may not be specific for GABA, since DPA also increased the size of the DR responses elicited by β-alanine and taurine applications.

The anticonvulsant depressed and shortened dorsal root potentials elicited by DR or ventral root stimulation. This effect may be caused by the ability of DPA to reduce (40%, 10 min, 10^{-5}M) the K^+-induced efflux of [^{3}H]GABA from cord slices and to the decreased release of K^+ induced by tetanic DR stimulation (as measured with K^+-sensitive electrodes).

These effects of DPA may contribute to its anticonvulsant properties. (Supported by V.A. funds, MRIS 1769).

270.10 METABOLISM OF L-DOPA AND DEUTERATED L-DOPA IN RAT BRAIN: CONVERSION INTO METABOLITES OF NOREPINEPHRINE. David J. Edwards and Marguerite Rizk*. Dept. of Pharmacology-Physiology, Univ. of Pittsburgh, Sch. Dent. Med., Pittsburgh, PA 15261.

The therapeutic effects of L-DOPA in Parkinson's disease is generally attributed to its conversion to dopamine (DA), thereby overcoming the deficiency of this neurotransmitter in the basal ganglia. However, several studies show effects of L-DOPA on norepinephrine (NE) metabolism in brain, and other studies suggest that NE may play a role in some of the behavioral effects of L-DOPA. We have developed techniques utilizing gas chromatography/mass spectrometry for analyzing the neutral deaminated metabolites of DA, NE and related amines (Edwards, et al., J. Chromatogr. Biomed. Applic. 164, 407, 1979), which we now have applied to studying the effects of L-DOPA and its deuterated analogue in the rat. Urine metabolite studies revealed that the excretion of 3,4-dihydroxyphenylglycol (DHPG), a NE metabolite, was elevated 2.3-fold in male Sprague-Dawley rats (240-300 g) injected i.p. with 150 mg/kg L-DOPA as compared to control animals (22 ± 3 µg/24 hr in experimental vs. 9.4 ± 1.1 µg/24 hr in controls, $p < .01$), but the excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG) was not significantly changed (63 ± 8 vs. 60 ± 8 µg/24 hr in experimental and control animals, respectively). In separate experiments, brain levels of MHPG and DHPG were measured in brain samples of rats injected with L-DOPA. Both MHPG and DHPG as well as the corresponding DA metabolites reached a maximum 1 hr after injection. MHPG and DHPG concentrations in whole brain were increased by 78% ($p < .001$) and 134% ($p < .02$), respectively, 1 hr after an injection of 150 mg/kg L-DOPA. MHPG and DHPG concentrations were also increased in each of the specific brain regions examined. Since the increases in MHPG and DHPG could be due to either an increase in NE synthesis (via conversion from the exogeneously administered L-DOPA) or to an increased turnover of the endogeneous NE content, experiments were carried out using deuterium-labeled L-DOPA (L-DOPA-d3) to distinquish between these two possibilities. MHPG and DHPG and the corresponding d3-labeled analogues were measured in the cerebellum and "rest of brain" of rats injected with either 50 or 150 mg/kg L-DOPA-d3 one hour before sacrifice. The concentrations of unlabeled MHPG and DHPG showed small, but nonsignificant, changes in the two brain parts. MHPG-d3 concentrations in the cerebellum and "rest of brain" of rats injected with 150 mg/kg L-DOPA-d3 were 77 and 80 ng/g, respectively; and DHPG-d3 concentrations were 82 and 61 ng/g, respectively. These amounts of deuterated metabolites in tissues account for almost all of the increases in MHPG and DHPG observed in experiments using unlabeled L-DOPA. Thus, these results demonstrate that the increases in MHPG and DHPG produced by L-DOPA is primarily due to the conversion of L-DOPA to NE. (Supported by grant MH-28340 from NIMH).

270.11 INTERACTIONS BETWEEN HARMALINE(HAR), RESERPINE(RES) AND APOMORPHINE(APO) ALTER CENTRAL AND PERIPHERAL CATECHOLAMINE(CA) METABOLISM. S.G. Speciale*, F.Karoum and R.J. Wyatt(SPON: R.M. Stewart). Dept. Psychiat., U. Tx. Hlth. Sci. Cntr., Dallas, TX 75235 and Lab. Clin. Psychopharm., NIMH, St.Elizabeths Hosp., Wash., D.C. 20032

The mammalian sympathetic ganglion is a useful model of neuronal circuitry and transmitter interaction. It contains cholinergic preganglionics that synapse on noradrenergic (NE) cell bodies (sending postganglionic fibers to target organs) and interneurons, which, in some species, are dopaminergic (DA) and possibly peptidergic. We have used pharmacological approaches to manipulate CA metabolism and compare effects in the celiac ganglion (cg) with several brain regions. We examined the interactions of a monoamine oxidase (MAO) type A inhibitor, HAR (since ganglia contain type A almost exclusively and brain contains types A and B), the DA agonist APO and RES, a CA depletor.

Male Sprague-Dawley rats (200gms) were utilized in all experiments. Drug dosages were adjusted to standardize sacrifice time to 150 minutes. HAR (1mg/kg,ip) elevated NE, but not DA or its metabolite, dihydroxyphenylacetic acid (DOPAC) in the cg. APO (2.5 mg/kg, ip) similarly increased NE without changing DA or DOPAC. RES (1mg/kg,sc) lowered cg NE 75% and DA 56%, while DOPAC rose over 5-fold. APO+HAR combination did not differ significantly from that of HAR or APO alone. RES+HAR resulted in less amine depletion (NE 38% Control (C), DA 76% C) and completely antagonized the RES-induced increase in DOPAC (expected by blocking catabolism).

CA metabolism was also measured in the hypothalamus (hpth) and caudate (cn) of the same animals. In the hpth HAR did not change DA or its metabolites, but reduced NE and its metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG). APO did not alter NE, DA or their metabolites. RES significantly depleted NE (20% C) and DA (7% C), while DA metabolite homovanillic acid (HVA) and MHPG only changed slightly. APO+RES antagonized the CA depletion (NE 56% C, DA 60% C) and metabolites remained unchanged. HAR+RES even further reversed depletion (NE 83% C, DA 139% C), while metabolites were unchanged.

In the cn HAR did not alter CA concentrations but DOPAC and HVA decreased 40%. Apo produced very similar changes. RES alone depleted NE (14% C) and DA (3% C), while DOPAC and HVA increased over 2-fold. As in hpth, HAR+RES increased DA 30% and restored NE levels, however metabolites were significantly reduced (>60%).

Brain and cg CA metabolism responded differently to these drugs. HAR antagonized RES-induced metabolite increase but HAR did not elevate amines greatly in any tissue, as MAO inhibition should do. At these doses HAR and APO had similar actions, alone or combined with RES, in cg and cn, suggesting a common DA-like mechanism, different from MAO inhibition. Decreases in cn DA metabolites without amine changes suggest HAR and APO reduce DA turnover.

270.12 DIFFERENTIAL EFFECTS OF APOMORPHINE ON DORSAL AND MEDIAN RAPHE NEURONS. E.H-Y. Lee* , and M.A. Geyer. Dept. of Psychiatry, Univ. of California at San Diego Sch. of Med., La Jolla, CA 92093.

In an effort to assess the functional inter-relationships between monoaminergic systems in brain, we have used quantitative cytofluorimetry to measure changes in cellular serotonin produced by the dopaminergic agonist apomorphine. A measure of fluorescence fading using a computerized microspectro-fluorimeter enables us to discriminate changes in serotonin from changes in catecholamines and to discriminate intracellular from extracellular amines (Geyer et al., 1978. J. Pharm. Exp. Ther. 207:650-667). Thirty minutes after intraperitoneal injections of saline, 1.0 or 10.0 mg/kg apomorphine, male rats (125-150 gms) were sacrificed, the midbrain raphe area was removed, freeze-dried, treated with formaldehyde, and embedded in paraffin. Microscopic measures were made in both the dorsal (B7) and median (B8) raphe nuclei in 8 micron sections. Both doses of apomorphine significantly increased intracellular serotonin levels in B7, without significantly affecting the cells in B8. This regional difference in apomorphine's effect may be related to the differential amounts of catecholaminergic innervation to these two nuclei. We have reported similar regional difference in the effects of amphetamine on serotonin fluorescence in B7 and B8 (Geyer et al. 1975, Brain Res. 85: 135-139), although amphetamine's effect was to decrease rather than increase serotonin. Apomorphine also produced a dose-dependent increase in the fluorescence outside the cell bodies, particularly in B7. This change appears to be due to increases in both serotonin and catecholamines in the extracellular spaces. Further studies are in progress to clarify the changes in catecholamines within the raphe and to determine the mechanism of apomorphine's effect on the serotonergic cell bodies.

270.13 INHIBITION OF PROTEIN SYNTHESIS IN RAT STRIATUM BY AMPHETAMINE TREATMENT: PARTIAL REVERSAL BY HALOPERIDOL PRETREATMENT. Robert Cohen*, Susan Chung*, and Lawrence Roel (SPON: Mary F. Orr). Dept. of Anatomy, Northwestern Univ., 303 E. Chicago Ave., Chicago, IL 60611.

Previous studies have indicated that intraperitoneal (i.p.) administration of d-amphetamine sulfate causes polysome disaggregation in rat brain (Proc. natn Acad. Sci. U.S.A. 72: 779, 1975) which is accompanied by reduced rates of brain protein synthesis (J. Neurochem. 31: 341, 1978). Doses of amphetamine which are effective in causing whole brain polysomal disaggregation inhibit protein synthesis in all areas of the brain that were investigated.

We now have undertaken studies to determine whether lower doses of amphetamine (subthreshold for producing whole brain polysomal disaggregation) might produce changes in protein synthesis in selective areas of the brain. In the first series of experiments, amphetamine sulfate (1 mg/kg) or an equal volume of saline was administered i.p. to 140 g male Sprague Dawley rats. Seventy five min later, animals were injected intravenously (i.v.) with 20 μCi ^{3}H-lysine, and killed 30 min thereafter. In the second series of experiments, rats were injected i.p. with haloperidol (1 mg/kg) or 0.025 M citric acid diluent, and 1 hr later injected i.p. with 1 mg/kg amphetamine or saline diluent. One hr after amphetamine (or saline) administration, animals were injected i.v. with 20 μCi ^{3}H-lysine and killed 30 min later. Brains were rapidly removed, dissected into striatum, cerebral cortex, hypothalamus, cerebellum, and "rest of brain", and incorporation of ^{3}H-lysine into trichloroacetic acid precipitable protein determined.

Synthesis of protein in striatum is inhibited 25-40% 105 min after administration of 1 mg/kg amphetamine sulfate. At this time after amphetamine administration, protein synthesis did not appear to be altered in cerebellum, cerebral cortex, hypothalamus, or "rest of brain". When haloperidol was administered 1 hr prior to amphetamine injection, the selective inhibition of protein synthesis in striatum was blocked about 50%. Haloperidol treatment alone did not alter protein synthesis in striatum, or any of the other brain regions studied. Supported by a starter grant from Northwestern University (NIH RR05370).

270.14 SPECIFIC INTERACTION OF ATROPINE WITH α-ADRENERGIC RECEPTORS IN RAT BRAIN. Elinor H. Cantor, Shlomo Abraham* and Sydney Spector*
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Several reports in the literature have suggested that the antimuscarinic drug atropine inhibits catecholamine-induced contractions in isolated vascular tissue. We have recently described a hypotensive response to atropine in conscious rats which is apparently mediated by postjunctional α-adrenergic receptor blockade. The present study was undertaken to determine directly if atropine interacts with α-adrenergic sites. Binding of the α-adrenergic receptor ligands [^{3}H]WB-4101 (WB) (0.2 nM) and [^{3}H]prazosin (PRAZ) (0.1 nM) and the α_2-adrenergic receptor ligand [^{3}H]clonidine (CLON) (1.0 nM) was measured in homogenates of brains of Sprague-Dawley rats. Binding was measured in the presence and absence of various concentrations of phentolamine, l-norepinephrine, and atropine. IC_{50} values were determined by linear regression analyses of log-probability plots of the displacement curves obtained with each competitor. K_i values were calculated according to the formula $K_i = IC_{50}/(1 + C/K_d)$, using the following K_i values determined in our laboratory: K_d(WB) = 0.5 nM, K_d(PRAZ) = 0.1 nM, and K_d(CLON) = 5.0 nM. All assays were performed in duplicate and the data are presented as mean ± SEM. Total binding (fmol/mg protein) was 21.3 ± 1.7 (n=11) for WB, 15.6 ± 1.3 (n=7) for PRAZ, and 10.2 ± 2.3 (n=8) for CLON. Phentolamine was the most potent of the agents tested, with K_i values of 2.4 ± 0.5 nM (n=3) for displacement of WB, 2.2 ± 0.3 nM (n=4) for PRAZ, and 9.7 ± 2.4 nM (n=3) for CLON. Norepinephrine was more active in displacing CLON binding (K_i = 24 ± 5 nM, n=3) than WB (K_i = 840 ± 140 nM, n=3) or PRAZ (K_i = 560 ± 40 nM, n=4) binding. Atropine was equally as active as norepinephrine in displacing α_1-adrenergic ligands with a K_i value of 860 ± 90 nM (n=5) for WB binding and 740 ± 50 nM (n=4) for PRAZ, but was essentially inactive against CLON binding (K_i = 200 ± 3 μM, n=4). These data indicate that atropine interacts specifically with the classical postjunctional α_1-adrenergic site as defined by radioligand binding studies. This property is not common to all antimuscarinic drugs as methylatropine and scopolamine failed to displace either α_1- or α_2-receptor ligands at concentrations less than 100 μM. Furthermore, the ratio of the K_i values for displacement of α_1- and α_2-ligands by atropine is of the same order as that generally obtained with α-adrenergic receptor antagonists. These data, together with the observed effect in vivo, suggest that atropine acts as an antagonist at the α_1-site.

270.15 ANTAGONISM OF BEHAVIORAL AND ELECTROPHYSIOLOGICAL RESPONSES TO SEROTONIN BY CLONIDINE. William D. Matthews. Department of Biological Research, Smith Kline and French Laboratories, Philadelphia, PA 19101.

The head shake response in rats after systemic administration of the serotonin (5HT) precursor 5-hydroxytryptophan (5HTP) is a useful model for central 5HT receptor activation (Matthews and Smith, Life Sci. 26:1397,1980). Head shakes induced by 5HTP are particularly sensitive to intraperitoneal administration of known 5HT antagonists. Metergoline inhibited head shakes with an ED_{75}= 0.07 (0.02-0.27) mg/kg as did cyproheptadine, ED_{75}=0.15 (0.09-0.30) mg/kg. Recent studies revealed that clonidine also produced a dose-related antagonism of the head shake behavior, ED_{75}= 0.005 (0.002-0.014) mg/kg. Electrophysiological experiments were then conducted to determine if antagonists of 5HTP-induced head shakes could inhibit responses to 5HT applied iontophoretically to hippocampal neurons.

Rats were anesthetized with urethane and standard electrophysiological techniques used to record the activity of individual hippocampal neurons (pyramidal and granule cells) while applying drugs by microiontophoresis. Hippocampal neurons respond to 5HT in a uniform manner. Ninety-one percent (103 of 113) of all cells encountered were inhibited (≥ 50% reduction in spontaneous firing rate) by iontophoretic application of 5HT (0.05M, pH4, 50-100nA). Metergoline (0.04M, pH4, 20-40nA) applied for up to 5 min had no effect on spontaneous neuronal activity but blocked the inhibitory actions of 5HT in 60% (9 of 15) of the cells tested. Clonidine (0.01M, pH4, 5-20nA) reduced or abolished the inhibitory effect of 5HT in 100% (9 of 9) of the cells studied. Some cells increased their rate of discharge in the presence of clonidine. A clonidine analog (2-3,4-dihydroxyphenylamino) imidazoline (DPI) was also studied in the electrophysiological experiments. DPI (0.01M, pH5, 10-20nA) reduced or abolished the inhibitory response to iontophoretic application of 5HT in all cells examined (5 of 5).

The data suggest that inhibition of 5HTP-induced head shakes by clonidine may result from an interaction between clonidine and 5HT at the level of a central 5HT receptor. Further studies are in progress to definitely characterize the clonidine-5HT interaction.

270.16 NEUROPHARMACOLOGICAL CHARACTERIZATION OF CNS PURINERGIC P_1 RECEPTORS. G. G. Yarbrough and J. C. McGuffin-Clineschmidt*.
Merck Institute for Therapeutic Research, West Point, PA 19486.

Purines exert pronounced influences on the excitability of many types of tissues, including the CNS, and the existence of subclasses of the "purinergic" receptor has been inferred (Burnstock, 1978). While there is a substantial body of literature describing the effects of purines on central neuronal excitability and various biochemical parameters, there is a relative paucity of data concerning the in vivo behavioral effects of adenosine (ADO) and related compounds. Administered intracisternally, ADO, 2-chloroadenosine (CADO), adenosine-5'-cyclopropylcarboxamide (ACC) and adenosine-5'-ethylcarboxamide (AEC) caused dose-related increases in hot plate reaction times in rodents. The rank order of potency was AEC = ACC > CADO > ADO. ADO itself was more potent than AMP, ADP, ATP and several other related compounds of interest. Theophylline and caffeine antagonized the antinocisponsive effect of CADO or ACC. Papaverine (an adenosine uptake blocker) and erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA; an adenosine deaminase inhibitor) potentiated the effect of ADO. EHNA did not potentiate the action of CADO in this procedure. The antinocisponsive effect of CADO was not antagonized by a host of neurally active agents including naloxone, clonidine and RO 20-1724. Time course studies indicated that the antinocisponsive effect of ADO was transient with the peak effect occurring 5 min after injection and disappearing by 60 min, whereas the effect of CADO persisted for up to 4 hrs. Intracisternally-administered CADO also caused a pronounced hypothermia and loss of muscle tone in mice and exerted a hypotensive effect in anesthetized rats. CADO was also active in the mouse writhing test. Taken together, these data demonstrate that purines exert potent in vivo behavioral effects and are consonant with the existence of a central purinergic P_1 receptor which is amenable to selective pharmacological manipulation.

Burnstock, G. (1978) In: Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach. eds. L. Bolis and R.W. Straub, Raven Press, New York.

270.17 BINDING OF THE ANTITUSSIVE DRUG DEXTROMETHORPHAN TO GUINEA PIG BRAIN. G. L. Craviso* and J. M. Musacchio. Dept. Pharmacology, N.Y.U. School of Medicine, New York, NY 10016.

We have studied the binding of the non-narcotic antitussive drug dextromethorphan (DM) to guinea pig brain homogenates. ^{3}H-DM at a final concentration of 4 nM is incubated with brain tissue for 2.5 hrs at 0°C in the presence and absence of 10 µM unlabeled DM. Bound ^{3}H-ligand is separated from free drug by rapid filtration. The binding of DM is saturable and reversible and displays stereospecificity since levomethorphan is 20 fold less effective than its dextro isomer as an inhibitor of ^{3}H-DM binding. Scatchard analysis demonstrates the presence of two binding components, a high affinity component with a K_d of 30 nM and a low affinity component. Subcellular distribution studies indicate that the low affinity component is localized in the mitochondrial fraction whereas the high affinity component is found in both "microsomal" and "membrane fractions". In regional distribution studies, the greatest proportion of high affinity binding sites is in the cerebellum and lower brain stem.

Various classes of centrally-acting antitussive drugs have been tested for their ability to alter ^{3}H-DM binding. Opiate analgesics and opiate antagonists with antitussive activity (butorphanol) have negligible effects on binding when used at concentrations up to 10^{-5} M. The non-analgesic antitussive drugs (+)-methadone and (-)-propoxyphene produce significant inhibition at 10^{-5} M (73% and 28% respectively) and dextrorphan has an IC_{50} value of 2.5×10^{-6} M. Clonazepam, an antitussive benzodiazepine, does not alter binding at any concentration tested. Noscapine, a phthalideisoquinoline antitussive drug, produces a dose-dependent (10^{-7} M to 10^{-5} M) increase in ^{3}H-DM binding. Saturation studies indicate that this drug increases the affinity of ^{3}H-DM for the high affinity binding sites 3 fold while the low affinity binding component is not affected. The antitussive noscapine analog hydrastine also increases ^{3}H-DM binding but only when used at higher concentrations.

High affinity, stereospecific and saturable binding of ^{3}H-DM has also been detected in liver and other non-neural tissue. However, the high affinity DM binding studied in the liver is not increased by noscapine, suggesting that the nature of the binding sites is different in neural and non-neural tissue.

Whether or not the binding of ^{3}H-DM described is related to the mechanism of action of the drug cannot be determined at this time. The antitussive activity of DM is not naloxone reversible and therefore not mediated by opiate receptors. The results of our binding experiments are consistent with this finding. (Supported by PHS grants #1 RO1 DA02013, MH17785 and a Pharmaceutical Manufacturers Association Predoctoral Fellowship to G.L.C).

270.18 METHOTRIMEPRAZINE, A PHENOTHIAZINE ANALGESIC, LACKS DIRECT ENKEPHALINERGIC ACTIVITY. T. A. Robert, A. N. Hagardorn*, and E. A. Daigneault. Dept. of Pharmacology, East Tennessee State University College of Medicine, Johnson City, TN 37601.

The drug methotrimeprazine (MTM) is unique in that it possesses both clinically useful neuroleptic and analgesic activities. These actions are stereospecific in that the levorotatory isomer is the pharmacologically active specie. Since stereospecificity and potency are characteristics associated with receptor-specific interactions, it was proposed that MTM produces analgesia as an opiate receptor agonist. This hypothesis was tested by in vitro opiate receptor binding studies and in vivo rat prolactin (rPRL) release experiments.

Opiate receptor studies were performed upon crude homogenates of the calf caudate nucleus utilizing tritiated methionine enkephalin, dihydromorphine, and naloxone as ligands at concentrations of 2, 1, and 1.5nM, respectively. Various concentrations of both levorotatory and dextrorotatory MTM were tested for their abilities to inhibit specific opiate receptor binding. Specific binding was defined as the difference between that which occurred in the absence or presence of 1 µM levallorphan. The effects of various test drugs upon rPRL release were determined in groups of five male rats (300 ± 27 g) at 0, 30, and 60 min post treatment. Treatments employed were: saline control (vehicle); chlorpromazine HCl, 10 µmole/kg; morphine sulfate, 10 mg/kg; (-)-MTM, 10 µmole/kg; all administered i.p. Plasma samples from blood obtained by heart puncture under light ether anesthesia were analyzed for rPRL concentrations using the NIAMDD radioimmunoassay kit (supplied by Dr. A. F. Parlow of the Rat Pituitary Hormone Distribution Program).

In all the opiate receptor studies with endogenous or exogenous agonists or the antagonist, MTM failed to show stereospecific inhibition of radioligand binding at concentrations less than 10^{-5}M. Furthermore, the observed release of rPRL induced by (-)-MTM was not significantly different ($P<0.05$) from chlorpromazine or morphine sulfate. These results suggest that (-)-MTM does not interact directly upon enkephalinergic neurons in the central nervous system.

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270.19 THE PHARMACOLOGICAL EFFECTS OF NICOTINE IN THE UNANESTHETIZED DOG. S. G. Kamerling, J. G. Wettstein*, and W. R. Martin. Dept. of Pharmacology, University of Kentucky, Coll. of Med., Lexington, Ky 40536.

Six unanesthetized beagle type dogs (weighing 8.5-11.0 kg) participated in a study of the effects of nicotine on heart and respiratory rates, electroencephalographic amplitude and frequency, pupillary diameter, heat evoked skin twitch latency, body temperature and behavior. The release of methionine-enkephalin into ventricular perfusates was also studied in these dogs concurrently and will be reported elsewhere. Each dog participated in three experiments conducted at weekly intervals using a crossover design. The experiments were (1) intravenous nicotine infusion (40 µg/kg/min) for 20 minutes, (2) intravenous saline infusion for 20 minutes, and (3) no treatment. Observations were made at 5 minute intervals for 60 minutes. For statistical purposes, observations were divided into three 20 minute epochs. During the second epoch the nicotine or saline infusion was given. The data were expressed as a percent of the mean of the first epoch for each physiologic parameter. During the nicotine infusion skin twitch latency, heart rate and respiratory rate were significantly increased, while pupillary diameter and EEG amplitude were significantly decreased. The decreased EEG amplitude and pupillary diameter persisted into the third epoch. Nicotine generally produced behavioral arousal, vomiting and moderate degrees of salivation, rhinorrhea, and lacrimation during the second epoch. Nicotine analgesia, as measured by prolongation of the skin twitch reflex latency, was not antagonized by naltrexone (2 mg/kg) or by mecamylamine (1 mg/kg) pretreatment. However, the mecamylamine experiment was confounded by the fact that mecamylamine alone produced a significant degree of analgesia.

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270.20 NEUROPHARMACOLOGICAL STUDIES OF THE PARASITIC TREMATODE, SCHISTOSOMA MANSONI, USING ELECTROMYOGRAPHY. T. N. Mellin, R. D. Busch* and C. C. Wang. Dept. of Biochemistry, Merck Sharp & Dohme Research Labs, Rahway, NJ 07065.

A dual in vitro test system was developed which combines a 1 hr. visual motor assay with suction electrode electromyography for quantitation of the electrical activity produced by contracture of the worms musculature. Studies were conducted on adult male and female worms maintained in 50% heat inactivated horse serum/Earles balanced salts at 37°C. This media was buffered at pH 7.4 with 0.02mM Hepes. Surface electrical activity was measured as the absolute integral of positive and negative input signals, obtained by full wave rectification, and expressed in mV seconds. Male worm length was also measured. All changes in motility were confirmed by electromyography.

5-Hydroxytryptamine (5-HT-0.01mM), a putative neurotransmitter in schistosomes, caused a marked increase in motor and electrical activity. Tryptamine (0.1mM) and the 5-HT agonist 2-(p-chlorophenyl)-ethylamine, were equivalent to 5-HT in activity. Acetylcholine (20mM), a putative inhibitory neurotransmitter in schistosomes, and the cholinergic agonist, carbachol (1mM), produced a flaccid paralysis. Worm length was unaffected. The cholinergic antagonists, atropine (muscarinic-0.01mM) and mecamylamine (nicotinic-1mM), increased worm motility and blocked the action of exogenous acetylcholine (10mM). Worm activity was decreased by the cholinesterase inhibitor, neostigmine (1mM). GABA (1mM) and its agonists, piperazine (1mM) and muscimol (0.1mM), had no effect on schistosome activity, nor did the GABA antagonists, picrotoxin (0.1mM) and bicuculline (0.1mM) or the Cl^- channel blockers, Na-penicillin-G (1mM) or pentylene tetrazole (1mM). L-glutamate (1mM), and its agonist, kainic acid (1mM), glycine (1mM) and avermectin B_{1a} (0.01mM) were also without effect. Dopamine (10mM) and apomorphine (10mM) caused little or no decrease in worm activity, but produced 76% ($P<0.01$) and 36% ($P<0.01$) increases in worm length, respectively.

The novel antischistosomals, praziquantel (PZQ-0.001mM) and clonazepam (CLZ-0.025mM), caused a rapid and total loss of motor and electrical activity, with a significant ($P<0.01$) shortening in length. Other benzodiazepines, diazepam (valium-0.1mM) and chlordiazepoxide (librium-0.1mM), were without effect. The activity of PZQ and CLZ was blocked by the Ca^{+2} transport/binding inhibitors, ruthenium red (1mM) and lanthanum (1mM). These inhibitors did not block the spastic paralysis produced by the general cation ionophore, lasalocid (1mM), suggesting that PZQ act by a non-ionophore mechanism.

270.21 INTERACTIONS BETWEEN ANTIDEPRESSANT DRUGS AND β -ADRENERGIC BINDING AND FUNCTION IN NORMAL MOUSE ASTROCYTES. L. Hertz* and J.S. Richardson, Dept. of Pharmacology, University of Saskatchewan Saskatoon, Saskatchewan S7N 0W0 Canada.

The demonstration by Sulser (Trends Pharm. Sci., 1, 92, 1979) that chronic treatment with any type of antidepressant drug leads to a "down-regulation" of β-adrenergic receptor function has led us to study the effect of antidepressant drugs on cyclic AMP level and on the binding of [^{3}H]dihydroalprenolol, a β-adrenergic ligand, in normal mouse astrocytes in cultures; such cells are known to possess β-adrenergic receptors (McCarthy and de Vellis, J. Cycl. Nucl. Res. 4, 15, 1978; Van Calker and Hamprecht, J. Neurochem. 30, 713, 1978). The labeled ligand was bound in large amounts by a saturable, high-affinity binding (K_D approximately 50 nM) which was efficiently displaced by the β-adrenergic blocker propranolol but, peculiarly enough, not by the β-adrenergic agonist isoproterenol. The antidepressant drugs studied were amitriptyline and desipramine (two monoamine uptake inhibitors), tranylcypromine (a MAO inhibitor), and doxepine, inprindole and mianserin, which have little or no effect on these two parameters. All these drugs displaced the labeled dihydroalprenolol. They showed somewhat different potencies but half maximum displacement was in general observed at a concentration of about 5 μM, i.e., a concentration about 10 times the plasma levels observed in patients under chronic treatment with antidepressants. Binding studies were also made of the binding of one of the antidepressants, doxepin, which was bound specifically with a K_D value which likewise was a few μM. This binding could be displaced both by amitriptyline and propranolol.

In accordance with findings by Van Calker and Hamprecht (opus cit.) the level of cyclic AMP in the cultured cells was substantially increased after 10 min. of exposure (at 37°) to the β-adrenergic agonist isoproterenol. This increase was partly (about 50%) blocked by 5 μM amitriptyline, the only drug tested until now. In contrast, amitriptyline had no effect on the level of cyclic AMP in the absence of isoproterenol.

From these studies we conclude that antidepressant drugs show specific binding to cells from the central nervous system, that this binding may interact with β-adrenergic binding sites, and that antidepressants seem to have a beta blocking effect. The experiments were all carried out using astrocytes,and studies are in progress whether similar phenomena also occur in cultured neurons. We are also studying possible chronic effects of exposure of cultured astrocytes to antidepressants in the absence or presence of β-adrenergic agonists.

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270.22 EFFECTS OF YOHIMBINE ON DESIPRAMINE INDUCED ADRENORECEPTOR ALTERATIONS IN THE LIMBIC FOREBRAIN. Richard W. Johnson*, N.L. Weich*, R.C. Ursillo* and Henry I. Yamamura. Depts. of Biochemistry, Pharmacology and Psychiatry, Univ. of Ariz. HSC, Tucson, Ariz., 85724 and Merrell Research Center, Cincinnati, Ohio 45215.

Desipramine (DMI), along with several other tricyclic antidepressants, has been shown to be a potent inhibitor of neuronal norepinephrine (NE) reuptake, an effect which has been sugested to constitute its primary mechanism of action. Administration of DMI to rats results in down-regulation of β-adrenergic receptors; this effect is usually manifest only after chronic DMI administration, and has been postulated to be a neurochemical correlate of antidepressant action. The present study was undertaken to determine the effects of yohimbine, an α_2-receptor antagonist which would be expected to disrupt feedback regulation of NE release, on DMI-induced down-regulation of β-adrenergic receptors in the limbic forebrain of the rat. DMI induced alterations of α_2-adrenoreceptors, and the effects of yohimbine thereon, were also investigated in order to determine the involvement of these autoregulatory receptors in the actions of DMI. Our results indicate that after only 4 days [^{3}H]DHA binding to limbic forebrain areas of animals which were treated with DMI plus yohimbine was significantly ($p<.005$) reduced as compared to control (vehicle injected) animals. There was no significant decrease in binding after treatment for this period of time with either DMI or yohimbine alone. The observed reduction of [^{3}H]DHA binding was attributable to a decrease in receptor density (Bmax), with no significant change in affinity. An increase in α_2-receptor density, as measured by [^{3}H]clonidine binding, was also seen after 4-day treatment with DMI plus yohimbine. A similar increase was noted after treatment with yohimbine alone, which may be explicable on the basis of direct α_2-receptor antagonism; however treatment with DMI alone, which has very little affinity for α_2-receptors, also resulted in a significant, though less dramatic increase in α_2-receptor density.

In light of the cotemporality between DMI-induced β-receptor down-regulation and clinically observable reversal of depressive symptoms, the finding that β-receptor desensitization can be accelerated by co-administration of yohimbine suggests that this type of drug combination approach may lead to the development of clinically applicable treatment regimens which exploit this phenomenon to achieve a more rapid reversal of the symptoms of affective disorders. The implications of the observed α_2-receptor increases remain to be elucidated, however the possibility exists that alterations in α_2-receptor density play a role in the mechanism of action of a certain antidepressant drugs. The apparent reciprocity between these α_2 an β-adrenoreceptor alterations is discussed with respect to possible noradrenergic antoregulatory mechanisms within the CNS. Supported by grants from the NIMH.

270.23 [^{3}H] IMIPRAMINE BINDING TO RAT FOREBRAIN AND HUMAN BLOOD CELLS - INHIBITION BY CATIONS. J.L. Steinberg, J.T. Brauchi* and J.D. Lane. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Several groups have demonstrated tritiated imipramine binding to rat CNS and human platelets. Our laboratory investigated the specific high affinity binding of this ligand to these membrane preparations and also to membranes from human red blood cells. In addition, numerous cations and anions were added to the incubation media to determine their effects. Total particulate membranes were prepared from rat forebrain, human platelets and human red blood cells by lysing in water or Tris-acetate buffer, with repeated disruption using a Polytron^R and high speed centrifugation. The binding studies were carried out at 50 mM Tris-acetate buffer, pH 7.4. To this buffer were added 0.5-100 mM salts of sodium, potassium, lithium, ammonium, silver, chloride, acetate, citrate, bicarbonate and/or nitrate, and/or 1 mM calcium, cobalt, manganese or magnesium in the presence or absence of 100 μM desipramine. All three kinds of membranes had specific imipramine binding sites (total binding sites: platelets>rat forebrain>red blood cells), with dissociation constants circa 2 nM. Monovalent and divalent cations inhibited specific imipramine binding by as much as 80% (100 mM cations) in a concentration dependent fashion, while non-specific binding was not affected. Numerous anions had no effect on binding. The inhibition was additive if more than one cation was included in the incubation. The effects of guanine nucleotides and displacement of ligand by imipramine metabolites were also investigated. High affinity imipramine binding sites may be similar to opiate, adrenergic, benzodiazepine, and muscarinic cholinergic receptors which also show ion-dependent binding characteristics. (Supported by USPHS Grant #MH-31835)

270.24 COMPARISON OF RADIORECEPTOR AND CHEMICAL ASSAYS IN THE EVALUATION OF THE EFFECTS OF NEUROLEPTICS AND ANTIDEPRESSANTS IN MAN. C.H. Misra, H. Shelat*, J. Sammeta*, D.E. Leelavathi, J.C. Schoolar, and R.C. Smith, Behavioral Neurochemistry, Texas Research Institute of Mental Sciences, Houston, Texas 77030.

Multi-spectrum radioreceptor (RR) assays on the same plasma sample may provide a better correlation with differential clinical and side-effects of a psychotropic drug in man than specific chemical assays. We have been studying the utility of radioreceptor blood level assays in controlled clinical studies. Haloperidol and thioridazine levels in the plasma sample were assayed by displacement of three ligands -- spiperone, QNB and WB-4101. Antidepressants were assayed by displacement of QNB and WB-4101. Drugs were also assayed by specific chemical methods. There was a good correlation ($r = .80 - .90$) between levels of haloperidol obtained by radioreceptor assay of spiperone and GLC assay, although there was considerable interpatient variability. Hydroxy metabolites of imipramine, desipramine, and nortriptyline, as well as the parent drugs, were fairly potent in displacing QNB and WB-4101 (IC_{50} 10^{-5} to 10^{-6} M). Radioreceptor blood levels determined by QNB and WB-4101 displacement correlated highly with some side-effects of the drugs in patients. Detailed studies of the correlation of blood levels determined by RR and chemical assays in relation to clinical response are being analyzed.

270.25 DOPAMINERGIC CHARACTERISTICS OF A NIGRO-STRIATAL SYSTEM MAINTAINED IN VITRO. Catherine Mytilineou, William O. Whetsell and Gerald Cohen. Mount Sinai School of Medicine, New York, N.Y. 10029.

Explants from substantia nigra and striatum from newborn mongrel dogs were grown together in organ cultures for periods up to 2 months. The explants developed fiber outgrowth which connected the substantia nigra with the striatum. Glyoxylic acid histofluorescence demonstrated the presence of catecholamines in several cell bodies in the substantia nigra and in numerous fibers originating from that explant. Fluorescing fibers were also seen reaching and terminating in the striatal tissue. Both the substantia nigra and the striatal explants exhibited an active uptake for ^{3}H-dopamine which was completely inhibited by the specific dopamine uptake inhibitor nomifensine (10^{-5}M). The accumulated ^{3}H-dopamine could be released when the explants were incubated in a medium containing high concentrations of K^+ (50 mM). The synthesis of dopamine could also be followed in the living cultures by measuring the accumulation of homovallinic acid (HVA) into the feeding medium by high performance liquid chromatography with amperometric detection. The amount of HVA which accumulated in the 50 ul of feeding medium bathing the nigro-striatal cultures during 3 to 4 days ranged from 4-24 ng. The accumulation of HVA was inhibited by treating the cultures with 10^{-5}M pargyline. HVA was not present in feeding medium that was not exposed to the nigrostriatal cultures. In addition, there was no HVA present in the feeding medium from cultures that consisted of striatal tissue alone.

Supported by NIH Grant 11631.

270.26 DOPAMINE AGONIST ACTIVITY OF "PARTIAL" ERGOLINE MOLECULES. E. B. Smalstig,* E. C. Kornfeld,* and J. A. Clemens. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

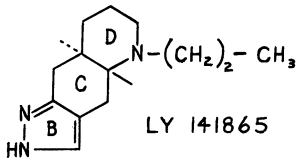
Based on the potent dopamine (DA) agonist activity of ergoline molecules such as lergotrile, pergolide, and bromocriptine, an attempt was made to identify the portion of the 4-ringed ergoline structure which imparts this DA activity. In vivo inhibition of reserpine-elevated serum prolactin was used to assess activity at pituitary dopamine receptors. In vitro dopamine agonist activity was tested in a paired hemipituitary incubation. Central nervous system DA agonism was measured by ability to induce "turning behavior" in rats with unilateral 6OHDA lesions of the nigrostriatal pathway and/or electrolytic lesions of the caudate nucleus. Specific DA receptor blockers (spiperone, haloperidol and pimozide) were used to verify the dopaminergic basis of the observed effects.

A comprehensive series of tri- and bicyclic compounds (containing various portions of the ergoline structure) was synthesized and tested. These compounds included tricyclic benz<cd> indols and tricyclic and bicyclic pyrrols and pyrazols. To avoid rapid metabolism by MAO, most compounds were secondary or tertiary amines with substitution of methyl, ethyl or propyl groups on the nitrogen corresponding to the 6 position on the ergoline molecule.

DA agonism was present in all of the classes of partial ergolines tested. Based on both prolactin inhibition and turning induction data the DA potency of the classes decreases in the following order:

tricyclic pyrazoles=tricyclic pyrroles>tricyclic benzindols>
bicyclic pyrazoles=bicyclic pyrroles

The tertiary amines were always more active than the primary or secondary amines and propyl substituted amines were the most active compounds in each class. The most potent of the partial ergolines was LY141865, a propyl substituted tricyclic pyrazole. The dopamine agonist activity of LY141865 approached that of pergolide in vitro, and in vivo, and could be blocked by spiperone and haloperidol. Isolated smooth muscle testing indicated that LY141865 has none of the serotonin agonist or α-blocking activities seen with many of the ergolines.



LY 141865

These experiments illustrate that the dopamine agonist properties of the ergolines reside in the pyrrolethylamine "substructure" rather than the phenethylamine portion of the molecule.

270.27 AMANTADINE SPECIFICALLY PROTECTS STRIATAL DOPAMINE RECEPTORS FROM HALOPERIDOL INDUCED HYPERSENSITIVITY. R.M. Allen, J.D. Lane, J.T. Brauchi*. Psychiatry Research Unit, Department of Psychiatry, LSU Medical Center, Shreveport, LA 71130.

Amantadine has been shown to present haloperidol induced dopamine receptor hypersensitivity using a stereotyped behavior model (Allen et al, Biol. Psychiatry, 14, 541). In this study we looked at the effects of amantadine on the development of striatal, mesolimbic and hypothalamic DA neuron hypersensitivity secondary to chronic haloperidol administration using both the apomorphine induced stereotyped behavior assay and [^{3}H] spiroperidol binding. Three groups of 18 Fisher rats were treated according to the following protocol for 21 days: Group I saline; Group II haloperidol 5 mg/kg; Group III haloperidol haloperidol 5 mg/kg and amantadine HCl 50 mg/kg; all intraperitoneally. Four animals were selected from each group and challenged with subthreshold apomorphine (.25 mg/kg I.P.) prior to treatment and following treatment and rated for stereotyped behavior at 5,15, and 30 min using a 4 point scale as reported previously (Ibid). The remaining animals were killed by decapitation and the striata, nuclei accumbens, and hypothalami dissected and frozen at -70°C. The areas from each group were separately pooled and membrane preparations made. The [^{3}H] spiroperidol binding technique of Fields et al (Brain Res.,136, 578) was used. The results of the stereotyped behavior assay, which assesses only striatal supersensitivity, revealed a significant reduction in striatal hypersensitivity in the amantadine group compared to the haloperidol alone group (P<.014 using Marin-Whitney single tail t-test). The results of the radioligand assay were similar and consistent for the striatal area-Group I Saline Bmax=748 fmol/mg protein; KDapp 73; Group II haloperidol Bmax=932 fmol/mg protein; KDapp=89 pM; Group III haloperidol and amantadine Bmax 789 fmol/mg KDapp 75 pM. All linear correlations on the Scatchard analysis were greater than 0.997. Because of limited tissue, binding was assayed at 100 pM concentration of [^{3}H] spiroperidol in the nucleus accumbens and hypothalamic areas for the 3 groups with the following results=Group I NA 72.7 fmol/mg protein; hypo. 24.4 fmol/mg; Group II NA 89.6 fmol/mg, hypo. 27.3 fmol/mg; Group III NA 95.4 fmol/mg, hypo. 33.7 fmol/mg. These results show that amantadine protects striatal but not mesolimbic or hypothalamic DA receptors from haloperidol-induced supersensitivity. These results correlated with clinical observations that amantadine is effective in treating neuroleptic induced extrapyramidal side effects without affecting antipsychotic activity (DiMassio et al, Arch. Gen. Psychiatry, 33, 599) and support Allen's hypothesis that amantadine might prevent neuroleptic induced tardive dyskinesia (Curr. Ther. Res., 22, 914).

270.28 ANTAGONISM OF DRUG-INDUCED ACTIVATION OF STRIATAL DOPAMINE SYNTHESIS BY LEVONANTRADOL. B. Kenneth Koe. Central Research, Pfizer Inc., Groton, CT 06340.

Dopamine (DA) synthesis in rat corpus striatum is accelerated in response to (1) the blockade of DA receptors by neuroleptics, and (2) the inhibition of impulse flow by γ-butyrolactone or baclofen. This phenomenon may be used to study agents which exert a modulatory effect on striatal DA neurons. Levonantradol is a novel cannabinoid-related benzo[c]quinoline with potent analgesic properties (Milne et al., The Pharmacologist 20: 243, 1978). I have found that levonantradol (1 μmole/kg s.c.) antagonized the increased synthesis of DA (dopa accumulation) induced by haloperidol (HA) (0.32 μmole/kg s.c.), γ-butyrolactone (8.72 mmoles/kg i.p.), or baclofen (178 μmoles/kg i.p.). Levonantradol itself did not affect dopa accumulation in striatum. This action of levonantradol may be related to the known anticholinergic properties of Δ^9-THC or its recently described GABAergic activity (Revuelta et al., 1979). These authors reported that Δ^9-THC (32 μmoles/kg i.v.) or cannabidiol (64 μmoles/kg i.v.) doubled GABA turnover rate in rat substantia nigra. I found that at these high doses Δ^9-THC, but not cannabidiol, diminished the enhanced dopa accumulation in striatum after HA. Levonantradol was effective at doses down to 0.1 μmole/kg i.v. This effect on HA showed the same stereospecificity that characterizes other pharmacological activities of levonantradol. The inactive enantiomer CP-53,870-1 was 30 times less active than levonantradol. These results suggest that levonantradol may modulate central dopaminergic mechanisms.

270.29 THE EFFECT OF LEVONANTRADOL ON RAT CEREBELLAR CYCLIC GMP LEVELS; POSSIBLE GABA-LIKE ACTION. J.P. Leader and A. Weissman (SPON: R.W. Caldwell). Central Research, Pfizer Inc., Groton 06340.

Levonantradol, a novel phenanthridine, produces stereospecific analgetic activity in animals 9-14 times more potent than morphine and yet does not act at the opiate receptor (Milne et al., NIDA Research Monograph Series 9-46, in press). More recent studies have also demonstrated its ability to block the emesis induced by antineoplastic agents in cats (Borison and McCarthy, The Pharmacologist 21: 205, 1979). Despite its stereospecificity of action, the biochemical mechanisms by which levonantradol produces these effects hasn't been elucidated. We report here that levonantradol alters rat cerebellar cyclic GMP in a manner similar to known GABAergic agents.

There is a known reciprocal relationship between cerebellar cyclic GMP levels and GABAergic activity. In the current experiments rats were sacrificed by microwave radiation two hours after intraperitoneal administration of levonantradol and cerebellar cyclic GMP levels were determined by RIA. Levonantradol decreased cyclic GMP levels by 50% at a dose of 1 mg/kg. In rats GABAergic activity can be decreased pharmacologically by the administration of the glutamic acid decarboxylase (GAD) inhibitor isoniazid, thereby increasing cerebellar cyclic GMP. Under these conditions levonantradol decreased cyclic GMP levels by 50% at a dose of .32 mg/kg. This enhanced potency in decreasing isoniazid-elevated cyclic GMP levels compared to the effect on basal cyclic GMP levels is characteristically seen with GABAergic agents. As reported for analgetic endpoints this effect was stereospecific since the analgetically less active d-isomer, CP-53,870, was ten fold less potent than levonantradol in decreasing cyclic GMP. Δ^9-tetrahydrocannabinol also decreased isoniazid-elevated cyclic GMP, but was one hundred fold less active than levonantradol. Scopolamine had no effect on isoniazid-elevated cyclic GMP levels, suggesting that the potent effect of levonantradol and Δ^9-THC reflects a GABAergic component of activity. In support of this we found that levonantradol protected mice against convulsions induced by the administration of the GAD inhibitor 3-mercaptopropionic acid.

In summary, these biochemical and behavioral studies suggest that levonantradol has GABA-like activity at relatively low doses. The relationship of the GABAergic component of activity of levonantradol to its pharmacologic effects is currently under investigation.

271.1 TRIETHYLTIN INTOXICATION INDUCES SELECTIVE DEFICITS IN SEROTONERGIC AND CHOLINERGIC NEUROTRANSMISSION IN RAT BRAIN SYNAPTOSOMES. J.J. Valdes, R.N. Cory*, G.G. Bierkamper*, A.M. Goldberg and Z. Annau. Division of Toxicology, The Johns Hopkins University, Baltimore, MD 21205.

The alkyltins are highly toxic compounds with widespread industrial applications. The toxicity of alkyltins increases to a maximum as the number of alkyl groups is increased to three, and, since the ethyl-tins are maximally toxic to vertebrates, triethyltin (TET) has been used to study the toxicity of alkyltins in the vertebrate nervous system. Symptoms of TET poisoning include cerebral edema, affective disturbance, and muscular weakness and are suggestive of dysfunctions of serotonergic and cholinergic neurotransmission.

Adult male Long-Evans hooded rats were individually housed and given ad libitum access to food and water. Rats in the acute exposure group received one injection of TET (10 mg/kg, i.p.) and were decapitated 24 hr after injection. Rats in the chronic exposure condition received TET in their drinking water (30 mg/L) and were sacrificed, along with matched controls, at 1, 2, and 3 weeks after initiation of exposure. TET-treated rats developed progressive symptoms of hindlimb weakness, penile erection, and aggressive response to handling. Whole brain P2 synaptosomal pellets were obtained, and high-affinity uptake of tritiated 5-hydroxytryptamine (3H-5-HT) and choline (3H-Ch), and spontaneous and potassium-stimulated release of 3H-5-HT and nonlabelled acetylcholine (ACh) were assessed. Uptake of 3H-5-HT and 3H-Ch were not compromised by either acute or chronic treatment, suggesting that the nerve terminals were functionally intact. Chronic, but not acute, TET intoxication resulted in time-dependent selective debilitation of the release systems, with increases in spontaneous, and decreases in potassium-stimulated, release of 3H-5-HT being the major finding. These data are consistent with the assertion that TET promotes cerebral edema by interfering with serotonergic modulation of cerebrovascular permeability, and suggests the possibility for the pharmacological manipulation of TET neurotoxicity. Rats that survive a normally lethal acute dose, or those that are removed from the chronic TET exposure regimen, show recovery of function over a period of weeks. Experiments are in progress to determine whether the neurochemical deficit recovers as a function of discontinuation of exposure.

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271.2 REDUCED HIGH-AFFINITY ACCUMULATION OF CHOLINE IN CULTURED FIBROBLASTS FROM PATIENTS WITH INHERITED TORSION DYSTONIA AND GILLES DE LA TOURETTE SYNDROME. Donald Kay Riker, Robert H. Roth and Xandra O. Breakefield. Depts. Pharmacology, Psychiatry, and Human Genetics, Yale University School Med., New Haven, CT 06510.

We have examined high-affinity ^{3}H-choline accumulation (HACA) in cultured skin fibroblasts from patients with two inherited movement disorders of unknown etiology: dystonia musculorum deformans (DMD) and Gilles de la Tourette syndrome (GLT). Central cholinergic dysfunction might contribute to the pathognomonic signs in these disorders; for example, intravenous physostigmine can abate the motor tics of GLT (Stahl & Berger, NEJM 302:298, 1980).

HACA in brain synaptosomes (K_T<5μM) has been implicated as the rate-limiting step in the synthesis of acetylcholine in septal-hippocampal neurons. Recently, Na^+-dependent HACA has been demonstrated in avian muscle and human skin fibroblasts (Barald & Berg, Dev. Biol. 65:90, 1978; Riker et al, Soc. Neurosci. Abstr. 5:757, 1979). The K_T of HACA in four control human fibroblast lines ranges from 5-11 μM. In a jewish family with DMD the V_{max} and K_T of HACA was compared in parallel in fibroblasts from an unaffected (16 yrs) and severely affected (19 yrs) sister. We observed (N = 3) a 100% elevation in the K_T of HACA in cells from the affected sister (24.8 μM ± 0.7 SEM) when compared to the unaffected sister (12.4 μM ± 1.4 SEM). The V_{max} of HACA was similiar in these siblings (∿225 pm/mg pro/4 min). A second unrelated dystonia patient (13 yrs) demonstrated a 90% increase in the K_T (18.5 μM), with little change in the V_{max} (+20%), when compared to parallel cultures of an age-matched control (9.8 μM). Finally, the comparison of HACA in a patient with GLT (16 yrs) compared to an age-matched control yielded a 146% greater K_T (19.2 μM ± 2.55 SEM vs. 7.8 μM ± SEM; N = 2) with similiar V_{max}'s (∿275 pm/mg pro/4 min).

These preliminary results in fibroblasts demonstrate a decrease in affinity of the transport carrier for choline in lines from patients with these inherited disorders. Such differences in the K_T for choline might result from a common polymorphism for the transport carrier in the human population, variable ratios of fibroblast cell types, or discrete biochemical alterations of the cell membrane. The concentration of choline in human CSF (∿5 μM) and plasma (∿12 μM) indicates that HACA normally operates near its K_T. Under physiologic conditions a doubling of the K_T would reduce the fractional velocity of HACA to 25% of its V_{max}. This reduction in affinity of the choline transport carrier could impair cerebral cholinergic function in brain regions strongly dependent on reuptake of choline, such as the candate-putamen. (Supported by The Dystonia Medical Research Foundation).

271.3 DIPIPERIDINOETHANE MIMICS KAINATE CONVULSIONS AND BRAIN DAMAGE. J.W. Olney, T. Fuller, R. Collins, T. de Gubareff.* Departments of Psychiatry & Neurology, Wash. Univ. Sch. Med., St. Louis MO 63110.

Levine & Sowinski recently described a pattern of brain damage due to dipiperidinoethane (DPE) intoxication which resembles that we have observed in adult rats treated systemically with kainic acid (KA). We undertook the present experiments to explore possible parallelisms between DPE and KA neurotoxicity.

DPE was neutralized with acetic acid and administered sc in aqueous solution to adult male rats in single doses up to 400 mg/kg or was injected (400 nmoles) directly into several brain regions. In some rats, CNS utilization of 2-deoxyglucose (2-DG) was studied after sc DPE injections (400 mg/kg) and in others, the possibility that diazepam pretreatment (20 mg/kg) might protect against DPE neurotoxicity was explored.

Although Levine and Sowinski did not observe convulsions in their DPE-treated rats, ours exhibited the temporal lobe seizures and status epilepticus typically seen in KA-treated rats; moreover, the type and pattern of brain lesions and of 2-DG utilization induced by DPE appeared remarkably similar to that we have reported in KA-treated rats. Diazepam pretreatment, which protects rats from both the convulsions and brain damage induced by KA, also protected DPE-treated rats against both phenomena. Paradoxically, whereas intra-amygdaloid injection of 4 nmoles of KA results in convulsions and extensive local, as well as "distant" neuronal loss, injection of 400 nmoles of DPE into the amygdala caused neither convulsions nor significant histopathological changes locally or elsewhere in brain over a 24 hr post-treatment interval. Since some of our rats treated sc with DPE developed both status epilepticus and a diffuse pattern of limbic brain damage within 2-8 hrs after treatment, it is possible that DPE owes its convulsogenic and brain damaging properties to an active metabolite generated in the periphery. The molecular structure of DPE is not closely related to KA, but bears some resemblance to tremorine, a putative cholinergic agonist. Thus, studies are needed to assess the possible role of cholinergic mechanisms in DPE or KA neurotoxicity (or both). We suspect that a seizure mechanism (status seizures, not seizures per se) underlies the limbic pattern of brain damage sustained in rats treated sc with either KA or DPE.

Our DPE findings, although quite preliminary, are reported for their implications in relation to epilepsy, epileptic brain damage and unresolved questions regarding the mechanism of KA neurotoxicity. Supported by USPHS grants NS-09156, NS-148434, DA-00259, RSA MH-38894 (JWO) and Huntington's Chorea and Wills Foundation Grants.

271.4 ALTERATIONS IN SEROTONERGIC RECEPTOR BINDING IN AN ANIMAL MODEL OF MOVEMENT DISORDER. A.C. Sconzert, S. Gabay, and B. Haber. Marine Biomedical Institute, UTMB, Galveston, TX. and V.A. Medical Center, Brockton, Mass.

The ECC syndrome may be induced in rats by the daily intraperitoneal administration of β-β'-iminodipropionitrile (IDPN; 300 mg/kg) for one week. The symptoms are expressed immediately thereafter and are stabilized within seven days. Once induced, the syndrome is permanent and requires no further injections. The ECC syndrome is a behavioral complex of motor abnormalities which include a heightened level of general excitability, circling, and choreiform head and neck movements. Thus the ECC syndrome is a potentially useful model system in which to explore some of the neurochemical parameters underlying hyperkinetic behavior. We have previously demonstrated alterations in both the GABAergic and the cholinergic neurotransmitter systems in the expression of this syndrome. Results from pharmacological studies utilizing Quipazine (2-(1-piperazinyl)quinone maleate), a 5-HT agonist, (Langlais and Gabay 1977) implicated a possible role of serotonin receptors in the expression of the ECC syndrome. We chose to clarify the role of serotonin receptors in the ECC syndrome by measuring the ^{3}H-5HT binding in various regions of the brain of ECC animals. Female syndromized rats were sacrificed two months post injection of IDPN, and the brains were analyzed for ^{3}H-5HT binding (Bennett and Snyder 1976) on a regional basis (olfactory bulb, frontal cortex, occipital cortex, striatum, hippocampus, hypothalamus, thalamus, cerebellum, pons, and medulla). In IDPN treated rats, levels of ^{3}H-5HT binding are generally decreased, particularly in the pons (37 % of control), striatum (52 % of control), and the frontal cortex (36 % of control). These changes in ^{3}H-5HT binding resemble those reported for post-mortem brains of Huntington Disease patients. The depression of ^{3}H-5HT binding in the striatum can be due to either destruction of terminals originating in the raphe or neuronal cell loss in the raphe per se. These possibilities, in tandem with those alterations in the GABA and acetylcholine systems seen in the striatum of the ECC rat are under further investigation.

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271.5 BIOCHEMICAL ALTERATIONS OF DOPAMINE RECEPTOR RESPONSES FOLLOWING CHRONIC TREATMENT WITH DOPAMINE AGONISTS. K. D. Wilner*, I. J. Butler, W. E. Seifert* and Y. C. Clement-Cormier (SPON: V. A. Lewis). Depts. of Pharmacology, Neurobiology, Neurology and Biochemistry, University of Texas Medical School, Houston, Texas 77025.

The effects of chronic dopamine agonist treatment on rat striatal adenylate cyclase and dopamine-receptor binding activities were studied using Sinemet (L-dopa/carbidopa) administered orally at a calculated daily intake of 150 mg/kg or bromocriptine (CB-154) given intraperitoneally (2 mg/kg). A four-fold increase was observed in the EC50 for dopamine on adenylate cyclase activity in homogenates of the caudate nucleus with no change in the maximal level of enzyme activity following Sinemet treatment. Conversely, the bromocriptine group showed an increase in the maximal level of enzyme activity with no change in the EC50. Binding studies using (^{3}H)-spiroperidol, a dopamine-receptor antagonist, revealed a decrease in the dissociation constant from 0.26nM in the control group to 0.069nM in the Sinemet treated group. In addition, the Bmax for (^{3}H)-spiroperidol in these animals increased by 300 fmoles/mg over that observed in the control group. There was no statistically significant change in the dissociation constant for (^{3}H)-spiroperidol binding in the bromocriptine group with only a slight increase in the Bmax. Overall these data reveal a differential effect of Sinemet and bromocriptine on the biochemical properties of the dopamine receptor which may in part explain the effectiveness of their use in combination drug therapy in the treatment of Parkinson's Disease. (Supported by the National Science Foundation 7816003 and the American Parkinson's Disease Association).

271.6 NEUROTRANSMITTER RECEPTOR BINDING AFTER VARIOUS INTENSITIES OF ELECTROSHOCK. A.S. Perumal, G. Connova* & A.I. Barkai. N.Y.S. Psychiatric Institute, New York, NY 10032. Electroshock treatment (EST) is most effective therapeutically for patients with certain forms of affective disorders. When applied to rats at extremely high current intensities (75-150mA) EST has been shown to decrease β-adrenergic receptor (β-AdR) sensitivity in cerebral cortex. However the relationship between the intensity of applied current and modification of receptor sensitivity in cortex and other brain regions have not been investigated. We have studied effects of EST at current intensities ranging from a subconvulsive level of 1-2mA to a seizure producing level of 20-30mA on the sensitivity of β-AdR and apparent dopaminergic receptors in membrane preparations of cerebral cortex and a combined thalamus-striatum-midbrain (TSM) region in rats. Animals were subjected to EST once a day through saline moistened ear-clip electrodes for seven days and sacrificed 1 hour after the last treatment. Specific binding of β-AdR was assessed with H^3-DHA and (-) alprenolol whereas apparent dopamine receptors were evaluated with hot and cold haloperidol. The larger decrease (35%) in β-AdR sensitivity was seen in the cerebral cortex with EST of 6-10mA which is not adequate to produce prolonged tonic-clonic seizures. The lower EST of 1-2mA resulted in 40% increase in β-AdR sensitivity in TSM with a slight (15%) decrease in cortical β-AdR. Haloperidol specific binding was increased nearly two-fold in cerebral cortex at all EST levels while a slight decrease was seen in TSM. These results indicate that changes in receptor sensitivity may be obtained with subconvulsive EST intensities. Hence the hypothesis that a seizure-producing EST exerts its therapeutic effects by modifying catecholaminergic receptors should be re-examined. (Supported in part by a grant from New York State #HRC1715)

271.7 NEUROCHEMICAL AND BEHAVIORAL CORRELATES OF ANTIDEPRESSANT DRUG ACTION. E. Mann* and S.J. Enna (SPON: I.J. Butler). Depts. Pharmacol. and Neurobiol. and Anat., Univ. Texas Medical School, Houston, Texas 77025

Experiments were undertaken on muricidal rats to better understand the neurochemical basis for this behavior and the modification of this activity by psychopharmacological agents. For the study, male Long-Evans rats were housed individually in isolation for 16 weeks, during which time 40% became muricidal. Following this, some animals were sacrificed while the remaining were divided into three separate groups, one of which received atropine (20 mg/kg), one imipramine (20 mg/kg) and the third saline once daily for 15 days, and their muricidal behavior tested at both 1 hr and 24 hr following each injection.

Neurotransmitter receptor binding assays in the cerebral cortical tissue revealed a change only in the 5-HT_2 receptor system, with binding being significantly increased (42%) in the isolated muricidal animals relative to the isolated nonmuricidal controls. No significant difference was noted in β-adrenergic receptor binding in these same animals. With regard to the drug treated animals, muricidal behavior was significantly inhibited in both the atropine and imipramine subjects at 1 hr after treatment, but not at the 24 hr time point during the first 4 days of treatment. Notably, however, there was an extinction in muricidal behavior in the imipramine, but not atropine, treated group at the 24 hr time point, commencing after 4-6 days of treatment and reaching a nadir at 7-9 days. Receptor binding analysis indicated that there was a reduction in both β-adrenergic (33%) and 5-HT_2 (55%) receptor binding in the imipramine treated group during this time compared to saline treated control animals. No change in receptor binding was noted in the atropine treated animals.

The results suggest that the acute (1 hr) response to antidepressants in muricidal animals may be due to the anticholinergic properties of these drugs but that, chronically, they induce changes in receptor sensitivity that cause a longer lasting alteration in behavior. Finally, from these data, it would appear that changes in the serotoninergic receptor system may be responsible for muricidal behavior in rats and their response to antidepressants. (Supported in part by USPHS grants NS-13803 and NS-00335).

271.8 REPEATED ANTIDEPRESSANT TREATMENTS INDUCE DOPAMINE AUTORECEPTOR SUBSENSITIVITY: ARE DAILY TRICYCLICS NECESSARY FOR THERAPEUTIC EFFICACY? S.M. Antelman* and L.A. Chiodo (SPON: G. Werner). Dept. of Psychiatry, Psychobiology Program, University of Pittsburgh, Pittsburgh, PA 15260.

Brain dopamine (DA) systems have received little attention in considering mechanisms which underlie antidepressant therapies. It has recently been proposed that antidepressants can induce a subsensitivity of DA autoreceptors (Serra et al., Life Science, 25, 415, 1979). This hypothesis was tested directly using single unit electrophysiological techniques. We now report that repeated tricyclic antidepressants (TCA), electroconvulsive shock (ECS) and amphetamine (AM, which has a brief antidepressant effect) treatments induce DA autoreceptor subsensitivity.

Male albino rats were anesthetized and single unit DA activity was recorded through micropipettes (2M NaCl, 4-12 megohm). DA autoreceptor sensitivity was determined by the administration of a presynaptic dose of apomorphine (0.004 mg/kg, i.v.). This dose was chosen because it has been shown to preferentially stimulate DA autoreceptors and thereby inhibit DA activity (Skirboll et al., Science, 206, 80, 1979). Animals were assigned to the following groups: two or ten days of either imipramine, amitriptyline or iprindole (10 mg/kg, i.p., twice a day); six days of ECS (105 V a.c., 0.7 sec), and six or fifteen days of AM (4 mg/kg, i.p.). Forty-eight hours later autoreceptor sensitivity was determined.

All treatments (TCA, ECS, AM) resulted in a highly significant attenuation of apomorphine's ability to inhibit the spontaneous discharge of DA neurons. Ten days of TCA treatment resulted in a greater degree of DA autoreceptor subsensitivity than two days of treatment.

Is the progressive autoreceptor subsensitivity seen between 2 and 10 days of treatment dependent on daily drug or the passage of time? To test this we administered imipramine for 2 days and waited 10 days before recording. This group showed an identical subsensitivity as the 10 day treatment group, but significantly more subsensitivity than animals given 2 days of drug followed by only 48 hrs. Similar time dependent changes were seen after only one ECS followed by a 7 day delay.

These findings clearly implicate DA autoreceptor subsensitivity as a potential mechanism underlying the clinical efficacy of a spectrum of antidepressant agents. More importantly, they suggest that the delayed therapeutic effects seen following TCA and ECS may depend on the passage of time rather than repeated treatment. Finally, the ability of pharmacological agents to initiate a process which then progresses in the absence of further treatment may necessitate a reevaluation of the most basic views of drug action.

RSDA-MH-00238, MH-23206

271.9 ENDOGENOUS INHIBITOR OF MONOAMINE OXIDASE (MAO) IN PLATELETS OF NORMALS AND SCHIZOPHRENICS. R.E. Becker,* C. Giambalvo, H. Elboim* and H. Lal* Rhode Island Psychiatric Training and Research Center, Cranston, RI 02920, and Dept. of Pharmacology and Toxicology, Univ. of Rhode Island, Kingston, RI 02881.

The activity of platelet MAO varies among subjects and is suggested to also vary with schizophrenic symptoms (Becker, R.E. and Shaskan, E.C., Am. J. Psychiat. 134: 512-517, 1977). We now report presence of an endogenous inhibitor in the plasma of normal volunteers and of schizophrenics, and this finding might explain the variation in MAO activity. The plasma from low, average and high MAO-activity groups among normal subjects and hospitalized schizophrenics was incubated with platelets obtained from the pooled normal blood using ^{14}C-tryptamine as MAO substrate. There was found a linear relationship between the amount of plasma added in vitro and the inhibition of MAO activity. The plasma inhibitory activity and the endogenous MAO levels were inversely correlated. In the schizophrenics with high MAO, their plasma showed no inhibition of MAO even at the highest amount (500 ul) of plasma tested. However, plasma from lower MAO schizophrenics inhibited MAO significantly. A number of neuroleptic and anti-Parkinsonian drugs tested failed to act as the inhibitors. Previous observations that hallucinations occur with greater frequency among schizophrenics with low MAO were also confirmed. The ability of differential MAO levels and the presently reported inhibitory activity in the plasma to predict clinical course of schizophrenic symptomology or drug therapy is being determined presently.

271.10 PROLONGED ADMINISTRATION OF L-DOPA DOES NOT INDUCE DEGENERATION OF NIGROSTRIATAL NEURONS IN THE MOUSE. E. Melamed, F. Hefti, J. Bhawan*, and R.J. Wurtman, Lab. Neuroendocrine Regulation, M.I.T., 56-245, Cambridge, and Department of Pathology, University of Massachusetts Medical School, Worcester, MA.

Autooxidation of L-DOPA gives rise to several cytotoxic products such as quinones, free radicals and also the catecholaminergic neurotoxin 6-hydroxydopamine (Graham et al., Molec Pharmacol. 14, 633, 1978). Toxic effects of L-DOPA on neuroblastoma and melanoma cells have been demonstrated in vitro. L-DOPA is currently the drug of choice in the treatment of many patients with Parkinson's disease. Theoretically, chronic administration of L-DOPA, its uptake by dopaminergic neurons and intracellular formation of cytotoxic substances, could cause neuronal loss in the nigrostriatal system. Therefore, in parkinsonian patients treated with L-DOPA, such L-DOPA toxicity and the underlying etiology of the illness could have a cummulative effect on the rate of the nigrostriatal degeneration and the progression of parkinsonism would be accelerated. This mechanism might also be involved in the emergence of side effects during long-term L-DOPA therapy, such as declining responsiveness to the drug and the on-off phenomenon.

We tested the hypothesis that prolonged administration of L-DOPA can destroy nigrostriatal dopaminergic neurons in an in vivo animal model. C57 black/6 mice received a diet containing 40 mg L-DOPA per g diet. Control animals were given the same diet without L-DOPA. Mice in both groups ingested approximately 5 g of diet per day and were kept on their corresponding diets for a period of 18 months. L-DOPA-treated mice were then transferred to the control diet containing no L-DOPA to allow complete washout of the drug. Eight days after discontinuation of L-DOPA, animals from both groups were decapitated. Corpora striata were assayed for tyrosine hydroxylase and DOPA decarboxylase activities. Striatal DOPA, dopamine and DOPAC levels were measured using high-performance liquid chromatography with electrochemical detection. DOPA was undetectable in striata of both control and L-DOPA-treated mice at the time of sacrifice, i.e.,8 days after withdrawal of the drug. Indices for the number of striatal dopaminergic terminals and the integrity of the nigrostriatal system, i.e. striatal dopamine and DOPAC concentrations and tyrosine hydroxylase and DOPA decarboxylase activities were equal in corpora striata of control animals and of L-DOPA-treated mice.

Our findings suggest that prolonged oral administration of large doses of L-DOPA (approximately 200 mg per animal per day during a period of 18 months) does not decrease the number of nigrostriatal dopaminergic neurons in the mouse. Therefore, long-term L-DOPA therapy in parkinsonism probably does not accelerate the degeneration of nigrostriatal neurons and the progression of the illness.

271.11 BIOCHEMICAL EVIDENCE OF DECREASED MUSCARINIC CHOLINERGIC NEURONAL COMMUNICATION IN AMYGDALA KINDLING. Richard Dasheiff, Mary Byrne*, Vincent Patrone*, and James O. McNamara Division of Neurology, Duke Univ. and VA Med. Center, Durham, N. C. 27710

Kindling is an animal model of epilepsy induced by periodic electrical stimulation of the brain. Pharmacologic evidence suggests that increased muscarinic cholinergic communication contributes to the development of amygdala kindling. Paradoxically, we have previously found decreased numbers of muscarinic cholinergic receptors in multiple sites of the limbic system of kindled rats. To obtain a more complete biochemical assessment of pre- and postsynaptic muscarinic cholinergic communication, we measured two presynaptic indices, choline acetyltransferase (ChAT) and sodium dependent high affinity choline uptake (SDHACU), the degradative enzyme acetylcholinesterase (AchE) and the muscarinic receptor. Rats were sacrificed one day following completion of kindling (defined as a single Class 5 motor seizure consisting of rearing and falling). Amygdala, dentate and hippocampal gyral tissues were dissected and the above indices measured with radiochemical assays. All three anatomic regions yielded similar results. Muscarinic receptors ([^{3}H] QNB binding expressed as pmol/mg $\pm$ SEM) in the dentate for kindled/control (electrode implanted, unstimulated) were (n = 9): left 893 $\pm$ 27, 1114 $\pm$ 45, right 785 $\pm$ 52, 1006 $\pm$ 24 ($p < .005$, paired t-test). SDHACU (pmol/mg/4 min $\pm$ SEM) in the dentate, K/C, was not significantly increased (n = 7): left 33.0 $\pm$ 2.0, 32.6 $\pm$ 2.7; right, 29.0 $\pm$ 2.6, 33.6 $\pm$ 2.1. The activities of ChAT and AchE were not significantly different from controls. Together these results are consistent with a net reduction of muscarinic cholinergic neuronal communication on the day following completion of kindling.

Measurement of muscarinic receptors at additional times disclosed that the reductions developed late (Class 4) during development of kindling and returned towards normal seven days following completion of kindling. Significant increases of choline uptake were never observed, despite measurements during development of kindling, during kindled seizures, and at multiple times after completion of kindling. This transient reduction of muscarinic cholinergic neuronal communication may represent the biochemical basis of an endogenous inhibitory process developing as a consequence of repeated limbic seizures.

272.1 AMPHETAMINE PRODUCES AN ENDURING RECOVERY OF LOCOMOTOR FUNCTION AFTER MOTOR CORTEX INJURY IN THE RAT D. M. Feeney, A. Gonzalez, H. M. Murray and W. G. Dail Depts. of Psychology & Anatomy, University of New Mexico, Albuquerque, NM 87131

Unilateral ablation of the entire motor cortex of the rat produces a marked contralateral hemiparesis. While the animals are able to locomote on a flat surface they are unable to walk normally on a narrow beam for several weeks after injury. We examined the effects of amphetamine and haloperidol on this recovery of locomotor function. Rats were trained to run a 122 cm long, 2.5 cm wide beam to escape a loud noise. Twenty rats were given three trials per day and within a week all animals were running rapidly. The entire motor cortex was then unilaterally removed by suction ablation. All animals were retested at 24-hours postsurgery and most could not maintain their balance on the beam and none could walk or run on the beam. The limbs contralateral to the injury hung off the beam. The animals were rated on ability to traverse the beam and movies taken of their performance for later blind ratings. The animals were assigned to one of four drug conditions A) 1 mg/kg D-amphetamine B) saline control C) 1 mg/kg D-amphetamine followed in 30 minutes by 0.1 mg/kg haloperidol D) 1 mg/kg D-amphetamine followed in 30 minutes by 0.3 mg/kg haloperidol. The single dose of amphetamine or saline was administered immediately after the 24-hour postsurgery testing. The animals were retested every hour for six hours and retested at 12 hours and every other day for 30 days. By one hour after amphetamine, the locomotor ability of the motor cortex injured, hemiparetic rats began to improve. Animals that previously had been unable to even stand on the beam after amphetamine could locomote the length of the board. This improved performance after a single dose of amphetamine was maintained through the subsequent weeks of testing. The saline control group's performance did not match that of the amphetamine group until three weeks postinjury. The 0.1 mg/kg haloperidol dose had no effect; however, the 0.3 mg/kg dose completely blocked the amphetamine-induced recovery of function. The data suggest that following unilateral motor cortex injury in rats, much of the locomotor deficit and contralateral hemiparesis is attributable to a depression of catecholamine function which can be reversed by amphetamine. This hypothesis is being directly examined using catecholamine histofluorescence and biochemical analysis of injured brain.

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272.2 CORTICAL AND HYPOTHALAMIC LESIONS DIFFER IN THE MOVEMENT DYSFUNCTIONS THEY PRODUCE. L. Misantone, M. Suokas*, A. Sassler* and J. Prendergast. Dept. Anatomy, Philadelphia College of Osteopathic Medicine and The Medical College of Pennsylvania, Philadelphia, PA 19131.

Unilateral lesions of either the dorsal cortex or of the lateral hypothalamus result in significant weight loss due to hypophagia and hypodipsia. Our findings suggest that dysfunctions in coordinated movement of forelimb and/or head musculature contributed to these weight losses, and that the 2 groups differ qualitatively in these movement deficits.

Rats (n=12) with unilateral lateral hypothalamic lesions (damaging lateral medial forebrain bundle and medial internal capsule at tuberal level) were compared to rats (n=8) with unilateral lesions of the dorsal aspect of the rostral cortex (sensorimotor fore- and hind-limb areas). Weight losses for both groups over the first 7 days after surgery were significant and equivalent. Subsequently, rats with cortical lesions recovered more quickly, and at 14 days were at their preoperative level, while rats with hypothalamic lesions were still significantly below preoperative. To ascertain the relative contribution of various movement dysfunctions to these ingestive behavior deficits, we examined head orienting responses to probing of the vibrissae and face; proprioceptive (joint deformation) placing and hopping responses, both without visual cues; and forepaw grasping responses. We found that 1 day after hypothalamic lesion, 11 of 12 rats did not orient to probing contralateral to the lesion, and 9 of 12 did not place in the forward direction contralaterally. By day 7 these responses were again present bilaterally, but were more difficult to elicit contralaterally. These two responses were largely unaffected by cortical lesions. On the other hand, backward grasping responses in the paw contralateral to the cortical lesion were nearly completely absent, and remained severely deficient until 7-10 days postoperative. Recovery occurred as a gradual increase of strength that moved from distal to proximal portions of the digits and paw. Alternatively, grasping was present in rats with hypothalamic lesions, and actually appeared initially to be more intense than usual.

Thus, deficits in sensorimotor integregation affecting head (orientation) and forelimb (placing) movements appear after hypothalamic lesions, while deficits in forepaw usage (grasping) appear after cortical lesions. Importantly, while both groups have in common an ingestive behavior deficit, the movement dysfunctions that probably underly the latter deficit differ qualitatively. (Supported by NS16101, EY03360 and NS15893).

272.3 ABNORMAL RELATIONS BETWEEN ISOMETRIC MUSCLE FORCE AND SURFACE EMG IN HEMIPARETIC SUBJECTS. A. Tang* and W.Z. Rymer. BME Program and Physiol. Dept., Northwestern Univ., Chicago, IL 60611.

The decerebrate cat with a dorsal hemisection of the spinal cord is a useful model of human spasticity[1]. A recent study using this model has shown that the slope of the isometric force-emg relation in the decerebrate state is radically increased after dorsal hemisection of the spinal cord. This change was attributed to the lowering of the average motor unit discharge rates following the cord lesion. This lowering of rates has been found in human spastic muscle and it is reasonable to expect that the force-emg relation of spastic muscle should be modified in a fashion similar to that described for the animal model.

Our study compared the force-semg relation of the biceps-brachialis and brachioradialis muscles in normal and spastic limbs of hemiparetic subjects. The muscles on the non-paretic side were used to provide a "control" for the paretic limb. The patient population consisted of subjects with stroke and traumatically induced hemiparesis. Muscular strength of the patients ranged from normal to 25% of normal strength.

Studies were performed on 10 hemiparetics and 7 normal controls. Subjects were seated with the elbow at 90 degrees, the forearm parallel to the floor, and the wrist in a semi-prone position in contact with an immovable handle. This handle was attached to a load cell. Semg was measured using 2 sets of 1 cm dia. platinum electrodes, located over the biceps-brachialis and brachioradialis. Location of the electrodes was identical on each limb. Semgs were amplified, rectified and filtered. The subjects generated a range of static forces, according to a computer-controlled display. Data analysis included plots of mean force vs. mean emg-each calculated over a stationary interval of about 1 sec. Regression lines were fitted to these points by the least squares method.

The relations between isometric force and semg of the biceps and brachioradialis were consistently linear. In 6 of 10 patients, the force-semg slope for the biceps on the paretic side was increased and statistically separable from that of the normal limb's. However, even in those cases where the slopes were not statistically separable, the semg at 2 kg. was usually systematically increased on the paretic side. Results from the brachioradialis were inconclusive. It is concluded that the force-semg relations are modified in the muscles of hemiparetic limbs and second, that this modification probably results from a reduction of motor unit discharge rates in the muscles.

1. Rymer, W.Z; Houk, J.C.; and Crago, P.E. "Mechanisms of the clasp-knife reflex studied in an animal model." Exper. Brain Res. 37:93-113 (1979)

272.4 PSYCHOMOTOR DYSFUNCTION OF PARKINSON PATIENTS. L. Z. Podbros* (SPON: A. Rosen). Dept. of Psych., SUNY at Stony Brook, Stony Brook, New York 11794.

Conventional group studies with parkinson patients have revealed performance deficits on a number of seemingly dissimilar tasks; however relationships among performance on the tasks have not been examined. The present investigation was designed to delineate more precisely some of the parameters operative in these performance deficits. Relationships among 20 clinical/subject variables and performance on a battery of 113 psychomotor measures were examined with 8 idiopathic parkinson male out-patients.

Although each subject shows a variety of deficits, subjects clearly differ from each other in their patterns of performance. After 4 subjects had been tested, Pearson product-moment correlations were computed to identify relationships among clinical and performance variables. Relationships of theoretical interest identified by this method were replicated by recomputation of correlations after each additional subject. Results indicate: 1) differences among subjects in the execution of relatively simple movements (e.g. arm flexion, crossing legs, simple reaction time) clearly relate ($r's \geq .80$) to differences in rigidity and tremor. 2) Rigidity and tremor do not relate as strongly to performance on more complex tasks. 3) Some aspects of psychomotor dysfunction relate strongly ($r's \geq .80$) to proprioceptive factors and other aspects to mnemonic factors; these dissociated deficits may or may not appear in the same parkinson patient. For example, proprioceptive difficulties (e.g. balancing on toes or on one leg) show strong relationships with bead transferring, pursuit rotor and purdue pegboard, as well as with a route-walking task requiring spatial operations. The latter relationship suggests that some of the parkinsonian "cognitive" deficits may be interpreted more parsimoniously than is usually done. However, proprioception can not account for all parkinson "cognitive" deficits. Deficits on a spatial task requiring rotation of stick figures seem to be dissociated from proprioception and related tasks and strongly related to mnemonic factors ($r=-.94$). These results support the role of basal ganglia in functions that involve sensory-motor rather than purely motor components. They also support the view that parkinsonian psychomotor dysfunction involves dissociated impairment of proprioceptive and mnemonic functions.

272.5 THERAPEUTIC EFFICACY OF L-DOPA CHELATES IN ANIMAL MODELS FOR PARKINSON'S DISEASE. K.S. Rajan, B.I. Diamond, R.L. Borison and J.M. Davis. IIT Research Institute, Mt. Sinai Hospital Medical Center, and ILL. State Psych. Inst., Chicago, Illinois.

The pharmacological activities of the Cu(II)- and Zn(II)-L-DOPA chelates were investigated in two different animal models of Parkinson's disease (PD). 6-Hydroxydopamine-lesioned Parkinsonian rats were treated separately with L-DOPA chelates and unchelated L-DOPA. All the chelate-treated PD animals showed significantly larger contralateral rotatory activity than the unchelated L-DOPA-treated PD animals. Experiments on the reversal of reserpine-induced Parkinsonism by L-DOPA and L-DOPA chelate treatments showed a substantial shift of the chelate dose-response curve to the left of the L-DOPA curve indicating substantially increased therapeutic efficacy. Further, treatment with Zn- and Cu-chelate resulted in a four-fold increase of the catecholamine content (DA and NE) in the striatum when compared to that obtained by mere L-DOPA treatment. Treatments with L-DOPA and L-DOPA + carbidopa merely increased striatal dopamine levels, whereas the chelate treatment produced increased levels of norepinephrine and dopamine. The clinical significance of these results is discussed.

272.6 THE EFFECT OF CHRONIC N-n-PROPLYNORAPOMORPHINE ADMINISTRATION ON 6-HYDROXYDOPAMINE LESIONED RATS. P.C. Chen*, R.E. Wilcox, and W.H. Riffee, Dept. of Pharmacology, College of Pharmacy, University of Texas, Austin 78712.

Specific lesions induced by 6-hydroxydopamine (6-OHDA) microinjected into the zona compacta of the substantia nigra (ZCSN) constitute a widely used animal model of Parkinson's disease. N-n-propylnorapomorphine (NPA) is of interest as a potent dopaminergic agonist and as an agent potentially useful in the treatment of various neurological disorders including Parkinson's disease. We have investigated the alteration of both behavioral and biochemical parameters due to 6-OHDA (8µg 6-OHDA base/4µl) induced unilateral lesions of the ZCSN or caudate nucleus (CN) of rats after chronic NPA treatment (5mg/kg i.p. once daily for 4 weeks).

Challenge with 5mg/kg apomorphine (APO) intraperitoneally showed that unilateral lesions of the medial ZCSN resulted in predominant contralateral turning, while lesions of the lateral ZCSN caused predominant ipsilateral turning. Most animals with lesions in the CN showed ipsilateral turning. H^3-DA uptake studies revealed that only rats with 80% or more depletion of dopamine nerve terminals exhibited significant contralateral turning upon APO challenge. After chronic NPA treatment, significant changes in turning behavior were observed in animals with contralateral or ipsilateral turning greater than 4 R.P.M.. H^3-spiroperidol saturability studies indicated that the affinity remained unchanged following chronic NPA administration. The receptor density (Bmax) increased by 70% after 6-OHDA lesions, but decreased by 30% after chronic NPA treatments with the lesioned side retaining more receptors than the unlesioned side. Behavioral alterations may serve as a supplement to neurochemical measures such as uptake and receptor binding to investigate the mechanisms of psychoactive drugs. (Supported in part by NIMH-MH 33443 to WHR and REW and UT-BRSG to REW).

272.7 HYPERTHYROIDISM, PARKINSONISM AND TRI-IODOTHYRONINE RECEPTORS. W. J. Nowack. Department of Neurology, Indiana University School of Medicine, Indianapolis, Indiana 46223.

The clinical association between Parkinsonism and disease of the thyroid has been noted in the past. A fifty four year old lady presented to the Indiana University Medical Center with clearcut clinical evidence of Parkinsonism. One year earlier, when she was seen at a local medical facility for weight loss and nervousness, a minimal tremor of the upper extremities, not thought to be Parkinsonian, was noted. T4 was elevated, a thyroid uptake was within normal limits, a thyroid scan showed cold nodules and nodular nontoxic goiter was diagnosed. Subtotal thyroidectomy was performed and areas of focal lymphocytic infiltration, but no evidence of carcinoma, were found. The patient was on thyroid replacement therapy when she was seen at the Indiana University Medical Center and found to be Parkinsonian. At that time T4-RIA was within normal limits and T3-RIA was elevated. Review of the records revealed no similar cases.

Nuclear receptors specific for tri-iodothyronine have been reported in the brains of rats. This case suggests that pathological stimulation of the tri-iodothyronine receptors in the brain can result in a syndrome clinically identical to Parkinson's disease. Elevations of T3 are usually seen in conjunction with elevations of T4. This case further suggests that the effect of the elevated T4 can mask the Parkinsonian symptomatology and thus, the infrequent observation of the simultaneous occurrence of hyperthyroidism and Parkinsonism may be a direct result of the rarity of isolated T3 thyrotoxicosis.

272.8 SIMILAR ISOELECTRIC PATTERNS OF LIPOAMIDE DEHYDROGENASE IN RAT BRAIN AND OTHER ORGANS. L. Möller*, D. M. Becker, C. Nissenson* and R. A. Pieter Kark. Ataxia Ctr, Reed Neurological & Jerry Lewis Neuromuscular Research Centers, UCLA Sch. of Medicine, Los Angeles, CA. 90024.

Defective activity of lipoamide dehydrogenase (LAD, EC 1.6.4.3) has been associated with inherited neurological disease including Friedreich's ataxia. These studies have largely been carried out in peripheral tissues and the assumption made that the findings may be extrapolated to the nervous system. However, several studies of electrophoretic patterns of LAD from mammalian mitochondria suggest the presence of 2 to 13 distinct bands on the gels, as identified by Coomassie-Blue or the diaphorase reaction. Two bands may represent conformational changes of a single LAD affected by two multienzyme complexes; the others may represent artifacts of partial proteolysis during purification or true distinct protein species. If these are true isoenzymes, and are tissue-specific, defects of LAD in peripheral tissues would not necessarily imply defective LAD in the nervous system. We have previously found a stain for LAD activity in gels dependent upon both dihydrolipoamide and NADH and more specific than diaphorase in identifying partially purified LAD.

In the present study, we investigated the number and pattern of isoelectric bands of LAD from four rat tissues. LAD was extracted from homogenates of brain, skeletal muscle, heart and liver. Extracts were adjusted to equivalent activity of LAD/volume and subjected to isoelectric focusing on a flat-bed, 6% polyacrylamide gel (pH 3-10). Protein was stained with 0.1% Coomassie Brilliant Blue, LAD by our specific method and diaphorase activity by NADH coupled to nitroblue tetrazolium dye.

Fresh samples from brain, muscle and heart had 2 major and 2 minor LAD bands, 4 major and 2 minor diaphorase bands, and over 40 protein bands, while liver had the same plus 4 additional minor diaphorase bands. Additional bands of LAD and yet others of diaphorase activity appeared in tissues after storage for weeks to months. The major bands were identical in all tissues, as were those minor bands that could be detected in each tissue.

The results suggest 1) that the speci(es) of LAD in each of these tissues is the same and 2) that some of the redundant bands may be artifacts arising during preparation and storage.

272.9 KINETICS OF LIPOAMIDE DEHYDROGENASE IN RAT BRAIN AND OTHER ORGANS. M. Jensvold*, S. L. Perlman*, S. Djordjevic*, R. A. Pieter Kark, D. M. Becker, M. R. Budelli*, D. Reeves* and N. Wheeler*. Ataxia Center and Reed Neurological & Jerry Lewis Neuromuscular Res. Ctrs, UCLA Sch. of Med. Los Angeles, CA.90024 & John Hopkins Sch. of Med. Baltimore, MD. 21205.

Abnormalities of lipoamide dehydrogenase (LAD, EC 1.6.4.3) in peripheral tissues have been associated with certain human neurological diseases, especially a recessively-inherited ataxia. The abnormalities appear to breed true in families in a pattern identical to that of the ataxia. However, it is not yet known whether LAD in the nervous system is the same protein species as in peripheral tissues. Kinetic properties can distinguish isoenzymes, and we have studied the kinetics of LAD from brain (B), skeletal muscle (M), heart (H) and liver (L) of 12 rats. To avoid possible selective loss of isoenzymes on purification, assays were in simple homogenates. These were assayed with their own optimal conditions: pH 7.3, 120 μM NADH and 8-12 concentrations of substrate in the range, 60-600 μM lipoamide, both at 30° and 37°.

Kinetic parameters were calculated with reiterative computer analysis by Eadie-Scatchard and Hill methods assuming a) linear and b) non-linear regression on Lineweaver-Burke plots. The two assumptions gave similar values for Km, Hill number and Vmax. Assuming the former, values for Km (in μM lipoamide) at the two temperatures were:

	B	M	H	L
30°	262 ± 37	140 ± 22	192 ± 25	235 ± 16
37°	205 ± 43	332 ± 72	288 ± 67	261 ± 94

Hill numbers ranged from 1.0 to 2.0 at both temperatures, varying with time of storage.

Neither Km nor Hill number varied significantly from tissue to tissue. Vmax values, as specific activity, were consistently high in heart and liver, intermediate in brain, and lowest in muscle ($p<0.005$). By these methods there is no evidence for tissue-specific isoenzymes of LAD in the four tissues of the rat.

272.10 IMINODIPROPIONITRILE NEUROPATHY IN CATS-PHYSIOLOGICAL CONSEQUENCES OF GIANT AXON FORMATIONS. B.G. Gold*, J.W. Griffin*, D.L. Price*, L.C. Cork* and H.E. Lowndes. Dept. Pharmacol., CMDNJ-N.J. Med. Sch. Newark, N.J. 07103 and Depts. Neurol. and Pathol., Johns Hopkins Med. Sch. Baltimore, Md. 21205.

β, β'-iminodipropionitrile (IDPN, 50 mg/kg) given i.p. once weekly for 5 weeks to cats produced a neuropathy manifested by ataxia, hindlimb weakness, swaying, hyperactivity and loss of righting reflexes. Morphologically the neuropathy was characterized by large (often > 100 μm) giant axon formations (GAF) in the proximal internodal region of virtually every spinal motor axon: these GAF remained for the duration of the neuropathy. Smaller GAF appeared more distally in almost all intraspinal axons and spread centrifugally with time. Additionally, scattered multifocal GAF were observed in tibial nerve branches. The consequences of these GAF on motoneuron and motor nerve function were investigated using standard electrophysiological techniques. Soleus (S) motor nerve conduction velocities were decreased an average of 35% 50 days and 49% 100 days after the first dose of IDPN. Renshaw inhibition was reduced from 31% in normal cats to 10.7% in test animals. Amplitudes of spinal monosynaptic reflexes (MSR), evoked by unconditioned (single) stimulation of dorsal roots (DR), S or medial gastrocnemius (MG) nerves were 1.69, 0.540 and 0.365 mV in normal cats but 1.00, 0.137 and 0.027 mV in IDPN-treated cats, respectively. Tetanic stimulation (500 Hz, 15 s) of the same afferents evoked a post-tetanic potentiation of MSR of 82, 111, and 125% in normal cats. Corresponding values in IDPN-treated cats were 157, 236 and 533%. A peculiar, double spike pattern of MSR response was observed in all treated cats, suggested altered spike formation in motoneurons (MN) with GAF. This was confirmed by intracellular recording of antidromically elicited spike potentials in lumbar MN. Spike potentials were normal, exhibited marked premature or delayed depolarizations or fired in doublet or triplet fashion upon single stimulation. These data suggest that IDPN neuropathy in the cat is an excellent model in which to study GAF and their consequences on neurophysiological function.

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272.11 IN VIVO AND IN VITRO EFFECTS OF NEUROTOXIC ACRYLAMIDE ON SELECTED ENZYME ACTIVITIES IN RAT BRAIN, SCIATIC NERVE AND LIVER, Ivy L. Vyas*, Richard D. Howland*, and Herbert E. Lowndes (Spon: Henry Brezenoff). Dept. Pharmacol., CMDNJ, N.J. Medical Sch., Newark, N.J. 07103.

Acrylamide causes a "dying-back" type of polyneuropathy characterized pathologically by degeneration beginning in distal portions of axons of large nerve fibers. It has been suggested that the metabolic basis for this type of neuropathy may be an interference with axonal energy metabolism (Sabri, et. al.: Neurotox. 1:285, 1979, J. Neurochem. 32:683, 1979 and Howland, et al.: Brain Res., in press). It is necessary to establish the precise pattern of effects on parameters of metabolism in neural and non-neural tissues if such a metabolic derangement is to be demonstrated for acrylamide and toxic compounds producing a similar type of neuropathy. The effect of acrylamide on activities of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme associated with axoplasmic transport in neural tissue, and phosphofructokinase (PFK), an important regulatory enzyme in glycolysis, was investigated in homogenates of rat brain, sciatic nerve and liver. Enzymatic activities were determined from initial velocity measurements in a post-nuclear fraction of tissue homogenates. For in vitro experiments aliquots of brain homogenates were incubated with or without acrylamide. When compared to controls incubated without acrylamide, incubation with acrylamide caused an inhibition of both GAPDH (I_{50}=0.41 mM) and PFK (I_{50}=5mM). For in vivo experiments, rats were treated with acrylamide 50 mg/kg/day i.p. to a cummulative dose of 350 mg/kg or 500 mg/kg. When compared to appropriate controls, activities of GAPDH were decreased by 31.9% in brain, 47.9% in sciatic nerve and 30.2% in liver homogenates from rats given 500 mg/kg and 27.8% in brain in rats given 350 mg/kg.PFK activity was decreased in sciatic nerve (23.2%), slightly decreased in brain (5.25%) but not different from control values in liver homogenates from rats given 500 mg/kg. In brain homogenates from rats given 350 mg/kg PFK activity was 10.8% lower than in controls. These preliminary results suggest that inhibition of GAPDH is a specific effect of acrylamide and occurs in both neural and non-neural tissue. The significance of the decreased activity of PFK in brain and sciatic nerve remains to be determined.

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272.12 NEURONAL AND NON-NEURONAL ENOLASE ACTIVITIES IN PERIPHERAL NERVE AND SKELETAL MUSCLES IN CATS WITH ACRYLAMIDE NEUROPATHY. Richard D. Howland* (Spon: Amos G. Gona). Dept. Pharmacol., CMDNJ, N.J. Med. Sch., Newark, N.J. 07103.

Current investigations of the etiology of acrylamide (ACR) neuropathy have focused on the role of inhibition of certain glycolytic enzymes, viz. enolase (EN), phosphofructokinase (PFK) and glyceraldehyde phosphate dehydrogenase (GAPDH). This laboratory has shown that the activity of the neuron-specific isoenzyme of enolase (NSE) is decreased in sciatic nerve and brain of ACR intoxicated rats (Howland et al. Brain Res., in press). In order to assess the significance of inactivation of NSE in the development of ACR neuropathy, the activity of total EN and NSE was measured in sciatic and tibial nerves of cats intoxicated with cummulative doses of either 150 or 300 mg/kg ACR. In addition, EN, PFK and GAPDH were measured in soleus and medial gastrocnemius muscles. (Enzyme activities are expressed as units where one unit equals the formation of one μmole of product per min. All values are $\bar{x} \pm$ SEM for at least 4 animals).

NSE activity in tibial n. was decreased 39 and 85% of control (0.013 ± 0.002 units/mg prot.) in cats receiving 150 and 300 mg/kg ACR, respectively. Total EN and NSE activities did not differ significantly in control sciatic and tibial nerves indicating no proximal-distal gradient for these enzymes. ACR, at either dose, had no effect on either enolase activity in sciatic n. In soleus m. of ACR treated cats EN, PFK and GAPDH activities were unchanged from control activities of 0.91 ± 0.05, 0.095 ± 0.004 and 1.11 ± 0.07 units/mg protein, respectively. The relative activities of these enzymes in gastroc. m. was much different: 5.97 ± 0.15, 0.004 ± 0.0005 and 6.92 ± 0.46 units/mg protein for EN, PFK and GAPDH, respectively. ACR (150 mg/kg) resulted in a 42% decrease in GAPDH activity and a 15% decrease in EN activity. No change was seen in PFK activity nor did the higher dose alter the results. These data suggest that the neurotoxicity of ACR is dependent upon the susceptibility of certain glycolytic enzymes to inactivation and that the susceptibility is tissue dependent. The data also suggest that the inactivation of NSE may be an initiating event in the development of ACR neuropathy.

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272.13 ASSESSMENT OF THE EFFECTS OF THREE NEUROTOXIC COMPOUNDS ON MOTOR FUNCTIONS IN MICE, S. G. Gilbert* and J. P. J. Maurissen. Dept of Rad. Biol. Biophys., University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Sensorimotor dysfunctions can be induced by a wide range of neurotoxic chemicals. Simple techniques to assess such motor disorders, often expressed as hindlimb weakness, have been developed. Rotarod performance and landing foot-spread were measured before, during and after the course of dosing, and the sensitivity of these techniques compared.

Thirty female BALB/c mice were divided into six groups. Acrylamide (250 ppm), 2,5-hexanedione (5000 ppm) and methylmercury chloride (10, 20 and 40 ppm) were dissolved in distilled water and administered via light-tight drinking bottles. Treatment ceased when the first signs of toxicity became evident.

Baseline data were collected before dosing started. Mice were placed twice weekly on an accelerating rod, and their retention time recorded. In the landing foot-spread test, the experimenter dropped mice from 15 cm on a flat smooth surface once a week. The hindlimb splay was then measured by the examiner. Both experimenter and examiner were unaware of the identity of each group during the testing period.

The effects of acrylamide in rats have been described previously with these two techniques (Kaplan and Murphy, 1972; Edwards and Parker, 1977). Decreased retention time and increased hindlimb splay were observed simultaneously in mice after 12 days of exposure. Recovery followed treatment cessation. Increased hindlimb splay preceded an obvious decline of rotarod performance in the group receiving the 10 ppm methylmercury solution.

Mice receiving the 20 and 40 ppm of methylmercury solutions did not display any changes in these tests before overt signs of toxicity. 2,5-Hexanedione did not produce any clear decline in performance although it induced subjectively observed changes (muscle weakness, reduced motor activity) leading to exposure termination.

Our data show that rotarod and hindlimb splay test in mice have approximately equal sensitivity to the effects of several neurotoxic chemicals. However, these techniques cannot always detect motor function disorders before overt signs of toxicity are apparent.

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272.14 PHENYTOIN, METHYSERGIDE, AND PENICILLAMINE IN HEREDITARY MUSCULAR DYSTROPHY OF THE CHICKEN. R.K. Entrikin, G.T. Patterson, and B.W. Wilson. Depts., of Pharmacology, Phys. Med. & Rehabil., and Avian Sciences, University of California, Davis, CA 95616.

Since 1975, improvements in hereditary muscular dystrophy of the chicken have been reported for phenytoin (DPH), methysergide (MES), and D-penicillamine (PEN). It has not been possible to conclude from the published reports which compound is most effective or the duration of treatment required to detect a beneficial drug effect. This is partly due to the fact that different aspects of the dystrophy were evaluated in each study. Even in the case of impaired righting ability, which was used in all the studies, a different method of assessment was used for each drug. Here we report the first direct comparison of these three drugs in a single, standardized test system.

Each drug was evaluated in 3 to 5 short-term trials (25 days duration) and one long-term trial (90 days duration) in male chicks of UC Davis lines 412 normal and 413 dystrophic. Drug doses were similar to those used in the original reports, and were administered i.p., b.i.d., beginning on day 1 ex ovo (PEN was also administered p.o., as originally reported). Righting ability was determined at 10-day intervals using the exhaustion score (ES), the consecutive number of times a chick could rise from the supine position in rapid succession during a single test period. Plasma creatine kinase (CK) activity was determined spectrophotometrically on venous blood.

Mean ES values (± SEM) and 29-day CK values are shown below for normal controls (412C), dystrophic controls (413C), and for one trial with dystrophics treated with DPH, MES, or PEN (n in parentheses).

	Day 15 ex ovo	Day 25 ex ovo	CK (% of 412C)
412C	18.8 ± 1.2 (55)	19.3 ± 1.2 (55)	100.0
413C	3.7 ± 1.2 (31)	1.7 ± 0.6 (31)	3018.2
DPH	7.7 ± 1.1 (9)	6.3 ± 1.9 (9)	1747.6
MES	7.4 ± 1.4 (10)	7.2 ± 2.3 (10)	2188.2
PEN	1.5 ± 0.4 (10)	0.0 ± 0.0 (3)	805.9

These data, as well as those obtained in other trials, confirm the earlier reports that both DPH and MES increase righting ability and decrease plasma CK in dystrophic chicks. As tested here, these drugs appear equally effective. PEN, on the other hand, did not increase righting ability. With respect to duration of treatment required, DPH and MES could be detected as beneficial at the first test period (10 or 15 days ex ovo) in all trials. Although the effects of DPH and MES decreased as the animals aged, both drugs still exerted a slight beneficial effect at 90 days ex ovo. (Supported by the Muscular Dystrophy Association, Inc.).

272.15 THE TURNOVER OF POLYPHOSPHOINOSITIDES IN ERYTHROCYTE MEMBRANES IN MYOTONIC MUSCULAR DYSTROPHY. R. B. Moore*, S. H. Appel and G. A. Plishker* (SPON: A. Coats). Dept. of Neurology, Baylor College of Medicine, Houston, Texas 77030.

Erythrocyte membranes possess a phosphodiesterase which in the presence of calcium cleaves the polar head groups from di- (DPI) and triphosphoinositides (TPI). The products of this reaction are 1,2 diacylglycerol and inositol di- and triphosphates. The diacylglycerol is converted in the presence of ATP by a phosphokinase to phosphatidic acid (PA). Recently, it has been reported that the calcium-dependent PA synthesis is altered in erythrocytes in myotonic muscular dystrophy (MyD) (Grey et al, Fed. Proc. 39:2495, 1980). In our studies calcium-dependent hydrolysis of DPI and TPI in erythrocyte ghosts and ATP depleted erythrocytes of normals and MyD patients was examined. A 40% loss of ^{32}P phospholipids was observed in ghosts incubated at 37° for 10 min. in the presence of 0.3 mM calcium. Thin layer chromatography showed that 94% of the ^{32}P was incorporated into DPI, TPI and PA. The loss in radioactivity resulted from a 70% decrease in DPI and a 30% decrease in TPI. PA remained unchanged. Calcium activation studies at pH 7 in the presence of 1 mM $MgCl_2$ revealed a half maximal hydrolysis of TPI and DPI at 0.8 μM calcium. Strontium was found to be 100 times less effective than calcium and barium was without an effect at 0.1 mM. Over the range of 1.5×10^{-7} to 1×10^{-3} M calcium the amount and pattern of polyphosphoinositide hydrolysis from 7 patients with MyD was normal. The hydrolysis of TPI as compared to DPI was higher in intact ATP depleted cells exposed to the calcium ionophore A23187. Cells incubated at 20° for 30 min with 1 μM A23187 and 3.6 μM free calcium showed a 65% loss in DPI and a 50% loss in TPI. Two patients showed similar patterns of TPI and DPI hydrolysis. In ^{32}P-labeled cells containing ATP more than a ten fold increase in ^{32}P-labeled PA was observed when they were incubated at 20° for 60 min in the presence of 3.6 μM calcium and 1 μM A23187. Half maximal synthesis of PA was observed in the presence of 0.4 μM calcium. A comparison of 7 patients with normal age, sex-matched controls revealed no significant differences in radioactive PA synthesis. A batch experiment demonstrated that the specific activity of the PA was the same for a normal and a myotonic. These results suggest that 1) the hydrolysis of TPI is higher in intact cells than in ghosts; 2) the hydrolysis of DPI and TPI in ghosts and ATP-depleted intact cells is normal in MyD; and 3) synthesis of PA in ATP containing cells is normal in MyD. (Supported by the Muscular Dystrophy Association and by a fellowship to RBM by the Muscular Dystrophy Association of Canada).

273.1 MORPHOLOGICAL AND QUANTITATIVE ANALYSIS OF ACETYLCHOLINESTERASE-CONTAINING NEURONS IN CAT NEOSTRIATUM. J. O'Reilly-Fromentin*, R. Boucher and A. Parent, Lab. Neurobiol., Fac. Med., Laval Univ., Québec, Canada.

The use of the Butcher's pharmaco-histochemical protocol (Butcher et al., '75) for the demonstration of acetylcholinesterase (AChE), which involves pretreatment with di-isopropylfluorophosphate (DFP), has allowed a clear visualization of AChE-producing neurons in cat neostriatum with minimal background staining. A quatitative investigation of these neurons could thus be undertaken with the help of a Zeiss modular system for quantitative digital image analysis. The AChE neurons in cat neostriatum represent only a small percentage of the total striatal cell population: 3-6 cells/mm^2 in caudate nucleus, and 14-15 cells /mm^2 in putamen. In caudate nucleus the strongly-stained AChE neurons were either fusiform with one typical thick process emerging from one pole of the cell body, or multipolar with numerous thinner processes. In putamen the AChE neurons were larger than those of the caudate and had a round or polygonal cell body with innumerable processes branching abundantly. These processes often intermingled with the myelinated fiber fascicles traversing the putamen. Measurements made on 138 caudate cells and 177 putamen cells reveal that the AChE neurons in caudate had a maximum diameter of 33.3 ± 0.7 μm (mean ± S.E.M.) and a surface area of 390.9 ± 0.7 μm^2. The AChE neurons in the rostral half of putamen had a max. diam. of 36.7 ± 0.7 μm and a surface area of 524 ± 15.5 μm^2, whereas those in caudal half of putamen were even larger: max. diam. 41.1 ± 0.6 μm and surface area 662.1 ± 15.1 μm^2. In caudal portion of putamen some AChE cells as large as 60 μm (max. diam.) were encountered. In the hope to find out if some of the AChE striatal cells are output neurons, HRP was injected in the entopeduncular nucleus or in the substantia nigra pars reticulata and retrorubral nucleus, in 4 cats, and the brain sections processed according to the combined HRP-AChE method of Mesulam ('76). After HRP injections a multitude of medium-sized (15-20 μm) labeled cells were found in both caudate and putamen, sometimes closely surrounding the larger AChE neurons. However, HRP granules could not be detected in the striatal AChE neurons themselves.

These findings suggest that, in cat, the AChE neurons of the putamen are morphologically different and significantly larger than those of the caudate nucleus, and support the view that the *striatal AChE cells are not output neurons.*

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273.2 COMPUTER-ASSISTED QUANTIFICATION OF DENDRITIC MATURATION IN THE KITTEN CAUDATE NUCLEUS. J.P. McAllister*, M.S. Levine, C.D. Hull, and A.M. Adinolfi. Mental Retardation Research Center, School of Medicine, UCLA, Los Angeles, CA 90024.

Several quantitative analyses have been employed to assess the dendritic maturation of spiny neurons in the kitten caudate nucleus. Tissue from kittens in 4 age groups (see Table) was impregnated using the Rapid Golgi method. Spiny neurons were drawn using a camera lucida and three dimensional analysis performed with a PDP 11/40 computer. Comparisons of mean values indicate: 1) the number of branches per dendrite remains constant, 2) total dendritic length increases approximately 20-25%, 3) branch length (Br), length of each dendrite and dendritic field radius all increase gradually during the first 10 days, then rise sharply between the second-third week to values observed in older animals. Since mean length of the first dendritic segment remains relatively constant at 18-22μm, linear growth occurs along intermediate and distal branches. These relationships were confirmed by comparing the same measurements from only the longest (optimally impregnated) dendrite of each cell, a procedure that corrects for branches that are truncated as they pass out of the section. The decrease in total dendritic length in older kittens may correlate with the finding that loss of primary dendrites occurs sometime after the third week. Counts of dendritic spines drawn at 1000x magnification indicate that these appendages are lost along proximal segments (P) but increase 228% on distal branches (D) with development. A concentric sphere analysis further demonstrates that nearly all dendritic branches (# crossings) are confined within 180μm of the soma origin at 2-3 days of age, but gradually shift to 270μm away by 90-143 days. Likewise, during the first two weeks of age, free endings are clustered between 60 and 150μm from the cell origin, but are distributed evenly up to 270μm in the older kittens. Branch order analysis indicates that the ordering of segments is not rearranged with development, and that with age dendritic branches lengthen uniformly beyond the primary segment. These results illustrate that the dendrites of most caudate neurons exhibit significant increases in length and distal spine density during the early postnatal period without changes in branch numbers, and suggests that apparent dendritic remodeling may be involved in neuronal maturation.

				Mean Length in μm				Spines/μm		
Age	#Cats	Cells	#Dend	Br	Ea.Dend	Radius	Total	P	I	D
2-3d	5	(81)	5.24	55	544	148	2708	.14	.28	.31
8-10d	3	(43)	5.38	60	598	154	3068	--	--	--
19-23d	2	(30)	5.43	69	680	181	3482	--	--	--
90-143d	5	(88)	4.72	74	725	204	3263	.07	.38	1.03

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273.3 TOPOGRAPHIC ORGANIZATION OF THE HABENULO-INTERPEDUNCULAR PATHWAY IN THE RAT. B.A. Flumerfelt, A. Contestabile* and A.W. Hrycyshyn*. Dept. of Anatomy, University of Western Ontario, London, Canada.

Using the HRP tracing method, the topographic organization of the projection to the interpeduncular nucleus (IPN) from the habenular nuclei (Hb) was investigated in the rat. Hand drawn micropipettes (O.D. 50μm) were filled with 50% HRP by capillarity and the solution was allowed to dry at the tip. The micropipette was then placed stereotaxically in the IPN and left in place for 10-30 mins., producing a discrete locus of HRP activity at the pipette tip. Using this method, HRP was deposited in various locations throughout the IPN in 32 rats. Following post-operative survival times of 2-3 days the animals were sacrificed and HRP activity was demonstrated in frozen serial sections through the brain using tetramethylbenzidine as the chromogen. The following pattern of organization in the Hb-IPN pathway was revealed: The main component arises from the medial Hb and follows a reversed caudorostral pattern, terminating throughout all but the caudalmost part of the IPN. The ventral two-thirds of the IPN receives a much heavier projection such that a large ventrolateral area of the medial Hb projects to the lateral part of the IPN in a completely bilateral way. An additional projection which is predominantly ipsilateral arises from the rostral half of the dorsolateral part of the medial Hb and terminates in the caudal IPN. The medial part of the medial Hb projects preferentially to central areas of the IPN.

The projection from the lateral Hb is quantitatively much smaller but appears to be distributed to the entire length of the IPN following a non-reversed, caudo-rostral arrangement, with the ipsilateral projection predominating. The projections from the medial and lateral Hb to the IPN were confirmed by tracing anterogradely transported HRP as well. No reciprocal connection from the IPN to the Hb could be demonstrated.

Additional observations revealed a small projection to the IPN with a strong ipsilateral predominance from the horizontal limbs of the nucleus of the diagonal band of Broca. Small projections from the premammillary and supramammillary nuclei were also demonstrated. Confirmatory data and some details of organization were also obtained for projections to the IPN from other areas, including the medial and dorsal raphe nuclei, the dorsal tegmental nucleus of Gudden and the adjacent dorsolateral tegmental nucleus. Very small projections from the ventral tegmental nucleus and the locus coeruleus were also found.

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273.4 THE INFLUENCE OF DOPAMINERGIC MECHANISMS ON TWO TYPES OF CERVICAL DYSTONIA IN THE CAT. F. Malouin and P. Bédard (SPON: L.J. Poirier). Lab. de neurobiologie, Université Laval, G1K 7P4.

Destruction of the dopaminergic nigro-striatal pathway in cats by injection of the neurotoxine 6-hydroxy-dopamine (6-OHDA) resulted in the appearance of an asymmetric static head position accompanied by spasmodic movements. These movements, which consisted of horizontal deviations of the head to the side ipsilateral to the lesion, were observed by the first post-injection day. In contrast, an electrolytic lesion dorsolateral to the substantia nigra (SN), which interrupted the striato-nigral efferents and destroyed part of the SN pars reticulata produced spasmodic horizontal deviations of the head to the side contralateral to the lesion. The influence of dopaminergic pharmacological agents on the asymmetrical cervical posture and the spasmodic head movements was described by means of concomitant recordings of electrogoniometric measurements of the neck movements and the electromyographic activity from neck muscles.

In the two types of cervical dystonia apomorphine (0.1 mg/kg s.c.), a dopamine agonist, corrected and subsequently reversed the asymmetry of the head, while pimozide (2.5 mg/kg I.P.), a dopaminergic antagonist, increased the spontaneous asymmetry. However while amphetamine (1.0 mg/kg I.M.) a dopaminergic agent increased the asymmetry caused by 6-OHDA lesion, it reversed the asymmetry resulting from the electrolytic lesion.

These results emphasize the role of the dopaminergic system in the modulation of head movements following the interruption of the nigro-striatal and striato-nigral pathways in the cat.

273.5 AXONAL ARBORIZATIONS OF NIGROTHALAMIC AND NIGROTECTAL CELLS: ANATOMICAL AND ELECTROPHYSIOLOGICAL STUDIES. J.M. Deniau*, I. Grofova*, D. Steindler*, S.T. Kitai (SPON: L.I. O'Kelly). Depts of Anatomy, Michigan State Univ., U.S.A., Univ. Oslo, Norway, Lab. Physiol. Ctr. Nerv., Univ P. et M. Curie Paris, France.

Patterns of intrinsic and extrinsic axonal arborizations of rat nigrothalamic and nigrotectal cells were studied by intracellular recording, intracellular HRP labeling and extracellular double labeling techniques.

Intrinsic Collaterals. Monosynaptic IPSPs were recorded in substantia nigra pars reticulata (SNr) following Thalamus (Th) and Superior Colliculus (SC) stimulation. These responses remained after hemitransection of the brain just rostral to Th, eliminating a possible source of inhibitory inputs from forebrain. Since there is no known connections from either Th nor SC to SNr, these monosynaptic IPSPs were considered to be induced by recurrent collaterals. Light microscopic examination of nigrothalamic and nigrotectal neurons intracellularly labeled with HRP revealed that axons of these neurons give off 1-3 collaterals within the SN. These collaterals arborize partly within and partly outside of the dendritic field of the parent cells and also reach the pars compacta. These morphological and electrophysiological observations indicate that intrinsic axon collaterals of SNr neurons are involved in collateral inhibition.

Extrinsic Branching. Previously we have demonstrated that antidromic activation of SNr neurons from Th, SC and Striatum indicate that many SNr cells project at least to two different target nuclei (i.e. Th and SC or Th and Striatum). We now demonstrate anatomically that branched axonal projections arise from SNr cells by the observation of double labeled neurons following conjugated extracellular injections of HRP in Th and tritiated wheat germ agglutinin in the Striatum or SC. Evidence for axonal branching of SNr projection cells is also provided by tracing the axons of nigral cells intracellularly labeled with HRP. An axon of a nigrothalamic neuron was seen to divide in four branches in the ventral mesencephalic tegmentum. One of the branches was followed rostrolaterally until reaching the ventral part of the subthalamic nucleus. The other three branches ran dorsocaudally in the direction of the SC and gave off several branches in the mesencephalic reticular substance. Some of them were of small caliber and arborize locally whereas two thick branches were seen to turn rostrally toward the Th. These observations favor the hypothesis that single SNr neurons may exert a synchronous influence on areas of CNS which are involved in the integration of sensorimotor activities.

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273.6 PROJECTIONS OF GLOBUS PALLIDUS NEURONS REVEALED BY LOCALIZED INJECTION OF RADIOACTIVE AMINO ACIDS IN MONKEY. J.L. DeVito and M.E. Anderson, Regional Primate Research Center and Dept. of Neurol. Surg., Rehab Med., and Physiol. and Biophys., Univ. of Wash., Seattle, WA 98195.

The destination and course taken by globus pallidus (GP) axons reaching thalamic and brainstem structures were traced by autoradiography in monkeys in which simultaneous injections of horseradish peroxidase (HRP) allowed the identification of potential afferents to the same pallidal sites.

The borders of GP were determined in 4 awake Macaca mulatta by recording the tonic discharge patterns characteristic of pallidal neurons. A mixture of radioactive amino acids and HRP (0.2-0.3 ul) was injected into GP via a microliter syringe. Survival times were 2-3 days and brain sections were processed with standard autoradiographic techniques. Major projections from the medial pallidal segment (Gpi) were traced to thalamic nuclei, ventralis anterior (VA) and lateralis (VL), the centromedian (CM) the habenula, and the pedunculopontine nucleus (PPN) as described by others. The clear topographic input from striatum into dorsal, central and ventral GPi as determined by retrograde transport of HRP was not reflected in orthograde transport of isotopes to the regions listed above. Efferents from a ventral GPi site extended somewhat more medially and efferents from dorsal GPi somewhat more laterally in the VA and VL thalamic nuclei. There was, however, considerable overlap of projections from the two regions. In contrast to some reports, GPi projections extended dorsally into the pars caudalis region of VL (VLc). This part of VLc seemed to correspond to areas free of degeneration following cerebellar lesions (G.Percheron, J.Hirnforsch, 1977), thus reinforcing the concept that pallidal and cerebellar fibers terminate independently in the thalamus. VLc connections were not observed following an injection centered in the lateral pallidal segment. Intralaminar connections were predominantly to the magnocellular part of CM and lateral part of the parafascicular nuclei. These regions are known to receive fibers from premotor and rostral area 4 cortex. The caudal and lateral parvocellular region of CM, which receives projections from caudal area 4 was only lightly labeled following GP injections. Pallidal axons took multiple pathways through the mesencephalon with the PPN as a major terminus. En route, the pattern of silver grains was suggestive of terminals in the prerubral field. Some fibers in the medial longitudinal fasciculus were also labeled although a final destination apart from the PPN could not be determined. Labeling of these pathways did not depend on involving substantia innominata adjacent to GP as has been suggested.

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273.7 THE VESTIBULAR PROJECTION UPON THE INTRALAMINAR THALAMUS AS DEMONSTRATED BY ANTEROGRADE TRANSPORT OF H^3-LABELED AMINO ACIDS. Charlotte M. McGuinness*, Michael Bixler*, and George M. Krauthamer (SPON: A. Hess). Dept. of Anatomy, CMDNJ-Rutgers Medical School, Piscataway, NJ 08854.

The presence of projections from the vestibular nuclei (VN) to the thalamus has been disputed, although electrophysiological responses to vestibular stimuli have been recorded in the intralaminar nuclei (IL), ventrobasal complex, and other areas. Studies using retrograde transport of horseradish peroxidase (HRP), in this laboratory, have indicated a projection from the medial VN and lateral VN to centrum medianum of IL (Brain Res., 1980, 184:255). Several recent autoradiographic studies of VN have not specifically examined the efferents of these two nuclei, and have therefore failed to unequivocally describe this projection.

Multiple small injections of H^3-labeled proline and leucine were made unilaterally in the medial and lateral vestibular n. of cats. Animals survived one week, and brains were processed according to standard procedures.

Autoradiograms of frontal sections taken through the thalamus showed that the injected nuclei project to a restricted portion of IL. Label was found, fairly uniformly, throughout centrum medianum; it was not present in abutting nuclei, nor in more anterior sections through centralis lateralis, indicating that the projection terminates at this level. The contralateral projection exceeded the ipselateral one, confirming our earlier HRP studies. Further experiments will examine possible differences between projection of the medial and lateral vestibular nuclei.

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273.8 GABA IS A PALLIDOTHALAMIC NEUROTRANSMITTER. J.B. Penney* and A.B. Young. Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

Research on the movement disorders has focused primarily on the nigrostriatonigral pathways. Much information about this neuronal circuit has become available. Despite the numerous studies, it is difficult to develop any theory of basal ganglia dysfunction based solely on this circuit. We are currently studying the more classical basal ganglia corticostriatopallidothalamocortical circuit in order to more adequately understand the neurochemical correlates of the movement disorders.

One of the major outputs of the basal ganglia is the pathway from medial globus pallidus (MPS) to ventrolateral nucleus (VL) of the thalamus. Neurophysiologic studies indicate this pathway tonically inhibits VL (Geogopoulous, A.P., Neurosci. Abst. 4:43, 1978; Uno, M., Brain Res. 99:377, 1975).

We have injected kainic acid (KA) into the murine counterpart of the MPS, the entopeduncular nucleus (EP), and measured neurotransmitter levels in VL. 1 nmole of KA is injected microelectrophoretically into one EP of anesthetized rats. At 2 weeks, animals are sacrificed, the brain rapidly removed and punches taken from VL bilaterally. Amino acid levels demonstrate a selective 54% decrease in GABA levels on the lesioned side. There are also 46% decreases in both glutamic acid decarboxylase, the rate limiting enzyme in GABA synthesis, and in high affinity synaptosomal uptake of GABA.

Thus, there is strong evidence that the pallidothalamic output of the basal ganglia utilizes GABA as its neurotransmitter. Since the striatopallidal pathway is also GABAergic, the presence of sequential GABAergic pathways may explain the failure of GABA agonists in movement disorders such as Huntington's Disease. Supported by USPHS grants NS00464-01, NS00420-01 and NS15140-02, the United Cerebral Palsy Research Foundation and Michigan Memorial Phoenix project grant 567.

273.9 THE PALLIDO-SUBTHALAMIC PROJECTION IN RAT: ANATOMICAL AND BIOCHEMICAL STUDIES. T. Hattori, Derek van der Kooy, Kathleen Shannak* and O. Hornykiewicz. Dept. Anatomy, Univ. Toronto, Toronto, Ont., M5S 1A8, Human Brain Lab, Clarke Inst. Psych, Toronto, Ont., M5T 1R8, Inst. Biochem. Pharmacol., Univ. Vienna, Austria

Horseradish peroxidase injected into the rat globus pallidus was transported retrogradely to subthalamic nucleus neuronal cell bodies and anterogradely to axon terminals in the subthalamic nucleus. Electron microscopic observations revealed that the labeled axon terminals made symmetrical axosomatic and axo-dendritic synaptic contacts with labeled subthalamic nucleus perikarya and dendrites. Injection of kainic acid in the globus pallidus several days prior to the horseradish peroxidase injection abolished the anterograde but not the retrograde transport of the tracer. This suggested the anterograde labeling observed in the subthalamic nucleus originated from neuronal cell bodies in the globus pallidus.

Kainic acid lesions identical to those employed in the above anatomical studies resulted in a loss of neuronal cell bodies throughout the globus pallidus and caused a drop in glutamic acid decarboxylase and choline acetyltransferase levels in the globus pallidus. Levels of these two enzymes were not changed in the subthalamic nucleus after the globus pallidus kainic acid lesions, but both showed small, statistically significant decreases in the substantia nigra. It was concluded that there is a massive pathway from the globus pallidus to the subthalamic nucleus, which terminates on subthalamic nucleus neurons projecting back to the globus pallidus. Neither γ-aminobutyric acid nor acetylcholine is the major neurotransmitter in the massive pallido-subthalamic pathway.

273.10 SOMA-DENDRITIC MORPHOLOGY OF THE SUBSTANTIA NIGRA PROJECTION NEURONS INTRACELLULARLY LABELED WITH HRP. I. Grofova* J.M.Deniau* and S.T.Kitai. (SPON: M.B.Rheuben) Depts. of Anatomy, Mich. State Univ., U.S.A.: Univ. Oslo, Norway, Lab. Physiol. Ctr. Nerv., Univ P. et M. Curie, Paris, France.

Rat substantia nigra pars reticulata (SNR) neurons were activated antidromically following thalamic or tectal stimulation and subsequently intracellularly labeled with HRP. After fixation the brains were cut in a sagittal or frontal plane, processed for HRP histochemistry, osmicated and flat-embedded in plastic following the method of Wilson and Groves (J.Neurosci. Methods, 1:383-391, 1979).

Light microscopic examination of 25 labeled cells revealed three types of projection neurons. The most frequently encountered was a medium-size cell in the dorsal part of SNR. Three to five thick primary dendrites arise from the elongated cell bodies and immediately branch to form secondary dendrites which course primarily anteroposteriorly and dorsoventrally, but also having a considerable spread in the mediolateral direction. The dendritic fields extend up to 1400μm anteroposteriorly, 700μm mediolaterally, and 500μm dorsoventrally covering almost the entire anteroposterior and dorsoventral extents of SN. Thin beaded branches, spines, and protrusions of various sizes and shapes appear on all dendrites and are more frequent distally. Ventral dendrites terminate in complex arborizations, "thickets", composed of several branches covered by numerous pleomorphic appendages. The medium-size cell lying in the ventral part of SNR has a strikingly different dendritic organization. The dendrites course mediolaterally and anteroposteriorly, and completely remain within the ventralmost part of SN. Distally, the dendrites branch more frequently and are covered by numerous pleomorphic appendages. Some terminal dendrites are varicose, other form complicated thickets. Large cells are characterized by voluminous somata and sparsely branching dendrites which gradually taper toward the end and course mainly anteroposteriorly. The dendritic field is relatively narrow mediolaterally and dorsoventrally. Thin tortuous branches which are often beaded arise from all the dendrites but spine-like appendages and terminal thickets are absent.

The nigrothalamic and nigrotectal cells were intermingled in SNR and their appearance was not related to whether they were identified as nigrothalamic or nigrotectal. On the other hand, the dendritic morphology of medium-size neurons was related to their position within SN. The observations suggest that, regardless of the precise topography of the striatonigral fibers, the nigrothalamic and nigrotectal neurons may receive afferents from wide areas of the striatum.

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273.11 COMBINED PUSH-PULL CANNULA AND ELECTROCHEMICAL DETECTOR IN CAT CAUDATE. C.R. Freed* and R. Lane. Depts. Med. & Pharmacology, Div. Clin. Pharmacology, U. Colorado Health Sciences Center, Denver, CO 80262; Dept. Chemistry, U. Oregon, Eugene, OR 97403.

To evaluate the specificity of the response of the in vivo electrochemical detector, we have correlated the detector response to actual catecholamine measurements made on the outflow of a push-pull cannula implanted in the same cat. Cats of either sex weighing 4-6 kg were anesthetized with 1-1.5 gm/kg of urethane. A 200 micron carbon paste electrochemical detector electrode was placed stereotaxically in the left caudate. Signal was measured at a fixed potential of 0.7 volts using a model LC-2A amplifier (Bioanalytical Systems). A 1.5 mm O.D. stainless steel push-pull cannula was inserted in the right caudate and the caudate perfused at 25 microliters per minute. The outflow passed through a second electrochemical detector and then to a fraction collector. One-half hour fractions were collected and assayed for catecholamine content by high performance liquid chromatography with electrochemical detection. A drug challenge of L-dopa 200 mg/kg I.P. was given together with the peripheral decarboxylase inhibitor carbidopa 50 mg/kg. Electrochemical signals and push-pull cannula fractions were monitored for 16 hr thereafter. Fractions were assayed for L-dopa, dopamine, DOPAC, HVA and 3-O-methyldopa. Results show that the in vivo electrochemical detector and the detector in the outflow stream of the push-pull cannula gave similar responses over time. Furthermore, the peak shape of the electrical response could be reconstructed from the catecholamine concentration data. The electrochemical detector was most sensitive to dopamine (response = 1.00) and less sensitive to L-dopa (.21), DOPAC (.04), HVA (.03), and 3-O-methyldopa (.03). Because of this differential sensitivity, most of the electrochemical detector response, both in vivo and in the push-pull cannula outflow, was due to L-dopa and dopamine. These data show that the in vivo electrochemical detector set at fixed potential is responding to catecholamines and metabolites that are soluble and that can be eluted from tissue. The electrochemical response correlates well with the concentration time curve for catecholamines assayed from push-pull cannula fractions.

273.12

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273.14 MOVEMENT DISORDERS ASSOCIATED WITH KAINIC ACID LESIONS IN PARTS OF THE AVIAN "BASAL GANGLIA". Garl K. Rieke. Dept. Anatomy, Texas A&M University, College of Medicine, College Station, TX 77843.

The pigeon paleostriatal complex(PC) is composed of three parts: the paleostriatum augmentatum(PA), the paleostriatum primitivum(PP) and the nucleus intrapeduncularis(INP). The PA may be the avian analogue of the caudatoputamen, while the PP-INP may be analogous to the mammalian globus pallidus. The PP projects to the ipsilateral nucleus spiriformis lateralis(SpL), the nucleus tegmenti pedunculopontinus(TP), the nucleus of the ansa lenticularis(AL) and the nucleus dorsointermedius posterior thalami(DIP) by way of the ansa lenticularis. The SpL may be the analogue of the pars reticulata of the substantia nigra(SNr), and the TP may be the pars compacta of the substantia nigra. The analogies are based upon comparative neuroanatomical, neurohistochemical observations and one recent behavioral study. If the PC and those nuclei that receive inputs from the ansa lenticularis comprise the avian analogue of the mammalian basal ganglia, then can neurochemically induced lesions in the avian brain be associated with movement disorders similar to those induced in mammals by treatment of analogous structures with the same neurochemical agent?

One microliter volumes of kainic acid (KA, 2.5-0.213µg/µL, pH 7.4-7.6) were intracerebrally injected into parts of the avian basal ganglia on one side. Equal volumes of vehicle alone were injected into the opposite side, and the birds were allowed to survive for 17hrs. to 5 days. Unilateral injection of KA into the PC destroyed the large neurons of the PP and reduced the numbers of neurons in the PA. The birds showed unusual postures of the head, irregular arrhythmic movements of the head and rotatory episodes. Birds rotated toward the side of injection as did rats with KA injections in the corpus striatum. Kainic acid destroyed neurons in the SpL, the AL and part of the TP. The birds again demonstrated unusual postures of the head, arrhythmic movements of the head, increased resistance to passive movement of the lower limbs and the neck, and rotatory episodes. Birds rotated away from the side of injection particularly when the SpL was involved in the lesion. The contralateral rotation occurred at frequencies as high as 24 turns/minute. The direction of rotation in birds following SpL lesions was the same as that in rats after KA lesions to the pars reticulata of the SN. Sham controls did not rotate, nor were postural problems present. In conclusion there appears to be a striking similarity in movement disorders in both birds and rats following KA induced lesions of the analogous components of the basal ganglia of the respective brains. The pigeon may therefore serve as another useful experimental model to assess the biochemical, neuropathological and behavioral changes associated with movement disorders.

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273.15 FURTHER STUDIES OF THE NIGROSTRIATONIGRAL MECHANISMS AND THEIR ROLES IN MORPHINE SUPPRESSION OF CAUDATE NEURONAL ACTIVITIES. Edward P. Finnerty and Samuel H.H. Chan. Dept. of Life Sciences, Indiana State University, Terre Haute, IN 47809.

A population of substantia nigra zona reticulata (SNR) neurons which appears to play an inhibitory role in the striatonigral feedback pathway was recently identified (Finnerty and Chan, Neurosci. Abst. 4:443, 1978; Eur. J. Pharmacol. 59:307, 1979; Grace and Bunney, Eur. J. Pharmacol. 59:211, 1979). This population of SNR neurons is thought to project to the substantia nigra zona compacta (SNC), and is involved, via an inactivation, in the morphine (MO) suppression of spontaneous caudate nucleus (CN) activities. Based on electrophysiologic evidences, we suggested that the chemical transmitter involved may be glycine (GLY) (Finnerty and Chan, Neurosci. Abst. 5:71, 1979). The present study is a further analysis of the role of the SNC and SNR neurons in the MO suppression of CN activities.

Spontaneous extracellular single-neuron activities were recorded from the SNC and CN by means of stereotaxically placed tungsten microelectrodes in pentobarbital (50 mg/kg, i.p.) anesthetized rats. Direct microinjection to the SN, at a volume of 1 µl, was performed by a stereotaxically positioned 27-gauge syringe needle attached to a microinjection device. Systemic injections were achieved via a cannulated jugular vein or intraperitoneally.

Microinjection of MO (100 µg) into the SN produced a suppression of the spontaneous activities of the CN, coupled with a simultaneous increase in the SNC discharges. The suppressive effect of MO on CN activities was not observed, however, in rats pretreated with the dopamine (DA) receptor blocker haloperidol (HAL, 0.5 mg/kg, i.p.). These results provided confirmation of our previous postulation that MO suppression of CN activities is at least in part produced via a direct activation of the DA-containing nigrostriatal pathway.

Intranigral microinjection of GLY produced an inhibition of the SNC neurons. An excitation of the CN cells, at a parallel time-course, was also observed. GLY microinjected into the SN following HAL pretreatment resulted in only a suppression of the SNC with no effect on the CN units. These data further suggested that GLY may well be the transmitter agent of the inhibitory SNR neurons that are interposed between the CN and the SNC DA-containing neurons in the striatonigral feedback pathway.

(We gratefully acknowledge the generous supply of haloperidol by McNeil Laboratories, morphine sulfate by Eli Lilly & Co. and naloxone HCl by Endo Laboratories used in these studies).

273.16 AUTORADIOGRAPHIC OBSERVATIONS ON THE ORGANIZATION OF THALAMOSTRIATAL AND CORTICOSTRIATE PROJECTIONS IN CATS AND KITTENS. J.A. Cospito, M.S. Levine and A.M. Adinolfi. Mental Retardation Research Center; Dept. of Anatomy, UCLA, Los Angeles, CA 90024.

The present study describes the organization of intralaminar thalamic projections within the caudate nucleus and putamen of kittens and cats and contrasts these observations with our earlier findings on the organization of precruciate corticostriate connectivity at comparable ages. Two adult cats and nine kittens (3 each at 2, 6, and 21 days) were used. In each instance, large single or multiple injections (0.2-0.7ul for total radioactive concentration of 5-35uCi) of equal mixtures of tritiated leucine and proline reconstituted in sterile saline filled the intralaminar complex on one side and extended into lateral portions of the mediodorsal nucleus. Animals were sacrificed between 24 hours and 5 days depending on their ages. In adult cats, thalamic projections fill the ipsilateral head of the caudate nucleus and putamen. The clustering or patchy nature of these projections (which has been described by Royce, Brain Res. 146: 145-150, 1978) was seen as irregular zones of density of the transported label within rostral neostriatum. Zones of intense labelling were also seen in the caudate body. In kittens, the organization of thalamic inputs is well-established by 21 days within the rostral neostriatum where the patchy nature of terminal fields is evident but less pronounced than in adults. However, projections into the body of the caudate nucleus were not found in kittens. From earlier studies, we note that the extent and patchy nature of precruciate corticostriate projection fields also becomes evident by three weeks of age. These terminal fields are bilateral and are confined laterally in the caudate head, a small projection into the body, and dorsally in the rostral putamen. In 2 day kittens these projections are bilateral and very prominent throughout the entire neostriatum. These observations suggest that the major inputs from neocortex and intralaminar thalamus differ within the neostriatum and that they become well-established by three weeks of age.

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273.17 SUBSTANCE P: LOCALIZATION WITHIN PALEOSTRIATO-TEGMENTAL PATHWAYS IN BIRDS AND REPTILES. Anton Reiner, Harvey J. Karten and Gary E. Korte. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, N.Y. 11794.

Immunohistochemical techniques (indirect immunoflorescence method or immunoperoxidase bridge method) were used to study the localization of substance P (SP) within the paleostriatal complex (PC) and within the projection targets of the PC in pigeon and turtle. Monoclonal antibodies against SP were used (generously supplied by A.C. Cuello). The neuropil of the small-celled zone of the PC (equivalent to the mammalian caudate-putamen) showed heavy SP-like immunoreactivity. Numerous medium-sized neurons containing SP-like immunoreactivity were observed within the more medial subdivision of the small-celled zone of the PC (termed lobus parolfactorius, LPO, in bird and area d in turtle). Fewer neurons containing SP-like immunoreactivity were observed in the more lateral subdivision of the small-celled zone of the PC (termed the paleostriatum augmentatum, PA) and none were observed in the large-celled zone of the PC (termed the paleostriatum primitivum in bird and globus pallidus in turtle).

Two prominent tegmental catecholaminergic cell groups have been noted to receive input from LPO/area d: the ventral tegmental area of Tsai (AVT) and a caudal ventrolaterally situated cell group termed the nucleus tegmentipedunculopontinus (TP) in bird and the substantia nigra (SN) in turtle (Kitt and Brauth, Neurosci. Abs., 1979; Reiner, Neurosci. Abs., 1979). A dense neuropil showing heavy SP-like immunoreactivity that could be eliminated by knife cuts of the efferent outflow pathway of the PC was observed in both AVT and TP/SN.

The present data argue that, as in mammals (Jessel, et al., Br. Res., 1978), portions of the small-celled zone of the basal ganglia (PC) in birds and reptiles may utilize SP as a neurotransmitter or modulator in their projections to tegmental sites. Further, the present data suggest that LPO and PA together in bird and area d and PA together in turtle are to be considered equivalent to the mammalian caudate-putamen and nucleus accumbens. Previous studies had only emphasized the similarity of PA to the mammalian caudate-putamen (Karten and Dubbeldam, JCN, 1973). This research was supported by NS 12078 to H.J.K. and F32 NS 05682 and T32 EY 07039 to A.R.

274.1 RIGHT-SIDE OUTPUT SUPERIORITY EFFECT IN WORD PROCESSING, WITH AND WITHOUT VERBAL EXPECTANCY. Y. Guiard ✱ (SPON : J. Requin). Département de Psychobiologie Expérimentale, CNRS-INP 3, 31, chemin J. Aiguier, 13009 Marseille, France.

In a previous study (Guiard, 1980), it was shown that right-handers display a right-side advantage in motor performance when responding to Stroop stimuli on the basis of the carrier-word, but not on the basis of the colour of ink. Furthermore, this output asymmetry effect of verbal processing was shown to consist both in a right-hand advantage - regardless of the movement parameters - and an advantage of movements towards the right side of space - regardless of the hand involved - (Guiard and Giraudo, 1980).

The present experiment was aimed at estimating the role of verbal expectancy (pre-stimulus processes) as distinct from verbal processing (post-stimulus processes) in the manifestation of this output asymmetry phenomenon. Non-coloured colour names (GREEN or RED) and coloured non-words (crosses printed in green or red ink) were presented in central vision, in random succession. On separate conditions, the probabilities of word processing and colour processing were .75/.25 and .25/.75 respectively. The choice response was a pointing movement either with the left hand towards a left-sided target, or with the right hand towards a right-sided target.

No effect of verbal versus non-verbal expectancy on performance asymmetry was observed. By contrast, the actual processing of the word, as compared with that of the colour, was found to result in a right-sided motor bias exerted in part on reaction-time processes, and in part on movement processes. The results are discussed in terms of the current model of cerebral lateralization of function.

Guiard, Y. Effect of processing mode on the degree of motor asymmetry in the manual Stroop test. To be published in Cortex.

Guiard, Y. and Giraudo, M.D. Right-hand and right-side advantages in the verbal processing of Stroop stimuli. Submitted.

274.2 HEMISPHERIC SPECIALIZATION IN SPLIT-BRAIN MONKEYS. B. A. Vermeire and C. R. Hamilton. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Split-brain rhesus monkeys were tested for differences in the abilities of their two cerebral hemispheres to process visual stimuli. Tests of visual preferences, i.e., what each hemisphere likes to look at, utilized color photographs of naturalistic stimuli such as monkeys, people, other animals, and outdoor scenes. In general these tests revealed an overall greater preference by the left hemisphere. Furthermore, for some types of photographs the hemisphere with the greater preference correlated with each monkey's handedness; the hemispheric ipsilateral to the preferred hand, possibly equivalent to the human non-dominant hemisphere, showed greater preferences.

The monkeys' ability to make same-different judgments about pairs of sequentially-presented geometrical visual stimuli was also assessed. Overall there was no generalized advantage for either the left or right hemisphere. There was a significant correlation between each monkey's dominance index and handedness, however, which indicated that the hemisphere contralateral to the preferred hand, possibly equivalent to the human dominant hemisphere, learned the task more readily.

These results and other examples of left-right differences and of correlations of hemisphericity with handedness in rats, monkeys, and humans reported in the literature suggest that the basis for hemispheric specialization, particularly in infrahuman mammals, may be quantitative asymmetries in emotional and/or attentional processes.

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274.3 BOTH SYNTACTIC AND SEMANTIC FACTORS AFFECT THE TASK-EVOKED PUPILLARY RESPONSE DURING SENTENCE PROCESSING. J. Beatty and M. Schluroff, Human Neurophysiology Laboratory, Dept of Psychology, University of California, Los Angeles, CA 90024

The performance of complex cognitive functions has been shown to elicit widespread momentary increases in sympathetic and decreases in parasympathetic activity that reflect the processing demands imposed by the task. These changes have been interpreted as peripheral signs of activity in the ascending reticular activating system. Orderly pupillary responses have previously been demonstrated during sentence processing. We now report that both syntactic and semantic organization act to reduce information processing load as indexed by the magnitude of the task-evoked pupillary response.

Twenty-four people listened to 3 sets of 12 6-word sentences and repeated them at the rate of 1 word/sec. Standard Sentences were semantically meaningful and varied in syntactic construction. Anomalous Sentences utilized these same syntactic structures but the actual words were exchanged between sentences to minimize meaning. In the third group, Scrambled Sentences, words were randomly ordered to eliminate syntactic structure.

For all sentence types, pupillary diameter increased while listening, dilated markedly before the first word was being repeated, then constricted to baseline by the end of sentence repetition. The magnitude of this response, averaged over the entire period of listening and repeating differed significantly for the 3 types of sentences ($F(2,46)=20.52$; p .001), being largest for the Scrambled Sentences and smallest for the Standard Sentences. A secondary analysis showed that pupillary diameter was significantly larger ($F(1,23)=10.44$; p .001) during repetition than during listening and that this effect interacted with sentence type ($F(2,46)=9.49$; p .001). This means that syntax and semantic structure exert their effects on processing load primarily during sentence production.

A final analysis contrasted syntactically simple and complex Standard and Anomalous Sentences. Both the effect of complexity ($F(1,23)=37.27$; p .001) and its interaction with input vs. output period ($F(1,23)=37.84$; p .001) were significant, indicating that syntactic complexity primarily effects production.

274.4 EVENT-RELATED SLOW POTENTIAL FROM RAT FRONTAL CORTEX IN RESPONSE TO AN AUDITORY CUE PRECEDING ELECTRICAL BRAIN STIMULATION: ALTERATION BY DEXTROAMPHETAMINE. James H. Pirch. Department of Pharmacology and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, Texas 79430.

Negative event-related slow potentials are generated in rat frontal cortex by auditory cues given two seconds prior to delivery of food reinforcement, to extension of a lever which can deliver food reinforcement or to delivery of a discriminative cue which signals food reinforcement. Dextroamphetamine reduces the amplitude of these food-reinforced slow potentials (SPs) (Pharmacol. Res. Comm. 9: 669, 1977; Pharmacol. Biochem. Behav. 6: 697, 1977; Neuropharmacol. 19: 365, 1980). In order to examine the effect of amphetamine on SPs associated with another type of reinforcement, rats were implanted with monopolar stimulating electrodes in the medial forebrain bundle (MFB) as well as SP recording electrodes. The "active" SP electrode was a chlorided silver wire in contact with the dura via an agar-saline bridge. The location was 2 mm anterior to bregma and 1.5 mm to the right of midline, ipsilateral to the MFB electrode. The reference was a similar electrode placed into a bone indentation drilled at the midline immediately behind lambda. SPs were recorded with d.c. amplifiers and analyzed by computer. The warning cue (S^W) was a click and the initial training sequence consisted of 100-150 habituation trials (click only) followed by 30 trials each at S^W-MFB stimulation intervals of 0.5, 1, 1.5 and 2.0 seconds. Trials were presented at variable intervals of 15-50 seconds. During test sessions two sets of 50 trials each were obtained with saline or d-amphetamine injected subcutaneously 15 min before the second set. The SP response to S^W had two negative components; the first wave (N1) reached maximum amplitude (70-130 μV) at 190 to 350 msec and the second (N2) achieved maximum amplitude (80-120 μV) during the last 250 msec of the 2 sec interval following S^W. Amphetamine (0.25, 0.5, 1 mg/kg) produced a dose-related suppression of the SP with a greater effect on N2 than N1. For example, 0.5 mg/kg depressed N1 to 71% and N2 to 38% of the pre-injection amplitudes (saline responses for N1 and N2 were 102% and 101% of preinjection amplitudes respectively; 4 animals). The results indicate that the effect of amphetamine on frontal cortex SPs associated with brain stimulation reinforcement is similar to the effect on SPs associated with food reinforcement. (Supported by USPHS MH29653 and by the Tarbox Parkinson's Disease Institute at Texas Tech University Health Sciences Center.)

274.5 EFFECTS OF RESPONSE SPEED ON EVOKED POTENTIALS TO AUDITORY PROBES DURING A VISUAL LETTER-MATCHING TASK. A. J. Nash and C. S. Williams*. Department of Psychology, Florida Atlantic University, Boca Raton, FL 33431.

Evoked potential (EP) paradigms which require reaction time (RT) responses from the subject raise the possibility that non-specific effects of arousal associated with the RT response itself may restrict the reported effects of other stimulus/task variables on the EP to relatively high-arousal states only. In the present study, two groups of seven subjects each were tested in a Posner and Boies paradigm (Psychol. Rev., 78: 391, 1971) consisting of a (primary) visual letter-matching RT task and a (secondary) RT task to an auditory probe stimulus which occurred on 50 per cent of the trials at selected temporal positions within the "Warning Letter/First Test Letter/Second Test Letter" sequence. Probes P-1 and P-2 followed the warning letter by 480 and 680 msec while probes P-3 and P-4 followed the first test letter by 480 and 680 msec respectively. Probes P-1 and P-2 were selected to occur at temporal locations in the trial sequence when the information processing demands of the visual task were low relative to the task demands within the interval containing probes P-3 and P-4. The control group received standard RT instructions to respond as quickly as possible without making errors. The experimental "High Response Speed (HRS)" group received instructions which placed greater emphasis on speed of responding to the visual task.

Baseline measures of EEG activity averaged over the 100 msec intervals preceding each of the auditory probes were in general found to be more negative for the HRS group than for the control group. Baselines also tended to be more negative at probe locations P-2 and P-4 which preceded the first and second test letters respectively. The N100 and P300 amplitudes of the EP to the auditory probes were both greater under the HRS condition but only the P300 showed an interaction between the response speed conditions and the probe locations, the response speed effects being greatest at location P-4, just prior to the second test letter. These effects were attributed to the non-specific effects of arousal associated with the different emphases on RT speed and the varying processing demands within the visual task sequence.

274.6 EFFECTS OF VIGILANCE ON CORTICAL PROCESSING. DD Daly, DM Daly, JW Drane*, JD Frost Jr*, MG Jones*, P Kellaway, Neurol.UTHSCD, Dallas, Tx. 75235; Statistics, SMU; Neurol. Baylor Col. Med., Houston, Tx.

Normal persons significantly deprived of sleep show impaired vigilance (VG) with slowing of motor responses, errors in cognitive processing and defects in recall. Pupillometry measures autonomic correlates of VG in both normals and narcoleptics. We report effects of fluctuating VG on cortical processing using sparse acoustic stimuli, SAS (Drane et al Comput.Biomed.Res. 12:351, 1979).

A 12 year old boy was unable to remain alert while reading, studying or watching TV. A sister, his mother and maternal grandfather were similarly affected. During 4 hrs we recorded EEG and continuously presented various sets of SAS. During intervals of alertness (activated EEG), he classified SAS precisely (G^2=175.6, DF=22) with appropriate variation in reaction times. With declining VG, as indicated by EEG, classifications of SAS with FM components became increasingly homogenous (G^2=85.5, DF=22) and reaction times fluctuated widely. Methylphenidate (MPD) 20 mg sublingually restored and sustained VG for an interval consonant with its $T_{\frac{1}{2}}$, paralleled by activation of EEG and appropriate perception and classification of SAS.

For many years a 50 year old woman had difficulty remaining alert while reading, driving and in social situations. A 14 hr polygraphic study showed normal sleep staging without excessive arousals. Throughout life she had difficulty in perceiving and speaking certain polysyllabic words. Two of 3 sons have impaired VG and 1 also has similar auditory problems. When alert she classified anomalously certain sets of SAS. With declining VG she showed increasingly homogeneous classifications for all sets save vowels. MPD repeatedly restored and sustained VG and stable, even if anomalous, classifications for a time appropriate to $T_{\frac{1}{2}}$.

Alterations in perception involving FM components of SAS and sparing CF are consonant with their representations in auditory cortex (Daly et al J.Neurophysiol. In Press). With declining vigilance, increasing homogeneity, engulfing parametric extremes, in perception of FM components reflects altered cortical activity. Spontaneous fluctuations and pharmacologic reversability demonstrate that VG modulates cortical processing. SAS can be used to monitor continuously cortical states and quantify levels of VG.

Testing contributed and done by inventor who retains all proprietary rights and interests.

274.7 HEMISPHERIC DIFFERENCES IN EVOKED POTENTIALS: SELECTIVE ATTENTION TO THE LEFT AND RIGHT VISUAL FIELD. M. R. Harter,* C. Aine* and C. Schroeder* (SPON: R. Eason). Dept. of Psych., Univ. of North Carolina, Greensboro, NC 27412.

Changes in visual evoked potentials (VEPs) have indicated that selective attention influences the neural processing of visual information; however, it is not clear how early in the processing sequence this influence can occur. It is hypothesized that selective attention effects occur as early as in visual cortex. The fact that the left and right hemispheres of visual cortex are activated by stimulation of the right and left visual fields respectively provides an opportunity to test this hypothesis. This hypothesis predicts that attention to one visual field should selectively enhance the neural response in the hemisphere contralateral to the attended field.

Flashes of either a solid green circle or a white ring (diameters subtending 2.3°) were randomly presented in the left and right visual fields (20° from a central fixation point). The presentation rate was 1/520 msec. VEPs to these flashes were recorded over the left and right regions of occipital (O_1 and O_2) and central (C_3 and C_4) cortex. Attention was directed toward one of the visual fields by making the flashes in that field relevant to a reaction time task.

The results indicate that selective attention to one visual field enhanced a negative potential (onset about 160 msec post stimulation) in VEPs to flashes in the attended, but not ignored, visual field. As predicted, this enhancement was greater in occipital VEPs recorded over the hemisphere contralateral to the attended field. There was an additional tendency for this enhancement to be greater over the left hemisphere (occipital and central regions) regardless of the attended field. These findings were interpreted as indicating that selective attention influences the processing of information in visual cortex and that there is some degree of left hemisphere specialization in selective attention.

274.8 ENHANCED ATTENTION IN THE CONTRALATERAL VISUAL HEMIFIELD INDUCED BY UNILATERAL HYPOTHALAMIC STIMULATION. C. B. Pott* and M. F. Mac Donnell* (Spon: M.J. Weiss) Dept. Biology , Livingston College , Rutgers Univ. , New Brunswick , N. J. 08903

Electrical stimulation of particular areas in the lateral hypothalamus of cats evokes a set of discrete behavioral reactions that culminate in a predatory attack. Previous studies have demonstrated that lateral hypothalamic excitation regulates the utilization of tactile sensory information in the orientation to and capture of prey. The present investigation was undertaken in order to determine the role of the hypothalamus in directing visual attention during predatory behavior.

Ten adult female cats were prepared for electrode placement under Nembutal anesthesia (48 mg/kg ip). Stainless steel monopolar electrodes were placed postoperatively in unanesthetized freely moving cats to establish the behavior at each stimulation point. Biphasic square-wave pulses of 1-msec duration , 60Hz , and .05-0.8ma were delivered to unilateral electrodes , which were advanced in small steps until a well-directed quiet biting attack on a rat was elicited. After electrode placement the cats were painlessly immobilized in a headholder. Following the onset of hypothalamic stimulation a slowly moving visual stimulus (cotton probe) was brought manually into the visual field from either an ipsilateral or contralateral direction. One ipsilateral and one contralateral presentation constituted one trial. Each cat was tested for ten trials.The electrodes were confirmed to be in the lateral hypothalamus by histological examination.

Control trials without hypothalamic stimulation revealed no response to visual stimulation. Introduction of the moving target into the ipsilateral visual field of hypothalamically aroused animals produced no overt behavior. However , the introduction of a target into the contralateral visual field produced dramatic behavioral responses. All ten cats displayed an immediate orienting eye movement as soon as the target entered the field. Furthermore , once visual contact was established , all ten cats continued to follow the target until hypothalamic stimulation was stopped. Nine of the ten cats exhibited "facial following." As the visual stimulus moved toward the peripheral edge of the contralateral field these cats raised their upper lips on the contralateral side ; this effectively directed their whiskers laterally and backwards , keeping them in line with the stimulus.

The results of this study reveal sharply enhanced responses to previously ineffective stimuli moving in the contralateral retinal hemifield during hypothalamic arousal. These responses include saccadic orientation of the eyes , smooth pursuit , visual locking -on and facial movements , which can all be interpreted as promoting maximal sensory contact with a moving target.

274.9 CHARACTERISTICS OF ATTENTION-RELATED CELLS IN PREFRONTAL CORTICAL REGIONS. E. Bakay Pragay*, A.F. Mirsky, C.V. Mirsky* and B.H. Scales*. Lab. of Neuropsychology, Div. of Psychiatry, Boston Univ. Sch. of Med., Boston, MA 02118.

Bakay Pragay et al. (1978) have described attention-related units in the brainstem RF in monkeys working on a go - no go visual attention task. These "Type II" units showed changes either following or anticipating the stimulus in both go and no go trials. In contrast, Type I units showed changes only in go trials.

We are exploring other parts of an attention "system" in the brain. We have found Type II units in the prefrontal cortex. In addition, we varied the reward conditions and the length of the fixed interstimulus interval (ISI) in the basic go - no go task (Bakay Pragay et al., 1978). Units were tested with reward for both correct go and no go trials as well as with non-reinforcement for correct no go trials (NRNG); units were also tested under 3 different fixed ISI intervals: 1 sec., 2 sec. and 3 (or 4) sec. In addition, 2 extra-task conditions were applied: task-stimuli without access to task and reward; delivery of noncontingent reward.

A total of 50 task-related units were studied in the prefrontal cortical region of two monkeys, including the banks and depths of s. principalis as well as more ventral, inferior and caudal areas. The vast majority of units (43 out of 50) were of Type II. In most instances, the increase or decrease of firing rate began well before the end of the trial. Six units showed pre-trial, anticipatory changes in the basic task. Type II units only were tested under several conditions. NRNG: 30 units; ISI: 17 units; extra-task conditions: 34 units. In the NRNG condition, various degrees of loss of the original reinforced response, or the reverse form of response (i.e., from increase to decrease) were seen. In all, 12 units were changed by NRNG. These cells also responded to the noncontingent delivery of reinforcement. Varying the ISI affected 12 of the units tested. Increasing ISI typically increased or induced anticipatory activity. Decrease of ISI to 1 sec. prevented such activity, and in a number of cases disrupted task-related unit behavior. Extra-task administration of the CS usually evoked no systematic change.

Prefrontal units thus respond not only to the delivery of reinforcement (Rosenkilde et al., 1979) but also to CSs presaging reinforcement; and they adapt quickly to changes in reinforcing conditions. Plasticity was especially striking under ISI variations. However, apart from the high proportion of Type II (vs. Type I) cells, prefrontal units do not differ greatly from brainstem RF units in their attention related functions, as described by Ray (1979).

274.10 THE EFFECT OF BISECTING THE SUPERIOR COLLICULI ON THE CAT'S ORIENTATION TO PREY. J.S. Robinson and T.J. Voneida. UCSF, Brain-Behavior Res. Ctr., Sonoma State Hospital, Eldridge, CA 95431.

Sprague, et al. (J. Comp. Neurol., 172:441, 1977) have shown that bilateral removal of cortical areas 17 and 18 may leave the cat's orienting behavior undisturbed; our results show that a minimal strategically placed midline lesion, providing maximal destruction of relevant structures, i.e., commissural and decussating pathways connecting the deep layers of the superior colliculus (SC) with the reticular core (Edwards, S.B., J. Comp. Neurol. 173:23, 1977) may result in significant disruption of such behavior.

Two 4-day (6 min/day) tests of orientation to live mice were made before (T1) and after (T2) a 2 month interval for surgery and convalescence (all cross-midline pathways of the superior colliculi dorsal to the cerebral acqueduct were sectioned in 5 of 11 cats; 6 remained intact. All 11 were good "mousers"). The cats were tested in a semicircular double-walled surround, the inner wall of which was clear plastic. The live mouse prey was confined to a 2 1/2 in. space between the walls, but could move freely along the base of the surround, visible to the cat subject but protected from it by the plastic barrier. Video-taped records were made of the cat's and mouse's behavior and were analyzed to obtain separate scores showing 1) the amount of time a cat spent orienting to the mouse when it was motionless and 2) the time spent orienting to a moving mouse.

There was little change in the orienting behavior of any of the 11 subjects in the 4 test days of the T1 series, and the control subjects were as likely to show an increase as a decrease in orienting in T2 (as compared with the T1 level). There was no apparent qualitative change in the orienting behavior of the cats with the tectal splits, but they did show a quantitative one: there was a significant decrease in orientation to both the motionless and the moving mouse (Wilcoxon W=15, p=.03 for both decreases). A mouse could no longer elicit as much orienting from the most active of the operated animals as it could from the least enthusiastic orienter prior to its surgery.

274.11 STIMULATION OF NOREPINEPHRINE RECEPTORS WITH CLONIDINE BLOCKS DISRUPTION OF SELECTIVE ATTENTION BY APOMORPHINE. MaryLou Cheal. Neuropsychology Laboratory, McLean Hospital, Harvard Medical School, Belmont, MA 02178.

Evidence that amphetamine-treated gerbils responded selectively to a novel object even at a dose that resulted in continuous stereotypy was reported earlier (Soc. Neurosci. Abst., 1978, 4, 487). These animals spent little time actively investigating the object after injection, but a low rate of responding the next day indicated that they remembered the object. Apomorphine-treated gerbils, on the other hand, showed no memory of the object the second day even though they spent 11-12 sec licking, sniffing, or biting the object following an injection of 1 mg/kg (Psychopharmacol., in press). One difference in the actions of amphetamine and apomorphine is that amphetamine is an agonist of dopamine (DA) and norepinephrine (NE), whereas apomorphine is relatively selective to the DA system. To test the hypothesis that relative levels of NE and DA activity are necessary for selective attention and habituation, gerbils were given the NE receptor stimulant, clonidine (0, .01, .03, .3 mg/kg), followed 30 min later by the DA receptor stimulant, apomorphine (1 mg/kg). They were tested 10 min after the second injection in the 24-hour variation of stimulus-elicited investigation. Following adaptation trials, half of each drug group were given one 60 sec, nonreinforced trial in which to investigate a small cup. All of the gerbils were tested again the next day with no further drug treatment.

When gerbils were injected with both clonidine and apomorphine, the locomotor response to apomorphine was potentiated so that the animals were very jumpy and had a very low startle response. In spite of this, the gerbils showed memory the second day. For the gerbils given .03 or .3 mg/kg clonidine plus apomorphine, the responses were confounded because of residual effects of the drugs. Of greater interest, however, are those that received .01 mg/kg clonidine before apomorphine. Gerbils that had seen the object on Day 1 spent significantly less time investigating it than those that had not seen it previously. At this dose, both the exposed and the nonexposed groups of gerbils had approximately the same duration and frequency of responding on Trial 1 as those that received only apomorphine (1 mg/kg), but the Trial 2 responses indicated memory in those receiving clonidine and apomorphine, but no memory following just apomorphine. Thus, the hypothesis that selective attention and habituation are dependent on the relative amounts of NE and DA activity is supported.

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274.12 LEAD INGESTION AND ACTIVITY LEVELS IN THE RHESUS MONKEY. N. K. Laughlin*, P. J. Bushnell and R. E. Bowman (SPON: G. W. Kraemer). Primate Lab, University of Wisconsin, Madison, WI 53706.

In Exp. 1 rhesus monkeys were separated from their mothers at birth and maintained on a commercial milk diet and monkey chow. Cloth mother surrogates and regular social interaction with peers were provided during the first year of life.

Pb was administered as lead acetate added to the milk diet daily during the first postnatal year. Doses were adjusted weekly to maintain blood lead levels (PbB) of 90 µg/dl (High Pb, n=3) or 50 µg/dl (Low Pb, n=3). Controls (n=4) were given no added Pb and their PbB levels averaged 5 µg/dl.

Animals were tested for circadian locomotor activity at 12,13, 18, 22, 27 and 37 months of age. At each test, animals were housed singly for 24 hrs in a wire mesh cage quadrasected by 2 photobeams. The number of photobeam interruptions was cumulated at 3 hr intervals beginning at 1000 hrs. Lights in the test room went on at 0700 hrs for 12 hrs. Food was provided once during the test and water was available continuously.

The High Pb group was more active than controls from 18-27 months of age inclusive and the Low Pb group was more active than controls from 18-22 months inclusive. However, this hyperactivity was specific to the circadian test as dose-related locomotor hypoactivity was observed during the social tests with age-mates.

Subsequent experiments have failed to replicate the Pb-induced hyperactivity in the circadian test. These experiments have manipulated Pb dose (Exps. 2 and 3) and milk intake (Exp. 4). In Exp. 1, PbB levels were relatively constant across the year of Pb dosing; those in Exp. 2 rose gradually, reaching peak values of 150 and 70 µg/dl for High (n=4) and Low (n=4) dose groups, respectively. In Exp. 3, early elevated Pb dosing produced transient peak PbB values of 300 µg/dl followed by either chronic Pb intake (n=4) or no subsequent added Pb (n=4). An additional group received only chronic Pb intake (n=4). PbB levels averaged 90 µg/dl in the groups receiving chronic Pb. In Exp. 4, animals were dosed chronically with Pb in the presence of an ad lib milk diet (n=4) or a restricted milk diet (n=4). In all experiments, control monkeys received no added dietary Pb. In these experiments, hyperactivity did not result from early chronic Pb ingestion. The early transient Pb pulse and restricted milk intake each produced hyperactivity at certain ages. We conclude from these experiments that early chronic Pb exposure is not associated with hyperactivity in the rhesus monkey.

275.1 NEUROANATOMICAL FUNCTIONAL MAPPING OF THE VAGAL PRESSOR REFLEX USING THE AUTORADIOGRAPHIC [^{14}C] DEOXYGLUCOSE TECHNIQUE IN THE DOG. D.R. Kostreva and J.P. Kampine. Dept. Anesthesiology and Physiology, Med. Col. of Wis. and VA Med. Ctr., Wood, WI 53193.

The medullary and pontine projections of vagal afferent fibers that elicit a pressor response during electrical stimulation of the left vagus was studied in mongrel dogs (6-9 kg) using the [^{14}C] deoxyglucose technique of Sokoloff, et al. (J.Neurochemistry 28:897-916, 1977). The animals were fasted for 2 days and then anesthetized with sodium pentobarbital (35 mg/kg), intubated and placed on positive pressure ventilation. Systemic blood pressure from a femoral artery and the lead II electrocardiogram were recorded using a Grass polygraph. The cervical vagi were exposed bilaterally and sectioned transversely. The central end of the left vagus was desheathed, and placed on a pair of tungsten carbide electrodes connected to a constant current stimulator. The stimulus parameters were 10 Hz, 0.5 ms pulse width and a current strength of 0.5-10 ma. The current strength was increased until a pressor response of 15-25 mmHg could be elicited repeatedly. 1mCi of [^{14}C] Deoxyglucose (specific activity 57 mCi/mmol New England Nuclear) was injected as a single bolus into a femoral vein. The left vagus was stimulated repeatedly (45 sec stimulation with 15 sec rest) for a total of 45 minutes. The entire brain and part of the spinal cord was then removed and frozen in -40°C isopentane. 20 um sections were made using an AO Cryostat and dried on glass cover slips. The sections were covered with Kodak MR-1 film and stored in X-ray cassettes for 14 days. The autoradiographs were then developed and analyzed for the pathways having the greatest metabolic activity as indicated by the density of photographic emulsion. High cervical cord sections did not show any difference in density from the background activity. Sections in the region of obex revealed a marked increase in density in the left nucleus tractus solitarius and the tractus solitarius which is on the side of the centrally stimulated vagus. These structures could be seen as two distinct entities. The density of the right nucleus tractus solitarius and tractus solitarius was not distinguishable from the background density of adjacent structures on the right side. Also seen in these sections were an increase in density of the emulsion in the area of olivary nuclei bilaterally. Rostral sections continued to delineate the left nucleus tractus solitarius. Sections in the region of the 8th nerve showed a marked density in the nucleus olivarius superior. Sections in the rostral pons and midbrain revealed marked increases in density of the cerebellar peduncles and the interpeduncular nucleus. This study demonstrates that the deoxyglucose technique is useful for studying the central pathways of autonomic reflexes. (This study was supported by NIH Young Cardiovascular Grant HL 21042 and VA Medical Research Service).

275.2 CONVERGENCE OF SOMATIC AND VISCERAL INPUTS ONTO THORACIC NEURONS PROJECTING TO THE MEDULLARY RETICULAR FORMATION. R. Neal Weber,* Robert W. Blair and Robert D. Foreman, Dept. of Physiology and Biophysics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Spinoreticular neurons transmit nociceptive information from lumbar and cervical regions of the spinal cord into medullary, pontine and mesencephalic reticular formation nuclei. It is of interest to know if spinoreticular neurons of thoracic origin might constitute an afferent pathway for nociceptive input that is received from thoracic visceral and somatic receptive fields. The purpose of this study was to identify and characterize spinal neurons which originate in the gray matter of T_1 - T_5 spinal segments and project into the medullary reticular formation, and exhibit convergence of visceral and somatic afferent inputs. Such cells were located, using bilateral antidromic stimulation from the medullary nucleus gigantocellularis and tegmental region just anterior to the obex. Cell activity was recorded extracellularly in the left spinal gray matter of chloralose anesthetized and gallamine paralyzed cats. Of the total number of antidromic cells in the study, 46% exhibited both somatic and visceral inputs. These cells were selected for further characterization. Antidromic stimulation of the reticular formation from the contralateral side activated 61% of the cells, from the ipsilateral side 30% of the cells, and from both sides 9 % of the cells. The average antidromic conduction velocities were 36.3 M/s for contralateral and 25.9 M/s for ipsilateral neurons. Somatic input was determined by natural stimulation of skin and muscle in the cell's receptive field while visceral input was determined by electrical stimulation of the sympathetic chain between the left T_2 and T_3 segments. Ninety-one (91%) per cent of the somatic fields were classified as high threshold with the remainder being wide dynamic range. Of the cells that responded to sympathetic chain stimulation, 48% received C-fiber input as determined by minimum afferent conduction velocity and cell activation threshold. Eight cells out of a total of 20 tested for their response to intracardiac injections of bradykinin increased their discharge rate after an average latency of 10s. Injections of bradykinin into the femoral artery and vein only activated 2 cells that discharged after a longer latency and at a much slower discharge rate. This study demonstrates that sympathetic afferent fibers from the cardiopulmonary region and somatic afferents excite T_1 - T_5 spinal neurons projecting into the medullary reticular formation.

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275.3 PRESENCE OF GLUTAMIC ACID DECARBOXYLASE IN HINDBRAIN NUCLEI INVOLVED IN CONTROLLING PARASYMPATHETIC OUTFLOW TO THE HEART. B.L. Hamilton, S.C. Brown*, D.J. Williford, W.P. Norman*, M.J. Iadarola, K. Gale, and R.A. Gillis. Depts. of Pharmacol. and Anat., Schools of Medicine and Dentistry, Georgetown University, Washington, D.C. 20007.

Data has been accumulating to indicate that gamma-aminobutyric acid (GABA) may be an important neurotransmitter for controlling central vagal activity to the heart in cats. Drugs which block CNS GABAergic transmission (i.e. bicuculline, picrotoxin and isoniazid) increase vagal activity, whereas drugs which augment CNS GABA activity (i.e. GABA itself, muscimol, and THIP) decrease vagal activity. These effects appear to occur at nucleus ambiguus (NA). Additionally, we have demonstrated that GABA is present in NA in concentrations 2.5 fold higher than the GABA content in surrounding reticular nuclei. To obtain further evidence for GABA being a neurotransmitter at this site, measurements of the enzyme responsible for GABA synthesis, glutamic acid decarboxylase (GAD), were made and compared with values obtained for other hindbrain nuclei and substantia nigra. GAD activity was measured using the method of Sims and Pitts (1970). Results obtained are summarized in the table.

Brain Region Examined	GAD Activity (umole/g protein/hr) Right	Left
Nucleus Ambiguus (7)	97 ± 11	85 ± 8
Dorsal Nucleus of X (DMV) Plus Nucleus Tractus Solitarius (NTS) (7)	166 ± 5	148 ± 7
External Cuneate Nucleus (4)	45 ± 10	62 ± 7
Lateral Reticular Nucleus (4)	40 ± 5	40 ± 2
Substantia Nigra (4)	630 ± 132	600 ± 151

These results indicate a significant amount of GAD activity in NA as well as in the area encompassing DMV-NTS, and are consistent with the notion that GABA is an important neurotransmitter controlling central vagal outflow.

275.4 ALTERATIONS IN CARDIOVASCULAR REGULATION AND REFLEXES FOLLOWING 6-HYDROXYDOPAMINE LESIONS OF THE INTERMEDIATE NUCLEUS TRACTUS SOLITARIUS MEDIALIS. Healy, D.P.*, T.H. Williams, J. Jew and A.C. Black, Jr. Dept. of Anatomy, University of Iowa, Iowa City, Iowa 52242.

The catecholaminergic innervation of the nucleus tractus solitarius (NTS) has been implicated in central cardiovascular control. Snyder et al. (Circ. Res. 43:662, 1978) have shown that selective destruction of catecholaminergic nerve elements in NTS at the level of the obex using 6-hydroxydopamine (6-OHDA) resulted in increased arterial pressure lability. It has been reported from this laboratory that 6-OHDA lesions of the intermediate portion of NTS medialis (rostral to the obex) produced a bradycardia which persisted throughout 5 days of observation. The bradycardia was reversed by atropine, suggesting an increase in vagal activity following the lesion. We now sought to investigate: 1. whether chronically lesioned animals would also show a reduction in heart rate; 2. whether arterial pressure lability was altered either acutely or chronically; 3. whether the integrity of the baroreceptor reflex was altered; and 4. possible effect of atropine on heart rates of chronically lesioned animals.

24 - 28 hours prior to lesioning, animals were catheterized for recording of arterial pressure (AP) and heart rate (HR) in a conscious, freely-moving condition. 4.0 µg of 6-OHDA chloride in 1.0 µl vehicle was administered bilaterally into NTS medialis, 1.0 mm rostral to obex. Control animals received injections of vehicle. Chronic animals were lesioned as above and catheterized two weeks later. Measurements of AP and HR were taken for a period of a week in both groups. Quantification of the baroreceptor reflex response was carried out by giving graded doses of phenylephrine i.v. After one week animals were perfused and a glyoxlic acid catecholamine fluorescence technique used to verify lesion sites and to evaluate the extent of catecholamine terminal destruction.

1. There was a marked reduction in catecholamine terminals in intermediate NTS medialis. 2. Both acute and chronic animal groups displayed a significant bradycardia which lasted throughout the period of observation, (i.e. up to three weeks). 3. The bradycardia was reversed by atropine. 4. There was no change in arterial pressure lability. 5. The gain of the baroreceptor reflex response was decreased following lesions in both groups.

These results indicate that the catecholaminergic innervation of the intermediate NTS medialis is involved in inhibitory modulation of vagal activity. The decreased gain of the baroreceptor reflex response may be due to the already increased vagal activity observed following lesions. (This work was supported by N.I.H. grants NS11650 and HL21914.)

275.5 GIGANTOCELLULAR RETICULAR NUCLEUS: A COMMON NEURAL SUBSTRATE IN THE MEDULLA OBLONGATA FOR HYPOTENSION, BRADYCARDIA AND ANALGESIA. Samuel H.H. Chan and Julie Y. Hwa. Department of Life Sciences, Indiana State University, Terre Haute, IN 47809.

Investigations in our laboratory on the neural mechanisms involved in the central control of circulation and antinociception, as well as their modifications by antihypertensive and analgesic agents, have identified a common neural substrate for all these processes: gigantocellular reticular nucleus (GRN) in the medulla oblongata. The present communication summarizes the evidences that lead to this intriguing conclusion, based on experiments performed on anesthetized or decerebrate cats.

(1) Electrical stimulation of the GRN induced a significant reduction in heart rate (HR), accompanied mostly by a decrease in arterial blood pressure (ABP). The degree of suppression was related to the pulse frequency and intensity of the reticular stimulus train. Such GRN-elicited bradycardia interacted synergistically with reflex cardioinhibition and antagonistically with reflex tachycardia.

(2) Intravenous injection of the antihypertensive agent clonidine (10 µg/kg) failed to promote a reduction in HR and ABP after the GRN was electrolytically lesioned. Bilateral microinjection of clonidine (1 µl in vol.) directly into the GRN, at an ineffective intravenous dose (0.2-0.5 µg/kg), on the other hand, produced an appreciable cardioinhibition and hypotension.

(3) Intravertebral administration of clonidine (0.5-4.0 µg/kg) elicited a drastic suppression of the GRN-induced bradycardia.

(4) GRN activation invariably produced a suppression of the dental pulp-evoked field potential in the subnucleus oralis of the spinal trigeminal complex (oralis potential) and jaw-opening reflex (JOR), based on a conditioning-testing paradigm. The degree and duration of such inhibition were also related to the reticular stimulus parameters.

(5) GRN stimulation evoked negative potentials in the spinal trigeminal tract at the level of the subnucleus oralis that possessed electrophysiologic characteristics similar to the dorsal root potentials induced by comparable reticular activation in the spinal cord. The time course of these negative potentials paralleled the GRN-promoted suppression of dental pulp-evoked oralis potentials, implicating the possible involvement of a presynaptic inhibitory mechanism in the process.

(6) Microinjection of morphine (10 µg/kg) into the GRN elicited a significant inhibition of the dental pulp-evoked JOR, which was reversed by naloxone (1 mg/kg, i.v.).

It is concluded that the GRN in the medulla oblongata is a common neural substrate for the induction of hypotension, bradycardia and analgesia. (Supported in part by the American Heart Association, Indiana Affiliate).

275.6 BRAIN STEM INTERNEURONS WITH OROPHARYNGEAL AND VAGAL RESPONSIVENESS. D.A. Bereiter, H.-R. Berthoud* and B. Jeanrenaud*. University of Geneva Medical School, Geneva, Switzerland

The early metabolic and hormonal responses to food ingestion, the so-called cephalic phase responses, are dependent on oropharyngeal afferent input and a vagal efferent innervation of abdominal end organs. To study the brain stem organization of neurons possibly involved in such cephalic phase reflexes, we have used single unit recording techniques to identify the location and response characteristics of neurons dually responsive to chorda tympani (CT) and vagus nerve (X) electrical stimulation. Male rats anesthetized with α-chloralose/urethane were used. Oropharyngeal stimulation was mimicked by electrical stimulation (200-900µA, 0.2 ms) of the CT proximal to its junction with the lingual nerve. Cervical X stimulation (200-900µA, 0.2 ms) was applied distal to the superior laryngeal nerve. A total of 79 dually responsive (CT/X) neurons were identified, all responding orthodromically. The mean latency to CT stimulation (11.2 ± 1.2 ms) and to X stimulation (24.7 ± 2.4 ms) was highly correlated ($P < .01$ for this group of CT/X responsive neurons, suggesting that both stimuli activate interneurons at an equal relative distance from the stimulus source.

The location of these neurons fell into two fairly distinct regions : a) lateral nucleus tractus solitarius and b) the nucleus ambiguus region. However, many cells were found in the intermediate dorsoventral region between these two nuclear structures. These results extend previous studies showing lateral hypothalamic-oropharyngeal convergence onto nts neurons (Bereiter et al., Ex. Brain Res., 1980).

These results demonstrate that CT and X input to second-order interneurons show considerable overlap and that both nucleus tractus solitarius and/or nucleus ambiguus may integrate oropharyngeal-vagal information necessary for the expression of cephalic phase reflexes. This is corroborated by the fact that nucleus ambiguus stimulation will evoke a prompt rise in plasma insulin levels (Bereiter et al., 1980).

This work has been supported by NIH grants No. R01 AM 25220-01 and the Swiss National Science Foundation.

275.7 THE VENTRAL MEDULLA: DIRECT PROJECTIONS TO THE INTERMEDIOLATERAL CELL COLUMN AND THE HYPOTHALAMUS IN THE RAT. A.D. Loewy, J.H. Wallach and S. McKellar. Dept. Anat. & Neurobiology, Wash. Univ. Sch. Med., St. Louis, MO 63110

Previous physiological and pharmacological studies have reported that the ventral surface of the medulla is a functionally important region concerned with the regulation of respiration, blood pressure, blood glucose levels and possibly vasopressin release. We have therefore used both autoradiographic and horseradish peroxidase tracing techniques to elucidate the efferent projections of this region.

Injections of ^{3}H amino acids were made in the ventral medulla in rats and after a 5-7 day survival period, the brains and spinal cords were processed by the autoradiographic technique. A very dense area of anterograde labeling was observed bilaterally in the intermediolateral cell column in both normal and 6-hydroxydopamine treated rats. In addition, we found a projection to the cervical and thoracic ventral horn. Ascending labeled fibers were found to course through the reticular formation and entered the central gray matter. A relatively heavy, ipsilateral projection was seen in the posterior and medial parts of the paraventricular hypothalamic nucleus and ventral part of the supraoptic nucleus in both normal and 6-hydroxydopamine treated rats. Furthermore, a few labeled fibers were traced to the lateral hypothalamic area and perifornical region of the hypothalamus.

In other rats, 30-50 nl of a 0.1% solution of horseradish peroxidase-wheat germ agglutinin (HRP-WGA) conjugate was injected into the hypothalamus and after 48 hr. the rats were perfused and brainstem sections reacted by the tetramethylbenzidine procedure. Hypothalamic injections which included the paraventricular and supraoptic nuclei resulted in retrograde cell body labeling in the ventral medulla in an area near the exit zone of the XII nerve, an area dorsal to the lateral reticular nucleus, and in the region of the A1 catecholamine cell group.

These results confirm the existence of a group of neurons in the ventral medulla with direct projections to sympathetic and hypothalamic centers known to be involved in cardiovascular regulation and neuroendocrine control. (Supported by USPHS grant NS12751, NS07071, HL 07275 and a grant-in-aid from the American Heart Association #80-723).

275.8 DESCENDING HYPOTHALAMIC PATHWAYS TO THE MEDULLA OBLONGATA IN THE RAT. J.H. Wallach and A.D. Loewy. Dept. Anatomy and Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110

Although it has been previously demonstrated that the paraventricular nucleus of the hypothalamus (PVH) has direct connections with the dorsal medulla, relatively little information exists on the medullary projections of the other hypothalamic nuclei. Considering the importance of the medulla as a major center for cardiovascular, respiratory, and gastrointestinal integration, we have used both anterograde and retrograde neuroanatomical tracing techniques to further delineate direct hypothalamo-medullary connections.

Small (30 nl) injections of ^{3}H amino acids were placed in the dorsomedial (DMH) and perifornical area (PFA) of the hypothalamus, Fields of Forel (FF) and zona incerta (ZI) in 20 rats, and after a 7 day survival period the brains were processed with the autoradiographic method. Injections in the region of the DMH and PFA resulted in anterograde labeling in the magnocellular reticular formation dorsal to the superior olive, nucleus reticularis gigantocellularis pars α (Rgc), nucleus reticularis parvocellularis, nucleus tractus solitarius (NTS), area postrema, and raphe pallidus. After injections in FF, labeled fibers were traced to the nucleus reticularis pontis, central grey matter, nucleus reticularis pontis caudalis (Rpc), and Rgc. Injections in the ZI resulted in labeling in Rpc and Rgc.

Additional experiments were performed in which 30-50 nl of a 0.1% solution of horseradish peroxidase-wheat germ agglutinin (HRP-WGA) conjugate was injected into the NTS, raphe pallidus, and nucleus reticularis gigantocellularis. Following a 48 hr. survival period, the rats were perfused and brain sections reacted by the tetramethylbenzidine procedure. HRP-WGA injections confined to the NTS resulted in retrograde cell body labeling primarily in the PVH and DMH. Labeled cell bodies were also found in the DMH following a small injection into the raphe pallidus. Lastly, HRP-WGA injections in the region of the nucleus reticularis gigantocellularis labeled neurons in the FF, ZI, DMH, and the lateral hypothalamic area.

These results are consonant with physiological studies that suggest an important role of several hypothalamic nuclei in the modulation and/or control of medullary autonomic centers. (Supported by USPHS grant NS07071 and NS12751 and by a Grant-in-Aid from the American Heart Association (#80-723).

275.9 PARABRACHIAL NUCLEUS AS A MEDIATOR OF BRADYCARDIA IN RABBITS. Robert B. Hamilton, Howard H. Ellenberger*, David R. Liskowsky*, Marc D. Gellman* and Neil Schneiderman. Dept. of Psychology, University of Miami, Coral Gables, Fl. 33214.

Electrical stimulation(10 sec trains, 100 pulses/sec, 0.25 msec pulse duration, M current= 100μA) in medial and lateral parabrachial n. of 14 Urethane-anesthetized rabbits induced bradycardia (M=87 bpm, SD=±34), a small concomitant increase in mean arterial blood pressure (M=10 mmHg, SD=±2), and variable changes in respiration. The bradycardia was mediated by the vagus nerves, since it was abolished after bilateral transection. Bilateral lesions of the dorsal vagal n. abolished the bradycardia elicited by train stimulation of the parabrachial n., indicating that the heart rate decrease was mediated via the dorsal vagal nucleus. The bradycardia was not secondary to a blood pressure increase since the latency of the heart rate response was shorter (<1 sec vs ≈2 sec) than the pressor response. The bradycardia was not secondary to respiratory changes, since the heart rate response did not change following paralysis induced by injection of Decamethonium.

Single-pulse electrical stimulation of the aortic nerve (.1 to 1.0 msec pulse duration, 100-400 μA) activated units in the commissural and dorsomedial portions of the nucleus tractus solitarius (NTS) at a mean latency of 7.5 msec (SD= ±1.5) and throughout the parabrachial n. at a mean latency of 12.1 msec (SD= ±2.9). Stimulation of the parabrachial n. with single pulses activated units in the dorsomedial NTS at a latency of about 4.6 sec; these units were also activated by stimulation of aortic nerve. The connections between parabrachial n. and NTS appear to be monosynaptic rather than antidromic, although injection of horseradish peroxidase (HRP) into the parabrachial n. readily labeled cell bodies in the NTS. Similarly, injection of HRP into the dorsal medulla led to extensive labeling throughout the parabrachial n. Bilateral lesions of parabrachial n. did not attenuate the bradycardia response to train stimulation of the aortic nerve.

It is concluded that the parabrachial n. (a) plays a role in the mediation of bradycardia and (b) receives barosensory information, but (c) is not absolutely essential for the elicitation of the baroreceptor reflex.

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275.10 ROLE OF CENTRAL SEROTONIN IN THE PRESSOR RESPONSE TO HYPOTHALAMIC AND MIDBRAIN STIMULATION. P. Gauthier* and T.A. Reader. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada.

Electrical stimulation of the hypothalamus and the central gray area of the rat anesthetized with chloralose elicits a biphasic increase in arterial blood pressure resulting from the peripheral activation of the sympathetic vasomotor neurons and the adrenal medulla (Gauthier and Reis, unpublished). We sought to determine whether serotonin (5-HT) plays a role in the central mediation of this response. Rats were treated with either intraventricular 5,7-dihydroxytryptamine (5,7-DHT) (200 μg) or intraperitoneal para-chlorophenylalanine (PCPA, 500 mg/kg/ day for 2 days). The cardiovascular responses as well as plasma noradrenaline (NA) and adrenaline (A) changes evoked by stimulation of the anterior hypothalamus and the central gray area of treated rats were compared with those obtained in control animals. The destruction of central 5-HT terminals by administration of 5,7-DHT, two weeks prior to the experiments, did not modify the cardiovascular biphasic response to brain stimulation which also increased plasma NA and A to levels similar to those observed in sham treated animals. In 5,7-DHT-treated animals, serotonin stores were depleted by about 90% in the spinal cord, pons-medulla, midbrain, hypothalamus and the cortex. Endogenous NA and A contents of the brain and of the adrenal medulla were either not modified or increased. The inhibition of 5-HT biosynthesis by administration of PCPA decreased the adrenal component (more than 50%) of the pressor response to brain stimulation. The sympathetic vasomotor component of the response was only mildly affected by the treatment. The concomittant increase in plasma NA levels to stimulation was unchanged in these animals whereas plasma A increased only to about 50% of the levels observed in control rats. Serotonin stores were depleted by about 70% in all investigated areas of the brain. However, in contrast to 5,7-DHT treatment, it was found that PCPA treatment also depleted NA and A stores in selected regions of the brain, such as the hypothalamus and the spinal cord. On the other hand, catecholamine content of the adrenal medulla was either unchanged or increased. The present results first indicate the absence of a role for serotonin in the central mediation of the activation of sympathetic vasomotor neurons and adrenal medulla elicited by hypothalamic or midbrain stimulation. Second, the adrenal component of the response to brain stimulation, and to a smaller extent the vasomotor component, seem to depend upon the integrity of catecholaminergic systems in the hypothalamus and/or the spinal cord.

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275.11 A HIERARCHY OF RESPONSIVITY OF ARTERIAL PRESSURE DURING NATURAL BEHAVIOR IN THE RAT: EFFECTS OF LESIONS OF CENTRAL CATECHOLAMINE NEURONS. J.E. LeDoux, A. Del Bo, G.A. Harshfield, L.W. Tucker, W.T. Talman, and D.J. Reis, Laboratory of Neurobiology, Dept. of Neurol., Cornell Univ. Med. College, New York, NY 10021.

We have sought to characterize the direction and magnitude of changes in arterial pressure (AP) associated with a repertoire of natural behaviors in rat and to determine whether these changes are altered by lesions of the catecholamine containing neurons of the A2 region of the medulla. Male Sprague-Dawley rats were instrumented with an indwelling aortic cannula for computer-assisted recording of AP and heart rate (HR) during behavior. Some animals were instrumented for recording EEG and EMG. Behaviors were categorized by an observer who signaled the onset and termination of behaviors using a keyboard which was read by the computer.

In the normal rat (n=6), a hierarchy of responsivity of AP was observed. Higher average pressures were associated with eating (118±2 mm Hg) and drinking (118±3) than with grooming (110±4) and exploring (108±5) (p<.05), which were in turn associated with higher pressure than rest (95±5)(p<.05). Lability was lower during rest than during other behaviors (p<.05). Standard EEG and EMG recording techniques were used to segregate rest into quiet wakefulness (QW) and the phases of sleep (n=7). Pressures were higher during QW and desynchronized sleep than during synchronized sleep (p<.05). Animals with A2 lesions (n=10) showed lower AP than controls during grooming (99±11), exploring (95±13) and rest (85±11), as well as greater lability of AP during these behaviors. AP during eating and drinking was not different in lesioned and control animals, in spite of occasional elevations up to 80 mm Hg above resting baseline following A2 lesions. We conclude: (a) in the normal rat there is a hierarchy of responsivity of AP during natural behavior; and (b) destruction of the A2 catecholamine neurons, which normally modulate baroreceptor activity (Talman et al., Circ. Res., 1980), disrupts the mechanisms which normally maintain the level and variability of AP appropriate to certain behavioral activities.

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275.12 THE ORIGIN, EXTENT AND TERMINAL DISTRIBUTION OF DIRECT AMYGDALA CENTRAL NUCLEUS PROJECTIONS TO THE DORSAL MOTOR NUCLEUS AND THE NUCLEUS OF THE SOLITARY TRACT. James S. Schwaber, Bruce S. Kapp, Gerald A. Higgins* and Peter R. Rapp*, Departments of Anatomy and Neurobiology and of Psychology, Univ. of Vermont, Burlington, Vermont 05405

The central nucleus of the amygdala contributes direct descending input to medullary motor and sensory nuclei involved in autonomic regulation: the dorsal motor nucleus of the vagus (DMV) and the nucleus of the solitary tract (NTS). In the present studies we first characterize the origin and extent of this descending projection system and then examine its pattern of termination in the DMV and NTS.

Multiple injections of HRP (Boehringer, 25-50% in sterile water) were made in 8 rabbits at the border of the DMV and the NTS into the full rostro-caudal extent of the nuclei. Following a 48h survival period tissue was processed using a modification of the TMB procedure of Mesulam (1978). We found the central nucleus of the amygdala to contain a large number of labeled cells throughout its entire rostro-caudal length, but localized within particular regions of the nucleus at given anterior-posterior levels. At the rostral end of the central nucleus, labeled neurons extended dorso-medially beyond the limits of the amygdala into the subtentricular part of the substantia innominata and along the medial border of the internal capsule to the lateral part of the bed nucleus of the stria terminalis. The total field of labeled cells appeared unbroken, forming a continuum.

Stereotaxic injections of ^{3}H-leucine/proline were made in 17 rabbits into the central nucleus and/or bed nucleus of the stria terminalis. As demonstrated by autoradiography a projection from this area descends to dorsomedial, medial, ventrolateral and commissural subnuclei of the NTS, and also to encapsulate and partly innervate the DMV.

HRP labeling of vagus nerve and aortic nerve afferent terminals in the NTS in the rabbit show regions of afferent termination in the dorsomedial, medial, and commissural NTS to be partially coextensive with the regions recipient to the descending projections.

These observations suggest the basal forebrain-to-dorsomedial medulla projection as a possible anatomical substrate for forebrain control of cardiovascular and other autonomic function by direct input to both afferent and efferent nuclei of regulatory reflexes.

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275.13 FAILURE TO SUSTAIN DEOXYCORTICOSTERONE-SALT INDUCED HYPERTENSION AFTER PERIVENTRICULAR PREOPTIC (AV3V) ABLATION IN RAT. J. Buggy. Department of Physiology, University of South Carolina, Columbia, South Carolina 29208.

Normal regulation of arterial pressure represents the coordinated interaction of several control systems. While hypertension may result from a variety of dysfunctions in these control systems, it is not unreasonable to expect some degree of central nervous system participation in any chronic elevation of arterial pressure. Studies in goat, rat, and rabbit have identified the preoptic-hypothalamic periventricular region as a receptive or integrative area for cardiovascular and fluid regulatory responses elicited by electrical brain stimulation or peripheral or central chemical stimulation with hyperosmotic fluids, carbachol, and angiotensin. In rat, ablation within this region of the periventricular tissue surrounding the anteroventral portion of the third ventricle (AV3V) results in acute adipsia uncompensated by increased release of vasopressin, and after recovery, persisting deficits in thirst, renal sodium excretion and water conservation, and pressor response to chemical stimulation. AV3V brain lesions affect some but not all models of experimental hypertension; renal hypertension may be prevented or reversed once established whereas spontaneous genetic hypertension in Wistar-Kyoto rats is unaffected in development or maintenance. In this study, the effect of AV3V lesions on maintenance of hypertension already established by ongoing steroid-NaCl treatment was investigated. Systolic arterial pressure was measured twice weekly by tail-cuff sphygmomanometry in conscious, uninephrectomized, adult male rats given 1% NaCl as the sole drinking fluid and injected weekly with deoxycorticosterone pivalate (CIBA, 25 mg/kg, sc). When hypertension was evident after 4 weeks of treatment, rats were randomly selected for AV3V lesioning or a sham lesion procedure. To insure adequate salt intake, sucrose or saccharin sweetener was added to the 1% NaCl as necessary to sustain drinking. Blood pressure measurement and steroid-salt treatment continued for 4 weeks after the lesion when the brains were removed for histological verification of lesion placement. Lesioned rats had a fall in blood pressure and maintained lower blood pressure through the treatment period compared to sham lesioned rats. Thus, rats with AV3V lesions can neither sustain nor as previously reported develop hypertension normally induced by steroid-salt treatment. The consequence(s) of AV3V ablation responsible for the disruption of certain forms of experimental hypertension remain to be determined. Supported by the American Heart Association.

275.14 RABBIT CARDIOVASCULAR RESPONSES PRODUCED BY STIMULATION OF THE AMYGDALA CENTRAL NUCLEUS. B. S. Kapp, M. Gallagher, M. D. Underwood*, and C. L. McNall*, Dept. of Psychol., and D. Whitehorn, Dept. of Physiol. and Biophys., Univ. of Vermont, Burlington, Vermont 05405.

We have demonstrated that lesions or β-adrenergic blockade within the amygdala central nucleus attenuates vagally-mediated conditioned bradycardia in the rabbit (Kapp et al., Physiol. Behav., 1979; Gallagher et al., Pharmac. Biochem. Behav., 1980) and that the central nucleus projects directly to the region of the vagal dorsal motor nucleus and nucleus solitarius in this species (Schwaber et al., Submitted). Since these data suggest a central nucleus contribution to cardiovascular responding, the present investigation was performed to characterize heart rate and arterial blood pressure responses to electrical stimulation within this nucleus.

Twenty-six New Zealand rabbits were anesthetized with α-choralose, and either single or multiple electrodes (monopolar, 0.25-0.50 mm tip exposures) were lowered in 0.25 to 0.50 mm steps through the region of the central nucleus. Pulse duration and frequency (0.2-1.0 msec; 30-100 Hz), train duration (1.0 vs. 5.0 sec) and current intensity (50 μA-1.0 mA) were investigated. Stimulation at sites within the central nucleus produced bradycardia. Maximum bradycardia (up to 40% decrease) was observed from the anterior, medial region of the central nucleus, an area from which a major portion of the projection to the dorsal medulla originates. This response began within two to three beats of stimulus onset, reached peak magnitude within two seconds of stimulation onset, and was followed by a slight tachycardia following stimulus offset. Threshold currents were as low as 75-100 μA (100 Hz; 0.5 msec pulse duration) at the most active sites. The magnitude of the bradycardia increased with increasing current intensity, pulse frequency, pulse duration and electrode tip exposure. The response was observed to occur following artificial ventilation and immobilization by Flaxedil and was blocked by I.V. atropine methylnitrate. While bradycardia was also elicited from the posterior and lateral regions of the central nucleus, the responses were of markedly less magnitude, and of longer latency to peak magnitude, than those from the anterior, medial region. In all cases bradycardia was followed by depressor responses (up to 20 mm Hg). Pressor responses were never observed.

The results demonstrate that stimulation of the central nucleus produces significant bradycardia and are consistent with our previous data suggesting that the central nucleus contributes to conditioned bradycardia during aversive Pavlovian conditioning. (Supported by USPHS Grants RO1 MH31811 and KO2 MH00118)

275.15 CHARACTERISTICS OF SYMPATHETIC NERVE ACTIVITY AND SYSTEMIC BLOOD PRESSURE DECREASES FOLLOWING ELECTRICAL STIMULATION OF THE ANTERIOR CINGULATE CORTEX IN SHR AND WKY RATS. S. Morrison, D. Whitehorn, E.D. Hendley, M.M. Myers*. Dept.of Physiology and Biophysics, Univ. of Vermont, Burlington, Vermont 05405.

In this report, we describe a potent, cardiovascular inhibitory response that follows electrical stimulation of the deep layers of the anterior cingulate cortex and the cingulum. The characteristics of this response were examined in adult, male Spontaneously Hypertensive (SHR) and Wistar-Kyoto (WKY) rats. Animals were anesthetized with α-chloralose (100 mg/kg), and were paralyzed with curare (1.0 mg/kg) and artificially ventilated. A concentric, bipolar stimulating electrode (0.5 mm O.D.) was stereotaxically located (Pelligrino: A.P.:8.5,M.L.:0.7,D.V.:7.5), and three-second stimulations were delivered using 0.3ms pulses at various frequency and intensity combinations between 10-100Hz and 500-1500μA. Blood pressure was monitored from the femoral artery,while multifiber, sympathetic nerve activity was recorded from a branch of the pre-celiac, splanchnic nerve, using a bipolar, hook elecrode. Nerve activity was amplified, filtered (100-3000Hz),and recorded on magnetic tape. Following computer digitization, the nerve signal was quantified with an amplitude variance method.

Stimulation resulted in a decrease in spontaneous levels of sympathetic nerve activity (onset latency:150-200ms), followed by a decline in blood pressure with a latency to maximum decrease of approximately 15s . Although all stimulus parameters tested were effective in lowering blood pressure, maximum responses were obtained at 40Hz and 750μA. The blood pressure changes were often, but not always accompanied by decreases in heart rate. Neither the sympathetic activity nor the blood pressure declines were blocked by atropine, although the heart rate response was attenuated. Blood pressure declines ranged from 10 to 65 mm Hg, with maximum responses in 9 SHR (-47 ± 5 mm Hg) being significantly greater ($p<.05$) than the maximum responses in 6 WKY (-31 ± 4 mm Hg).

Although we have recorded sympathetic nerve activity responses in a limited number of animals, it is clear that when maximally activated (blood pressure declines greater than 50 mm Hg), stimulation of this brain region can result in a nearly complete (greater than 85%) inhibition of splanchnic nerve activity. It is not known what circumstances normally result in activation of this response; however, it would appear that activity emanating from this higher forebrain area constitutes an important contribution to CNS influences upon the cardiovascular system.

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275.16 LOCAL CEREBRAL GLUCOSE UTILIZATION IN SPONTANEOUSLY HYPERTENSIVE RATS. M. Kadekaro, H. Savaki*, L. Davidsen*, and L. Sokoloff. Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

Spontaneously hypertensive rats (SHR) have been used as an animal model for studies of essential hypertension in attempts to elucidate pressor mechanisms in clinical hypertension. In the initial stage of hypertension blood pressure rises as a consequence of increased peripheral vascular resistance, which leads later to miscellaneous pathological consequences similar to those found in man. Previous findings indicate that neurogenic factors are important in initiating the mechanisms of spontaneous hypertension. It is known that hypertension in SHR rats is maintained when the brain stem is transected at the level of the midbrain, and a marked fall of blood pressure is observed in both SHR and control rats when the transection is made at the upper portion of the cervical cord. The central mechanisms responsible for the increased sympathetic nervous system activity are still unknown. The [^{14}C]deoxyglucose method was employed in the present studies to identify on the basis of altered energy metabolism regions with possibly different levels of functional activity in the brains of SHR and control WKY rats.

Male SHR and WKY rats (78-87 days old) were studied. Local cerebral glucose utilization was determined as previously described. Blood pressure, hematocrit, body temperature, and blood gases were monitored throughout the experimental period.

Mean arterial blood pressure was found to be significantly higher in SHR rats (143 ± 2 mm Hg, n=7) than in the control WKY rats (112 ± 2 mm Hg, n=7). Of 36 nervous structures examined only the external cuneatus n., vestibular n., and fastigial n. of the SHR rats exhibited increased rates of glucose utilization compared to those of the WKY rats. The external cuneate n. receives an input from the aortic depressor nerve and mediates reflex cardiac slowing during arterial hypertension (Ciriello et al., Am. J. Physiol. 235:R286, 1978). Its higher rate of glucose utilization in the SHR rats may reflect its increased activity as a consequence of hypertension. The vestibular n. and the fastigial n. have been shown to be involved in the regulation of orthostatic reflexes in the cat and monkey. Whether or not, however, they are involved in the mechanism of the hypertension in SHR rats is a matter for further investigation.

276.1 DEVELOPMENT OF KITTEN BSER TO PURE-TONE STIMULATION. E. Javel, E.J. Walsh* and J. McGee*. The Boys Town Institute for Communication Disorders in Children, Omaha, NE 68131, and the Department of Physiology, Creighton University School of Medicine, Omaha, NE 68131.

Auditory Brainstem Evoked Response (BSER) potentials elicited by free field pure tone stimulation were studied from birth through the 90th postnatal day in intact, unanesthetized kittens. Signals of 250 Hz, 2000 Hz, and 16,000 Hz with a 17 msec duration were presented in 10 dB steps in order to define the dynamic range of the individual responses. Vertex recorded potentials referenced to the interorbital / supraturbinate position provided optimal records. Earliest responses were obtained for the 2-kHz signal, followed by the 16-kHz and finally the 250-Hz signals. Initial BSER responses to pure tone stimulation were obtained significantly later than responses to click stimulation. The 2000 Hz response was first noted on postnatal day 8 - 9, with the 16-kHz response and the 250-Hz response following at 10 - 11 and 14 - 15 days respectively. Latent periods to individual BSER peaks decreased exponentially as age increased. Once a response to a pure-tone was established, the latencies and amplitudes of BSER peaks as a function of age followed the same patterns seen using click stimulation.

276.2 DEVELOPMENT OF KITTEN BSER TO CLICK STIMULATION. E.J. Walsh*, J. McGee* and E. Javel (SPON: B. Warr). The Boys Town Institute for Communication Disorders in Children, Omaha, NE 68131, and Department of Physiology, Creighton University School of Medicine, Omaha, NE 68131.

Auditory Brainstem Evoked Response (BSER) potentials elicited by free field click stimulation were studied from birth through the 90th postnatal day in intact, unanesthetized kittens. Intensity series in 10 dB steps were recorded in order to define as much of the dynamic range of the response as possible. Vertex recorded potentials were referenced to an interorbital / supraturbinate position. This configuration was shown to produce optimal responses. Results indicate that auditory function can be clearly discerned at high intensities as early as the 8th postnatal day. At earlier ages, low voltage, slow activity occurred which appeared not to be synchronized to the stimulus. The earliest response was always the appearance of wave I. This was followed by a 3 to 4 day period during which 5 to 7 positive peaks were observed in the first 15 - 20 msec following signal onset. Latencies to individual BSER peaks decreased rapidly between 8 - 28 days postnatal, then decreased at a slower rate until 60 days postnatal at which time adult-like latencies were achieved. Interpeak latencies all decreased uniformly with increasing age, indicating that maturation of BSER latencies is cumulative throughout the brainstem pathway. At the same time that latency decreases, amplitudes of BSER peaks increase. Adult amplitudes were attained at ages somewhat earlier than adult latencies. Wave I displayed a split peak throughout maturation. Other waves in the response displayed variations that were not clearly related to maturation but which appeared to be intensity-dependent. Response variations among littermates as well as across litters will be presented.

276.3 DEVELOPMENT OF AN AUDITORY NERVE TERMINAL: THE ENDBULB OF HELD. D.M. Fekete* and D.K. Ryugo. Dept. Anatomy, Harvard Med. School, Boston, MA 02115 and Eaton-Peabody Laboratory, Mass. Eye and Ear Infirmary, Boston, MA 02114.

We began a study of the primary innervation pattern of the cochlear nucleus in the adult cat by injecting horseradish peroxidase(HRP) into the auditory nerve; anterograde movement of HRP completely stained individual auditory nerve fibers and their terminal endings. We were particularly impressed by the morphology of the large, axosomatic endings in the anteroventral cochlear nucleus(AVCN), yet puzzled because they did not correspond to the illustrations of Golgi-stained endbulbs(Brawer and Morest 1975; Held 1893; Lorente de No 1933,1976; Ramon y Cajal 1909) or to the classic description of "bulbs or irregular clubs which issue numerous short appendages"(p.783,Ramon y Cajal 1909). Our observations on endbulb development reveal that the apparent morphological disparity between Golgi-stained and HRP-filled endbulbs is due primarily to age differences.

Kittens of 2,5,10 and 20 days of age and adult cats were prepared by variants of the Golgi method, and analysis was confined to the anterior division of AVCN(Brawer et.al.1974). The classic description of the Golgi-stained endbulb is accurate only for the youngest cats(2,5, and 10 days). We also wish to point out that for these ages, preliminary electron microscopic observations reveal that the endbulbs are flattened in one dimension and appear more like a spoon than a bulb. During these first 10 days the endings enlarge to cradle as much as half of the surface of the postsynaptic cell body. At 10 days, fissures and fenestrations are evident in about half of the endings; the larger and more fenestrated endings no longer exhibit the hair-like extensions. Finally(20 days and adult), significant resorption and remodelling of the endbulbs are demonstrable: the ending is divided into several thick(2-4 um), gnarled branches that are linked together by a delicate reticulum of very fine strands and vericosities of assorted sizes. Maturation has transformed the neonatal endbulb from a large, continuous ending into a network of smaller but interconnected endings.

The multiplicity of developmental events occurring simultaneously with endbulb maturation limits the usefulness of relating the changes in endbulb morphology to any single event in the ontogenetic sequence of auditory functioning. Nevertheless, we cannot resist speculating that endbulb maturation, which produces a significant increase in membrane surface area of the terminal, enhances synaptic transmission by facilitating ion transport necessary to quickly repolarize the endbulb.

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276.4 ORGANIZATION OF THE DORSAL COCHLEAR NUCLEUS IN THE ADULT HAMSTER. L. Schweitzer* & N. Cant (SPON: W.C. Hall). Dept. of Anatomy, Duke University Medical Center, Durham, NC 27710

The hamster is well-suited for studies of the development of the mammalian nervous system because of its short gestation and relative immaturity at birth. Our findings suggest that the dorsal cochlear nucleus (DCN) is immature at birth, developing over a course of about ten days. Although the DCN has been described in other species, the adult hamster DCN has not.

In the adult hamster, four neuronal types were identified in Nissl-stained DCN. Fusiform cells, located in the fusiform cell layer, can be distinguished by their large ovoid cell bodies, large nucleus, granular cytoplasm, and prominent nuclear cap of Nissl substance. The giant cells are also granular and often exhibit a Nissl cap, but these cells can be distinguished from the fusiform cells by their larger size and location within the deep layer. The small cells, found primarily in the molecular and fusiform cell layers, have pale, uniform cytoplasm and are smaller than the fusiform cells. The smallest cells, granule cells, are found in all layers and contain several nucleoli in their darkly-staining nucleus.

The size, shape, and distribution of these cell types enabled their identification in Golgi preparations. The apical dendrites of the fusiform cells, which extend into the molecular layer, are oriented perpendicular to the surface of the DCN and ramify predominantly in the sagittal plane. These dendrites are covered with spines in contrast to the spine-free basal dendrites, which extend into the deep layer. The dendrites of the giant cells also ramify primarily in the sagittal plane. These dendrites usually remain in the deep layer but occasionally extend to the superficial layers where sparse thorny appendages may adorn them. The dendrites of the small cells are oriented parallel to the surface of the DCN and run perpendicular to the apical dendrites of the fusiform cells. In the deep layer these cells assume a more stellate appearance. The granule cells have two or three dendrites which are oriented parallel to the surface of the DCN. In conclusion, while the dendritic morphology and Nissl pattern of some of the cell types in the DCN of the hamster differ from other species, the nucleus is highly organized and has a well-established laminar appearance. We are planning to investigate the developmental sequence leading to this organization.

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276.5 ONTOGENETIC DEVELOPMENT OF SINGLE UNIT RESPONSES IN THE COCHLEAR NUCLEUS OF THE MONGOLIAN GERBIL. Nigel K. Woolf and Allen F. Ryan* Otolaryngol. Research Lab, VA Hospital, San Diego and Univ. Calif. at San Diego Medical School, San Diego, CA 92103.

Single neurons in the cochlear nuclei (CN) of mongolian gerbils (Meriones unguiculatis) were examined by conventional extracellular recording techniques at postnatal ages 10, 12, 14, 16, 18, 30, 60, 90 (young adult) days after birth (DAB), and adult. This range of chronological ages included the onset of neonatal hearing through achievement of mature auditory system characteristics. The onset of hearing was confirmed, in addition to single neuron CN recordings, by measurement of intra-cochlear potentials.

Single neuron recordings in the neonatal gerbil CN revealed clear ontogenetic trends. At 10 DAB none of the neurons recorded from within the CN responded to acoustic stimulation. At 12 DAB only a small percentage of the neurons encountered were responsive. For subjects 14 DAB, or older, the vast majority of neurons isolated were responsive to acoustic stimulation. Based upon data taken from neurons which were responsive, it was evident that with increasing chronological age: first, the characterstic frequency (CF) neural threshold values rapidly and monotonically decreased; second, the absolute frequency range of neural CF values increased; and third, the high frequency limits for CFs increased progressively with age. It should be noted that while neural CF thresholds exceeded 95 dB SPL at 12 DAB, by 16 DAB the CF thresholds of the most sensitive CN neurons closely approximated the adult behavioral thresholds for mid-frequency values. This indicates a very rapid developmental sequence of maturation.

In addition to single neuron response at 12 DAB, cochlear microphonic responses were also first recorded at this time. The N_1 compound action potential, however, was not detected until after 12 DAB. The absence of N_1, concurrent with observed neural responses, suggested the population evoked response was asynchronous. Direct support for this hypothesis came from data on neural responses to tone burst stimuli.

In response to gated tone bursts, all CN neuron responded throughout the stimulus period. When stimulus duration was lengthened from 25ms to 500ms even the 12 DAB neurons showed no evidence of habituation or intermittent responses. However, while the great majority of post-stimulus-time (PST) histograms for mature subjects exhibited well defined onset peaks, the great majority of PSTs for neonates did not. This reflects greater variability in initial spike latency and a less pronounced initial rate of firing for neotal responses than are characteristic of mature subject responses. Both of these features are consistent with the absence at 12 DAB of N_1.

276.6 AGE AND POSITION DEPENDENT EFFECTS OF ACOUSTIC DEPRIVATION IN THE AVIAN N. MAGNOCELLULARIS. John W. Conlee* and T.N. Parks. (SPON: M. Jacobson). Dept. of Anatomy, Univ. of Utah Coll. of Medicine, Salt Lake City, UT 84132.

The effects of a unilateral conductive hearing loss on the development of neuron size in nucleus magnocellularis (NM) was investigated. NM receives large calyceal endbulbs from the cochlear nerve. Silicone plastic earplugs which cause a 40dB broadband conductive hearing loss (Kerr et al, J.Exp.Psychol.,5:97,1979) were placed unilaterally in the external auditory meatus of chick embryos on the 18th day of incubation. Animals undergoing prolonged deprivation had their earplugs replaced every 8-12 days. All animals were housed in communal brooders and were sacrificed at predetermined ages: 7(n=3), 13(n=3), 28(n=3),and 63(n=2)days after the initial earplugging. Serial transverse paraffin sections were stained with thionin; NM neurons meeting a nucleolar criterion were drawn with a camera lucida at 1400X at several caudo-rostral percentile levels through NM. The cross-sectional areas of neurons in NM on the normal and deprived sides were compared at chosen caudo-rostral percentile levels; at least 700 cells per brain were drawn. Within-animal comparisons between the deprived and normal sides of the brain showed that the mean sizes in the two NM were not significantly different at 7 and 13 days of unilateral deprivation, but were significantly different ($p<.001$)at 28 and 63 days of deprivation. Neurons on the deprived side showed smaller cross-sectional areas, with differences ranging from 12% at 28 days to 25% at 63 days of deprivation. Neurons in the middle two caudal-to-rostral quartiles (i.e. 25-75%)of NM were significantly smaller ($p<.01$)than those on the control side at 28 days of deprivation. With 63 days of deprivation, the effect spread rostrally and neurons in the three rostral quartiles of NM were significantly smaller ($p<.01$)on the deprived side. Neurons in the caudal quartile of NM receive a substantial innervation from the lagenar nerve and are not included in this analysis.

These results indicate that a moderate unilateral acoustic deprivation results in an age and position dependent smaller mean cell size in NM on the deprived side relative to the control side. The presence of an effect on NM cell size only in those animals undergoing more prolonged deprivation may demonstrate the maintenance role that acoustic stimulation has on the normal growth of NM. The fact that broadband attenuation initially affects the middle region of NM and then expands rostrally with continuing deprivation suggests that there may be regional differences among NM neurons which cause them to be unequally responsive to identical acoustic deprivation.

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276.7 DEOXYGLUCOSE LABELING OF AUDITORY FOREBRAIN AREAS IN ADULT AND NEWLY-HATCHED GUINEA FOWL. H. Scheich, V. Maier* and B. A. Bonke*. Zoological Institute, Technische Hochschule Darmstadt, 6100 Darmstadt, FRG.

Adults and chicks at various stages after hatching were injected with 14-C-deoxyglucose (37 µCi/100g) and were stimulated for 45 min with pure tones, harmonic tones or FM. As previously shown in adult birds two areas in the forebrain exhibit tone-activated stripe patterns of labeling which give evidence of tonotopic organization (Scheich,H.,Bonke,B.A.,Bonke,D.,Langner, G.,Cell Tissue Res., 204:17, 1979). Among these areas field L of the neostriatum receives the primary auditory input from the diencephalon. It is a three layer structure with a functionally simple input layer L_2 in the middle. The tone-activated stripes and thus tonotopic organization cut across all three layers and reach dorsally into a lamina of the overlaying hyperstriatum ventrale. This structure receives input from field L (Bonke,B. A.,Bonke,D.,Scheich,H.,Cell Tissue Res., 200:101, 1979). Harmonic tones produce several stripes of labeling in field L each corresponding approximately to the tonotopic place of the particular harmonic frequency. The stripes are less demarcated however than those obtained with pure tones.

In young chicks L_2 is weakly labeled throughout its extend without acoustic stimulation, probably due to high spontaneous spike activity which is also known from adult birds. Immediately after hatching tone-activated stripes were found which form a cross pattern together with L_2, thus tonotopy is present at this stage. Only in chicks of several days of age and most distinctly in adults labeling of L_2 adjacent to the tone- activated stripe is reduced suggesting that the efficiency of inhibitory mechanisms increases with age. Lateral inhibitory areas in tuning curves are typical for L_2 neurones in adult birds (Bonke,D.,Scheich,H.,Langner,G.,J. Comp. Physiol., 132:243, 1979) Towards the end of the first day strong glucose uptake developes in the most lateral part of L_2 and of the overlaying hyperstriatum where frequencies below 1 kHz are represented. This may indicate that low frequency analysis is not fully developed in newly-hatched birds.

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276.8 SOMATIC AND DENDRITIC MORPHOLOGY IN AVIAN N. MAGNOCELLULARIS AND N. LAMINARIS AFTER UNILATERAL OTOCYST ABLATION. T.N. Parks. Dept. of Anatomy, Univ. of Utah College of Medicine, Salt Lake City, UT 84132.

The morphology of neurons in the 2nd-order avian brain stem auditory nucleus magnocellularis (NM) and 3rd-order nucleus laminaris (NL) was studied after unilateral surgical ablation of the right otocyst at 50-60 hrs of incubation in several hundred White Leghorn chick embryos. This procedure completely prevents the development of the inner ear and 8th nerve on the right side. After variable survival times, the animals were sacrificed and staged. Brains with verified otocyst ablation were stained by rapid Golgi (n=4, stages 36,37,41) and Golgi-Kopsch (n=10, stages 40,42,43,44) methods. Camera lucida drawings were made of neurons in NM and NL. In NM cells, the morphological transformations described by Jhaveri & Morest (Neurosci. Abstr.,3: 109, 1977) proceed in a relatively normal fashion without 8th nerve afferents. At stages 36 and 37, NM cells are multipolar, with long somatic processes; by stages 42-44, they are spherical, with somata covered by small spines. Cells with a short somatic process are also seen occasionally at this age. Thus, the highly-characteristic morphology of NM neurons may develop normally in relative autonomy from the cochlear nerve. Alternatively, non-cochlear afferents may, in the case of otocyst ablation, fill an interactive role normally played by cochlear afferents. The latter possibility is now under study.

Drawings of 40 NL neurons from five stage 43 embryos were used to measure the dorsal and the ventral total dendritic length for each cell (cf. Smith & Rubel, JCN 186: 213, 1979). These cells came from both sides of all five brains and about equally from all regions of the nucleus (the most rostral area being underrepresented). A quantitative analysis of dendritic lengths in these cells showed the "manipulated" dendritic fields (right side dorsal and left side ventral dendrites connected to the affected right NM) to have an average length 49% smaller than that of the "unmanipulated" NL dendrites connected to the normal left NM ($p<.001$). This effect in NL may be due to the previously reported trans-synaptic loss of NM neurons after otocyst ablation (Parks, JCN 183: 665, 1979; cf. Benes et al., Br.Res., 122:1, 1977)and/or the complete absence of acoustically-driven neuronal activity along auditory pathways leading from the right ear.

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276.9 NERVE-TARGET CELL INTERACTIONS DURING THE DEVELOPMENT OF THE AVIAN INNER EAR. Mark Whitehead and D. Kent Morest. Dept. of Anat. UConn Health Ctr. Farmington, Connecticut 06032.

Synapse formation by cochlear fibers and the differentiation of their hair cell targets of chick embryos have been studied with electron microscopy. Before innervation (3½-4 days) hair cells and supporting cells are not distinquished in the sensory epithelium. Apical microvilli extend into a flocculant matrix in the cochlear duct. While junctional complexes occur apically, large extracellular spaces extend transversely in the basal epithelium. During innervation (6-7 days) fibers encounter resistance as they penetrate the basal lamina. Within the epithelium bundles of sensory fibers enter the large basal spaces where they can spread freely in the transverse dimension. The fibers form attachment plaques anchoring them to the basal epithelial processes. Subsequently these processes and the attached fibers assume more superficial positions in the epithelium. Early in synaptogenesis (8-9 days) hair cells and supporting cells can be distinquished. From a nascent cuticular cone the hair cell cilia extend into a loosely commposed tectorial membrane, apparently derived from the flocculent material of earlier stages. Recepto-neural synaptic junctions have symmetrical or asymmetrical membrane densities and a certain dense material organizing in the cleft. At many of these junctions synaptic bodies, as well as dense-cored and coated vesicles gather in the hair cell, while a few clear vesicles and flocculent material appear in the branching endings. Midway in synaptogenesis (day 13) hair cells contain many synaptic bodies, not always located at recepto-neural junctions. The sensory endings form large growth swellings containing flocculent material, endoplasmic reticulum and vesicles. Late in synaptogenesis (16-17 days) the swellings disappear, while synaptic contacts expand into foot-like processes. Synaptic bodies unassociated with junctions disappear. Efferent endings appear at 16-17 days.

In conclusion, there is a parallel sequence of changes in the structure of the developing sensory nerve endings and their target cells. The observations suggest a role of the basal lamina and epithelial end-feet in excluding the ingrowth of all but specific fibers. Once within the epithelium, the fibers may be free to spread within a system of extracellular channels in the transverse direction. The fibers are anchored to the basal processes of presumptive hair cells. This arrangement may promote the radial growth of the fibers which accompany their target cells as the basal processes withdraw to the superficial epithelium. Further differentiation of the hair cells, including synaptic bodies and membrane desities, is accompanied by a transformation in the morphology of the nerve endings.

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276.10 REORGANIZATION OF CENTRAL PROJECTIONS AFTER REGENERATION OF THE AUDITORY NERVE IN ANURANS. H. Zakon and R.R. Capranica Section of Neurobiol. and Behav., Div. of Biol. Sci., Cornell Univer., Ithaca, N.Y. 14853.

The vertebrate central auditory system must be precisely organized in its neural connections. The fact that sharp "V" shaped excitatory tuning curves are maintained from the level of the eighth nerve to at least the midbrain suggests that all of the afferents which innervate a given cell share similar best frequencies (BF). Furthermore, binaural cells usually have identical BFs when stimulated by either ear; thus, neurons with the same BFs from each side of the brain must seek out a common target cell.

In order to determine how such highly specific connections might be achieved in the central auditory system, we have studied the frequency sensitivities of neurons in the superior olive (SO) of the leopard frog (Rana pipiens) during reinnervation of brainstem primary auditory nuclei by the regenerating eighth nerve. The nerve was severed unilaterally and the animals were then maintained in separate tanks at 20°C. Under these conditions the eigth nerve regenerates in 5-6 weeks. Single units were then recorded in the SO at 6 and 12 weeks after nerve transection; the response properties in these experimental animals were compared to those in normal animals.

In normal frogs most of the cells in the SO are driven by the contralateral ear: they all have narrow "V" shaped tuning curves. About one-third of these cells are binaural, with the ipsilateral ear usually inhibitory. The BFs of the tuning curves from both ears are similar (usually less than 1/4 octave apart).

Many of the cells in the SO of 6-week regenerates, which are strongly driven by the contralateral ear again (via the regenerated nerve), exhibit narrow "V" shaped tuning curves. Nevertheless, a number of cells were found which were broadly tuned or had multipeaked tuning curves. Furthermore, while some binaural cells had matched BFs from the two ears, there were many binaural cells which were poorly matched (some had disparities of well over 1/2 octave).

By 12 weeks after nerve transection, almost all cells exhibit simple "V" shaped tuning curves, although a few "W" shaped tuning curves remain. The BFs of the binaural cells are once again as closely matched as in normal animals.

These results suggest that, upon their earliest contact with the central auditory system, regenerating auditory nerve fibers may not necessarily return precisely to their former postsynaptic targets. With time, changes occur which lead to the eventual matching of binaural frequency sensitivity.

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277.1 DEVELOPMENT OF COMPLEXITY IN RAT NEUROMUSCULAR JUNCTIONS
C.D. Tweedle and K.S. Stephens*. Department of Anatomy, Michigan State University, East Lansing, MI. 48824.

Using a combined silver-cholinesterase stain, motor endplate morphology was examined in both slow-twitch (soleus and adductor longus) and fast-twitch (rectus femoris and plantaris) muscles of 2,3,4,5,6 and 11 week old male rats (N=37 rats). Categorization of endplates from each muscle at each age into defined morphological classes was carried out. The differences between the distribution of endplates among these classes between muscles and between ages was then analyzed statistically. Polyneuronal innervation of endplates dropped dramatically between 2 and 3 weeks. The number of "branched" endings (2 muscle fibers innervated by a single axon) significantly decreased between 3 and 4 weeks in all the muscle groups and did not change thereafter. At 4 and 5 weeks the predominant appearance of the endplates of all the muscles was similar - being basically a single "bare" axon going into the endplate area (with one axon being associated with one endplate per muscle fiber). Complexity of the endplates then developed with maturation. At 6 weeks of age in the slow twitch muscles only there was first seen a significantly greater percentage of double endplates (one axon giving rise to 2 endplates on a single muscle fiber). This difference increased significantly between 6 and 11 weeks. The number of more complex nerve endings with accessory axonal branches to the endplate from the parent axon increased steadily throughout maturation in all the muscle groups. At 3 weeks these made up less than 5% of the endplates. However, by 11 weeks they were seen in between 25 and 40% of the endplates. This study indicates that 1) there is still synaptic re-arrangement (loss of branched endings) between 3 and 4 weeks in all muscle groups: 2) there develops a significantly greater number of double endplates in slow twitch muscles around 6 weeks; and 3) complexity of endplate structure increases with growth of the animal by adaptations of the terminal axon. Thus, even under normal conditions, young adult rats spontaneously display significant evidence of synaptic remodeling and plasticity. Supported by NSF.

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Withdrawn by Author

277.3 MORPHOLOGICAL PLASTICITY IN THE VENTRAL LATERAL GENICULATE NUCLEUS OF THE CHICK.
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The neuropil lamina of the two-layered ventral lateral geniculate nucleus (GLv) of the chick receives topographic projections from both the retina and optic tectum. Based on findings in the visual pathways of certain mammalian species one could predict that removal of one set of these afferents would lead to an increase in the density of terminal arborizations of the remaining set.

To test this possibility a group of newly-hatched chicks was subjected to partial tectal removal. One day prior to sacrifice six weeks later, the eye contralateral to the tectal operation was injected with ^{3}H-proline. Following reconstruction of the GLv and optic tectum from autoradiographs, an area of sharply increased silver grain density was found in the GLv which was in retinotopic correspondence with the lesioned area in the optic tectum. Thus the zone of heightened grain density was located at the site of the degenerated tectogeniculate projection.

This finding suggests a competitive mechanism of terminal arborization growth similar to that reported in mammals. Whether the increased density results from the proliferation of retinotopically appropriate or inappropriate axons remains to be determined.

(Supported by PHS grant EY-01796.)

277.4 A GOLGI STUDY OF THE POSTNATAL DEVELOPMENT OF PYRAMIDAL NEURONS IN THE RAT VISUAL CORTEX.
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In the rat visual cortex pyramidal neurons predominate in layers II/III and V. All of these cells undergo similar changes during development to their adult forms. At birth, they appear to be essentially bipolar with a single aspinous dendrite reaching toward the pial surface, whereas the adult appearance, characterized by a prominent apical dendrite and a basal skirt of dendrites, is achieved by day 21. To determine how these cells develop in layers II/III and V, general morphological characteristics such as numbers of visible spines along the apical dendrite, dimensions of the cell body, and numbers of primary basal dendrites were examined. Pyramidal neurons impregnated by the rapid Golgi method were studied in albino rats of 0, 3, 6, 9, 12, 15, and 21 days of age. For all neurons the overall spine density along the apical dendrite increases exponentially with age from 0.20 and 0.22 spines/μm to 1.02 and 0.91 spines/μm for layer II/III and V neurons, respectively, although there is a pause in this increase between the 9th and 12th days. This pause coincides with the arrival of geniculate input (Lund and Mustari, 1977). Moreover at each age spine density increases exponentially with distance from the soma to attain a peak density at about 125 μm from the cell body. At any age apical dendrites of individual layer V cells may show as much as a 3-fold difference in their spine density at the level of layer II/III as compared to layer IV. The mean for all layer V cells, however, shows no significant difference between the two regions. For both populations of neurons the lengths, widths, and volumes of their cell bodies increase linearly with age. For layer V cells the increase in volume is from 1200 μm^3 at day 3 to 4110 μm^3 on day 21, whereas for neurons in layer II/III the gain does not begin until three days later and increases from 930 μm^3 on day 6 to 2510 μm^3 on day 21. This delay of maturation between the two populations is also reflected in the attainment of the number of primary basal dendrites characteristic of adult neurons. Layer V pyramidal neurons achieve a maximum number of 6.2 primary basal dendrites at day 6, but layer II/III cells do not reach a maximum of 4.9 until day 9. Consequently, this study provides quantitative evidence that layer V pyramidal neurons begin to differentiate at an earlier age than layer II/III cells. Apparently the maturation of the general morphology of pyramidal neurons is affected strongly by their time of arrival in the cortical plate (Berry and Rogers, 1965) and by external factors such as thalamic input. Supported by NIH grants NS-07016 and EY-07054.

277.5 SYNAPTIC PLASTICITY WITHIN THE INTERPEDUNCULAR NUCLEUS AFTER UNILATERAL LESIONS OF THE HABENULA IN NEONATAL RATS. G. S. Hamill* and N. J. Lenn. Carnegie Labs. of Embryol., Univ. Calif., Davis, and Dept. Neurol., Univ. Virginia, Charlottesville, VA 22908.

Medial habenular (MH) lesions in neonatal rats have been shown to cause synaptic rearrangements in the interpeduncular nucleus (IPN) which vary for different synaptic types and with the magnitude of the MH lesion. A unilateral MH lesion produces a reproducible 50% reduction of MH input to IPN, and also allows consideration of bilateral integretion at the synaptic level. After such lesioned pups reach maturity, injection of ^{3}H-leucine and demonstration of axonal transport by autoradiography or scintillation counting of punch biopsies, or destruction of the remaining MH with demonstration of degenerating synapses by electron microscopy allow analysis of the induced plasticity. Controls consist of normal animals, with and without ^{3}H-leucine injection or adult lesions.

The S synapses, the principal MH afferents to IPN, are cholinergic, have identifiable fine structure at all ages, and form 90+% of all IPN synapses. 95% of S synapses terminate in IPN ipsilateral to their MH of origin normally, in spite of the fact that their axons cross through the contralateral half of IPN one or more times. Neonatal unilateral lesions result in a symmetrical bilateral districution of S synapses as shown by all 3 methods.

The crest synapses, a less frequent termination of MH afferents to IPN, consist of 2 endings indistingushable from S synapses which contact a markedly narrowed, 75 nm dendritic crest with parallel and coextensive contact zones. They normally are formed by 1 axon from each MH in 98% of cases. After unilateral neonatal lesions they are formed by 2 axons from the remaining MH in approximately 2/3 of cases as shown by degeneration of both axons simultaneously after the adult lesion. However, some crest synapses are formed by 1 MH afferent axon, and 1 presynaptic process of other origin, including processes which form somatic synapses. Occasional bare hemisynapses are seen apposed to myelin or glia, especially at 1½ days post-lesion. A few crest synapses are also formed in this material by 2 presynaptic processes of non-MH but so far unknown origin, a finding which is commonly induced by bilateral neontal MH lesions, as previously demonstrated.

These diverse manifestations of synaptic plasticity imply a loss of specificity, and therefore probably of functional integretity, as well as alterations of bilateral integretation. The underlying normal synaptogenetic control mechanisms implied are lateralized, as well as localized to both pre- and post-synaptic sites.

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277.6 PLASTICITY OF ADULT PURKINJE CELL SPINE SYNAPSES FOLLOWING DECREASED AFFERENTS. D.E. Hillman and S. Chen, Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016

Double transection of a cerebellar folium in the adult rat, by separations of one to 2 mm, produced greater than 50% reduction in parallel fiber synapses on Purkinje cells between the cuts. Qualitative observation of Purkinje cell spines after 5 days showed a marked length increase in synaptic profiles such that the entire head of some spines was almost encapsulated by a synaptic site. The majority of spines were greatly enlarged and had elongated synaptic profiles. Multiple parallel fiber synapses were also occasionally seen on enlarged spines. Very noticeable was the enlargement of parallel fiber boutons which appeared to engulf the spine head with a presynaptic thickening in apposition to the postsynaptic site. A proportional increase in the number of synaptic vesicles was evident in the enlarged boutons. Actual shape and size change was determined by three-dimensional reconstruction from serial sections. Quantitation showed an average increase of 30-40% in synaptic profile length. Estimation of contact area (taking into consideration the cupped shape of the synapse) revealed a greater than 50% increase in size. Thus, there appears to be an inverse relationship between synaptic contact size and spine synaptic number as has been shown by afferent reduction due to malnutrition during development (Soc. Neurosci. 5: 100, 1979). Intrinsic determination of synaptic contact area by Purkinje cells give rise to plasticity in synaptic size and thus the integrity of this circuitry is maintained even though certain afferents have been lost. (Supported by NICHD-10934).

277.7 EFFECT OF PERIPHERAL NERVE SECTION ON VIBRISSAE RELATED SEGMENTATION IN THE CENTRAL NERVOUS SYSTEM OF THE RAT. A. Shinder* and H.P. Killackey (SPON: H. Koopowitz). Dept. of Psychobiology, Univ. of Calif., Irvine 92717.

Previously the effect of individual cauterization of all of the vibrissae which compose the five rows of mystacial vibrissae has been determined. In the ventrobasal complex (VB) following this procedure, the five rows of discrete clusters are replaced by five bands. In other words, there is a loss of boundaries between clusters within a given row while the boundaries between rows are maintained (Belford & Killackey, JCN, 1979). The present experiment was undertaken to compare the effect of nerve section on vibrissae related segmentation with that of total vibrissae cauterization on such segmentation.

For the present experiment, the maxillary branch of the trigeminal nerve of littermate rats was severed at the level of the infraorbital foramen on the day of birth (PND 0) or on one of the two following days (PND 1 and PND 2). Animals were then allowed to survive to PND 5 at which time they were sacrificed, their brains removed and processed for succinic dehydrogenase (SDH) activity.

Following this procedure, the brainstem, thalamus and cortex were examined for dense clusters of SDH activity related to afferent segmentation. In the brainstem there were uniform low levels of activity in the trigeminal nuclei and no segmentation was detectable. We attribute the low level of SDH activity to the fact that the peripheral processes of the primary trigeminal afferents are damaged. The results for thalamus and cortex were different from what we found in the brainstem but similar to each other; hence, we describe our findings only for VB. In this nucleus the level of SDH activity was normal. However, the amount of segmentation within VB varied with the age of peripheral nerve manipulation. Damage on PND 0 resulted in no detectable segmentation within the vibrissae related portion of VB. Damage on PND 1 results in some tendency towards segmentation with between-row boundaries being better defined than within-row boundaries. Damage on PND 2 results in even more segmentation, and both between- and within-row boundaries are detectable, although neither are clearly delineated as they are normally.

The present results indicate that the effect of early nerve section is more severe than that of cauterization of all vibrissae follicles on central vibrissae representation. We interpret these results as suggesting that afferent boundary information is "passed" centrally from the periphery during the early postnatal period and that it is a time dependent process.

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277.8 COMPARTMENTALIZATION OF PREFRONTAL PROJECTIONS: COMPARISONS OF CORTICAL COLUMNS AND STRIATAL ISLANDS IN OLD AND NEW WORLD MONKEYS. N.M. Bugbee* and P.S. Goldman-Rakic, Sec. of Neuroanatomy Yale Univ. School of Med., New Haven, CT. 06510.

According to several reports, the ocular dominance columns of striate cortex are organized differently in Old and New World monkeys. In Old World monkeys, these columns are most prominent in layer IV, while in New World species, they are confined to the supragranular layers. This difference raises the question of whether the distribution of fiber terminals in other brain regions also varies between these species. Previous studies of rhesus monkeys have shown that cortico-cortical afferents to prefrontal association, limbic, and motor cortex are distributed in sharply demarcated columns; moreover, efferent projections from neocortex to caudate and putamen also terminate in a complex pattern of spatially segregated patches or compartments.

To examine the possibility of species differences in the terminal distribution of cortico-cortical and cortico-striatal projections, we injected tritiated amino acids into the prefrontal cortex of New World squirrel monkeys (Saimiri sciureus) and Old World rhesus monkeys (Macaca mulatta) and prepared their brains for autoradiography. In the rhesus monkey, fibers labeled by such an injection terminate in distinct bands, which in transverse sections appear as vertically oriented columns 200-500 um wide. These columns extend across all layers of cortex. Such columnar-like patterns are equally apparent in the cortex of the squirrel monkey, where again, they traverse all cortical layers and measure 200-300 um across.

Terminal patterns observed in the caudate nucleus of the squirrel monkey are also very similar to those in the rhesus. Labeled fibers are not uniformly distributed, but are separated by territories in which grains do not exceed background; some of these patches of label surround an elliptically shaped island which is relatively grain-free. In addition to their likeness in shape, these caudate patterns are also remarkably similar in dimension, with the diameter of the grain-free island ranging between 300-400 um in both species.

Thus, differences in the distribution of afferent fiber systems in Old and New World monkeys appear to be specific to the geniculostriate system. Our findings demonstrate a marked species similarity in the terminal patterns of cortico-cortical and cortico-striatal projections. The dimensions of cortical columns and striatal compartments are similar in spite of considerable differences in cortical surface area and neostriatal volume. If there is a species difference in the compartmentalization of prefrontal fiber systems, it may be in the number, rather than the size or laminar organization, of the compartments.

278.1 NEOCORTICAL TRANSPLANTS IN THE CEREBELLUM OF THE RAT: AN AUTORADIOGRAPHIC ANALYSIS OF THEIR HISTOGENESIS. D.T. Ross* and G.D. Das* (SPON: L.J. Pellegrino). Dept. of Biol. Sci., Purdue University, W. Lafayette, IN., 47907.

It is well established that embryonic neural tissues, when transplanted into the brains of neonatal or adult hosts, survive, grow, differentiate and become anatomically integrated with the host brain parenchyma. An integral element of transplant growth is the histogenesis of neurons from transplanted neuroepithelial cells. This study was conducted to analyze histogenesis in neocortical transplants obtained from embryos of different ages.

Donor embryos were obtained from pregnant Wistar-albino rats on day 15, 16, 17, 18, 19, 20 or 21 of gestation. The cerebral hemispheres were dissected from these embryos, and $8mm^3$ of this tissue was transplanted into the cerebellum of 10-day-old host animals. Following this, each animal received a single injection of H^3-thymidine. At least one animal per donor age group was given the radiochemical 6 hours after transplantation and at least one animal per group was given H^3-thymidine on each succeeding day throughout the histogenetic period for the transplanted tissue. Ninety days after transplantation host animals were sacrificed and their brains processed for autoradiography. Cresyl-violet counterstained autoradiograms were analyzed for the presence of heavily labelled large pyramidal cells (perikarya 18-22 μm), small pyramidal cells (14-17 μm), large stellate cells (12-16 μm), and small stellate cells (8-11 μm). Labelled and unlabelled neurons were counted in each transplant to determine the percentage of heavily labelled cells for each of the four types. The onset, peak and termination of neurogenesis were determined for each cell type in transplants obtained from embryos of different ages.

Generally, the pattern of histogenesis in transplanted neocortex was similar to that seen for neocortex in situ. Transplants of 15 day embryonic neocortex exhibited the longest duration of histogenesis, 11 days, while transplants from 21 day old embryos showed the shortest duration of histogenesis, 3 days. The duration of histogenesis for transplants of intermediate ages fell between these two extremes in a graded fashion. Transplants from 15, 16, and 17 day old embryos contained heavily labelled pyramidal and stellate cells whereas transplants from older embryos had only labelled stellate neurons. Transplantation was seen to cause some subtle changes in the time course of neocortical histogenesis, delaying the onset and peak and prolonging the termination of neurogenesis for each of the four cell types studied.

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278.2 CULTURED FETAL RETINAE TRANSPLANTED TO SUPERIOR COLLICULUS. L. K. McLoon, S. C. McLoon and R. D. Lund. Dept. of Anatomy, MUSC, Charleston, S. C. 29403.

Recent work has demonstrated that fetal rat retinae transplanted adjacent to the superior colliculus (SC) of newborn host rats develop relatively normal cytology and connect only with those regions of the host brain normally retino-recipient (McLoon and Lund, 1980). We have examined here whether a period of growth in vitro would affect the organization of fetal retinae and their ability to form connections after subsequent transplantation to the host brain. On embryonic day 14 rat neural retinae were dissected from the embryo and cultured in MEM supplemented with 10% calf serum. After 2, 3, 5, 7, 10 or 14 days in culture, some of the retinae were fixed, and the rest were transplanted adjacent to the SC of unilaterally enucleated newborn rats. After one month, half of the host rats were sacrificed, fixed, and their brains examined with Nissl and silver stains. Some of these also received transplant lesions for degeneration studies. The second group of rats received an injection of HRP into the left SC 12 hours before sacrifice in order to identify cells in the transplant projecting into the host SC.

Examination of the retinae fixed at the time of transplantation showed them to retain a neuroepithelial appearance. Only a slight degree of differentiation was evident with no obvious lamination present. In the longer term cultures there was evidence of cell death in the center of the explants. Retinae that had been in culture 2 to 7 days when transplanted appeared one month later as rosettes or folded sheets. They developed relatively normal cytology showing three distinct cell layers separated from one another by plexiform layers. After 10 or 14 days in vitro the resultant transplanted retinae were smaller in size and more disorganized, although some distinct cell differentiation and lamination developed. As suggested by fiber patterns and degeneration studies, transplants from all the in vitro periods projected into the superficial layers of the host SC. The retrograde transport of HRP after injection of the host SC labeled cells in the transplant corresponding in position to the ganglion cell layer. Thus, even after 2 weeks in vitro, fetal retinae retain the ability to differentiate relatively normally and form appropriate connections with the host brain. This technique will allow the opportunity to assay the consequences of in vitro manipulation which may modify the developmental program of the retina. (Supported by NIH EY 03414. LKM is an NSF Fellow #14844.)

278.3 CYTOLOGY AND CONNECTIONS OF RETINAL TRANSPLANTS IN RATS. R. D. Lund and S. C. McLoon. Dept. Anat., Medical Univ. South Carolina, Charleston, S. C. 29403.

Fetal retina, when transplanted adjacent to the superior colliculus of newborn rats, develops organization and connections appropriate for normal retina (McLoon and Lund, Neurosci Soc. Abs 5:631). We have extended these results to include ultrastructural findings. Presumptive retinae were excised from the eye cups of rat embryos on gestational day 14 and transplanted adjacent to the superior colliculus of neonatal rats with unilateral enucleations. After 4-6 weeks survival, the transplants were removed from the host brains and prepared for histological examination. Light and electron microscopic examination of the transplants revealed cell and plexiform layers characteristic of normal retinae. The outer plexiform layer contained numerous invaginating ribbon synapses; however, there was a conspicuous paucity of conventional synapses. Dyad ribbon synapses and conventional synapses were found in the inner plexiform layer suggesting relatively normal associations between bipolar, amacrine and ganglion cells. Myelinated axons originated from the ganglion cells.

After removal of the transplants, the hosts were allowed to survive two days, after which their brains were processed by the Fink-Heimer technique and an adjacent slice of the superior colliculus (SC) was prepared for EM. Projections from the transplants were found only to nuclei in the host brain which are normally retino-recipient. Ultrastructural examination of the SC revealed degenerating terminals confined to the appropriate superficial layers of the SC. The terminals, like typical optic terminals, contained round vesicles and large pale mitochondria, and formed mainly axodendritic and some serial synapses.

To determine the cells of origin of the transplant projection, the SC in a number of host rats was injected with HRP. After an appropriate survival time, sections of the transplants were reacted using DAB. This revealed labeled cells in the ganglion cell layer as well as a few along the border of the inner nuclear layer of the transplant. Several of these cells were sufficiently filled with HRP to give a Golgi-like image. They had dendritic processes extending into the inner plexiform layer with many horizontal branches at several levels.

Since the retinal tissue used in this study was transplanted prior to differentiation of all but a few ganglion cells, these results suggest that fetal retinae have the ability to develop certain morphological characteristics even when transplanted to anomalous positions. Furthermore, the retinal transplant efferents not only enter the host SC, but also form synapses in it. (Supported by NIH EY 03414 and EY 03326)

278.4 ABNORMAL BRAIN DEVELOPMENT FOLLOWING FRONTAL OR TOTAL NEODECORTICATION IN INFANT RATS. Bryan Kolb, Arthur J. Nonneman, Robert Sutherland & Ian Q. Whishaw. Dept of Psychology, University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4

In the course of studying the behavior of rats with neocortical lesions as neonates we have noticed a number of abnormalities in brain development. This study examined the effect of bilateral or unilateral frontal or total neodecortications one year after surgery as infants or adults. Measurements were made of brain weight, external cerebral and cerebellar dimensions, neocortical thickness, and thalamic width. Note was also made of degeneration or distortion of subcortical structures.

Normal brains showed a consistent cerebral asymmetry. The right hemisphere was 5% longer and wider and 5-10% heavier than the left. This difference resulted primarily from a 5% advantage in neocortical thickness in the right hemisphere. This unexpected asymmetry had to be considered in the analysis of the operated cases and may be of significance to other investigators doing both behavioral and anatomical studies.

Rats with bilateral neonatal frontal lesions had 10-15% smaller brains than adult operates as measured by brain weight or external dimensions. Lesion size was equivalent, however, as brain weight in rats sacrificed following surgery was 90-95% of normal weight in each group. The chronic difference is accounted for by a 10% reduction in neocortical thickness and a 5% reduction in thalamic width as compared to adult operates. Cerebellar size and hippocampal thickness were equivalent in the two groups. Rats with unilateral neonatal frontal excisions had similar reductions in the hemisphere ipsilateral to the lesion but not in the hemisphere contralateral to the lesion. However, rats with unilateral neonatal neodecortication had a significant increase (10-20%) in neocortical thickness in the intact hemisphere.

Neocortical lesions in infancy thus alter subsequent brain development but the nature of the effect depends upon the size of the removal. These effects appear to be neocortical as neodecortication in infancy does not result in a significantly smaller brain than in adult operates. There are, however, abnormalities following neonatal decortication. In particular, the dentate gyrus may form several extra gyri and Ammon's horn is grossly deformed, the thalamus is distorted and many nuclei are unrecognizable, the caudate-putamen and globus pallidus suffer marked gliosis, nucleus accumbens and the septum are shrunken, and there are variable abnormalities in the midbrain tegmentum.

These data indicate substantial plasticity and variability in brain development following neocortical damage in infancy. The mechanisms that mediate these changes are as yet unknown.

278.5 RESTITUTION OF THE CENTRAL NERVOUS SYSTEM AFTER DESTRUCTIVE INJURY BY PRENATAL IRRADIATION IN RATS. Constance J. D'Amato and Samuel P. Hicks, Department of Pathology, University of Michigan, Ann Arbor, MI 48109.

Destructive effects of 150 or 200 R X-irradiation in fetal life in rats usually leads to malformation, but there is always some degree of restitution of the cell loss. Restitution after 150 R on the 10th (6-14 somite pairs) or 12th (26-36 somite pairs) fetal days is remarkable, the resulting animal often appearing to be normal. In mature rats irradiated with 150 R on the 12th day we have found only subtle abnormalities of corticospinal neurons, and diminution of the size of the spinal cord (D'Amato, Hicks 1980) and we are now studying the morphogenetic steps leading to the restitution. Various stages in the development of these rats from 6 hours after irradiation until maturity were studied in four litters. Successive intervals in fetal life were studied by removing members by "caesarean" section. Six hours after 150 R there was extensive necrosis of the G_1 and G_2 cells in the proliferative cell zone throughout the spinal cord, metencephalon and cerebral vesicle, with somewhat less destruction in the diencephalon and mesencephalon. Twenty four to 48 hours after irradiation the residual cells had proliferated and largely restored the cerebral mantle, diencephalon and metencephalon. Except for minute foci of rosette formation in the dorsal anterior and lateral mesencephalon and ventrally at the site of the future interpeduncular region, restoration of the mesencephalon approached normal. By 72 hours there was virtually no evidence of the earlier damage except for rare remnants of necrotic cells, and the minute rosette foci, which had not grown in size. In the spinal cord, as a rule, remnants of necrotic cells could still be seen at 72 hours. There was usually some central rosette formation in the cervical cord visible from 1 to 4 days after irradiation. Five or 9 days (21st fetal day) after irradiation the spinal cord was slightly smaller than normal in diameter, and the number of neurons in the ventral horns was substantially reduced, that is, the ventral horns were sparsely populated compared with normals. Evidence of the rosettes was a reduced or obliterated central canal and jumbling of neurons in the central gray region. The picture in adult animals corresponded to that seen in these fetal rats, but in some animals, there was no disorder of the central canal region, and the cord could not be distinguished from normal. (USPHS NS 10531)

278.6 THE DIFFERENTIAL NEURONAL RESPONSE TO PYRAMIDOTOMY IN THE DEVELOPING AND ADULT HAMSTER. T.E. Durica. Dept. Anat., Rush Coll. Hlth. Sci., Chicago, IL 60612.

The neuronal response to pyramidotomy was studied in the developing and adult hamster to determine if there was an age dependent response for intrinsic (pyramidal) neurons comparable to the response which has been described for developing extrinsic (facial motor) neurons of the hamster. Unilateral pyramidal tract lesions were made in the medulla, rostral to the pyramidal decussation, in 5, 10, 15 and 20 day old, and adult hamsters. The animals were sacrificed at 5 and 10 days postoperative (dpo) and processed for histological examination.

At 5 dpo, the pyramidal cells of the adult and 20 day old animals displayed a central chromatolysis, while the pyramidal cells of 15 and 10 day old animals had a more localized chromatolysis. In the 5 day old animals some pyramidal cells had a chromatolytic response typified by a rounding of the cell, paling of the Nissl substance, and an eccentrically placed nucleus; many of the remaining pyramidal cells were relatively normal in appearance, however, their Nissl substance was less coarse and paler than that of pyramidal cells of the control side (10 days of age).

At 10 dpo, the pyramidal cells of the adult and 20 day old animals continued to show a central chromatolytic response. In the 15 and 10 day old animals many of the cells still had the localized response, while some cells displayed a central chromatolytic response. In the 5 day old animals (at 10 dpo) some pyramidal cells had a response similar to that seen at 5 dpo, however, many of the cells appeared smaller and had less Nissl substance when compared to cells of the control side (15 days of age). It should be noted that these particular pyramidal cells of the operated side had an appearance similar to pyramidal cells of the normal 10 day old animal.

These findings have shown that at 10 dpo the chromatolytic response of pyramidal cells differs cytologically from that reported for facial motoneurons. Notably, the facial motor neurons of the 5 day old hamster do not survive axotomy, while the pyramidal cells not only survive but are apparently continuing to differentiate, though at a delayed rate. It has been reported by Kalil and Reh (Science, 225:2258, 1979) that these young neurons are able to regenerate their axons following pyramidotomy and establish connections in the spinal cord which may account for their particular histological appearance in this study.

278.7 THE EFFECTS OF PRENATAL X-IRRADIATION ON THE DEVELOPMENT OF THE CORPUS CALLOSUM AND THE SUPRAGRANULAR LAYERS OF THE NEOCORTEX. Karl F. Jensen. Lab. Developmental Neurobiology, Dept. Biological Sciences, Purdue Univ., West Lafayette, IN 47907

Neurons of layers II and III of the rat parietal cortex are produced primarily during embryonic days 18 thru 21 (Berry and Rogers '65, Raedler and Raedler '78). A single dose (200 R) of 250 KV x-rays applied at various times during this embryonic period prevents the development and reduces the depth of the supragranular layers with no appreciable reduction in the depth of the infragranular layers. The reduction in the depth of layers II and III decreases with embryonic age according to developmental gradients within the neocortex. On a given embryonic day the extent of the reduction of the supragranular layers decreases in a lateral (rhinal fissure) to medial gradient and an anterior (frontal) to posterior (occipital) gradient. The graduated interference with the late forming neurons of the supragranular layers is associated with the absence of or reduction in the corpus callosum. The pattern of reduction in the corpus callosum closely correlates with the developmental gradients observed in layers II and III. It is concluded that the cells of the supragranular layers are the major source of fibers of the corpus callosum.

278.8 EFFECTS OF MULTIPLE EXPOSURES TO INTENSE ACOUSTIC STIMULATION ON AUDIOGENIC SEIZURE (AGS) SUSCEPTIBILITY (S) AND INTENSITY (I) IN RATS. I. A DEVELOPMENTAL STUDY IN PROGENY FROM A GENETICALLY SUSCEPTIBLE COLONY. P.C. Jobe, R.D. Brown, J.W. Dailey, T.B. Ray*, T.W. Woods*, M.E. Mims* and S. Bairnsfather. VA Med. Center and Depts. of Pharmacol. and Psychiat., LSU Sch. Med., Shreveport, LA. 71130.

Sprague-Dawley derived progeny of the AGS S colony at the VA Med. Center in Shreveport, LA were utilized as experimental subjects. The sound stimulus which consisted of mixed tones was generated by two bells ringing simultaneously. The sound level obtained (115 dB) is sufficient to produce seizures in adult AGS S rats. Four groups of pups from AGS S parents were used. Beginning on day 13 after birth, 3 of these groups were exposed to the sound stimulus on the following schedules: group 1-once daily; group 2-once every 3 days; group 3-once every 6 days. The fourth group was exposed only at 22 days of age. Seizure I was assessed by determining an audiogenic response score (ARS) for each animal (0-9 on an ascending I scale, Jobe et al., JPET 184: 1-10, 1973). Seizure S was determined by recording the fraction (F/S) of rats which displayed an AGS (i.e., where ARS ≥ 1). As shown in the table, pups exposed to the sound stimulus at more frequent intervals became susceptible to AGS more rapidly and exhibited more severe seizures than those exposed at less frequent intervals. Also, the ARS for rats stimulated on a daily schedule reached a peak at day 22 and fell rather dramatically thereafter. Other data not reported in the table, show that in rats which are 40 days of age upon first exposure to the stimulus, the ARS does not significantly decrease as a result of multiple acoustic exposure. These results indicate that neuronal development in very young rats is altered by acoustic stimulation, by seizure activity or by acoustic stimulation plus seizure activity. Either these same neuronal alterations do not occur in more mature rats or they are masked by compensatory changes.

Group	Age of Rats in Days							
	13		19		22		25	
	F/S	ARS	F/S	ARS	F/S	ARS	F/S	ARS
1	0/13	0.00 ±0.00	11/12	2.3 ±0.3	12/12	5.83 ±0.98	12/12	2.75 ±0.25
2	0/13	0.00 ±0.00	not tested		12/13	3.62 ±0.91	13/13	3.31 ±0.75
3	1/13	0.08 ±0.08	6/13	1.1 ±0.4	not tested		9/13	2.54 ±0.58
4	not tested		not tested		9/21	1.33 ±0.47	not tested	

278.9 EFFECTS OF MULTIPLE EXPOSURES TO INTENSE ACOUSTIC STIMULATION ON AUDIOGENIC SEIZURE (AGS) SUSCEPTIBILITY (S) AND INTENSITY (I) IN RATS. II. A DEVELOPMENTAL STUDY IN STRAINS OF NONSUSCEPTIBLE RATS. R.D. Brown, P.C. Jobe, S. Bairnsfather, M.E. Mims* and T.W. Woods*. VA Med. Center and Depts. of Pharmacol. and Psychiat., LSU Sch. Med., Shreveport, LA. 71130.

Two types of rats were used for these investigations: (1) animals obtained directly from Sprague-Dawley, Inc.; and (2) animals obtained from the VA colony of rats which are selectively bred for non-susceptibility to AGS. (Adult rats obtained from Sprague-Dawley, Inc. are also nonsusceptible to AGS.) Type 1 (T-1) and type 2 (T-2) rats were subdivided into 4 groups (G), exposed to the sound stimulus and assessed for seizure I as described in the abstract of Jobe et al. in this volume. Pooled data from both types of animals are presented in the table. At 31 days of age, stimulation on the every 3 day schedule produced a level of S which was greater than that occurring in rats stimulated on the daily or on the every 6 day schedule. The rates of appearance of S in G-1 and G-2 were equivalent until after 28 days of age, at which point S continued to increase in G-2, whereas it showed a tendency to deteriorate in G-1. Responses of T-1 rats were similar to the pooled data reported in the table. However, T-1 responses differed from those of T-2 in two ways: (1) increases in S in T-2, G-1 did not appear to deteriorate after day 28 as they did in this G in T-1 rats; and (2) responses from G-3 were less dramatic in T-2 than in T-1 rats. In all G's in both types of animals, 44 of the 46 rats which became S exhibited an ARS = 1. These observations indicate that in very young rats multiple exposures to intense acoustic stimulation cause the appearance of S but do not alter seizure I. This is in sharp contrast to the results obtained in progeny from AGS susceptible parents where multiple exposures to the acoustic stimulus caused marked increases in seizure I (see the abstract of Jobe et al. in this volume).

Group	Age of Rats in Days							
	13		22		28		31	
	F/S	ARS	F/S	ARS	F/S	ARS	F/S	ARS
1	0/38	0.00 ±0.00	3/37	0.08 ±0.05	6/36	0.17 ±0.06	4/36	0.11 ±0.05
2	0/38	0.00 ±0.00	3/37	0.08 ±0.05	8/36	0.22 ±0.07	15/36	0.47 ±0.11
3	0/11	0.00 ±0.00	not tested		not tested		1/11	0.09 ±0.09
4	not tested		2/36	0.06 ±0.04	not tested		not tested	

278.10 MORPHOLOGIC DEVELOPMENT IN THE BRAINSTEM RETICULAR CORE IN REHABILITATED, PRENATALLY UNDERNOURISHED RATS. R. P. Hammer and E. van Marthens*. Dept. Anat. and Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Prenatal exposure of the rat fetus to undernutrition induced in the mother prior to and during the entire gestation period results in a delayed course of development of brainstem reticular cells of the neonatal rat (Hammer, 1980, Inter. Congr. of Anat. Abstr., in press). However, as these animals are rehabilitated on a normal maternal diet to 20 days, their brainstem neuronal milieu tends toward its normal configuration. Developmental status is assessed by comparing gradually decreasing numbers of protospiny appendages and dendritic varicosities upon dendrites in proximal and terminal regions of the reticular arbor. Quantitative measures of greatest dendritic extent, number of dendrities, branchedness, and somal size reflect the state of the neuropil into which these cells extend.

Comparison of these quantified parameters in control and experimental conditions demonstrates the effects of nutritional rehabilitation. A significant increase of proximal dendritic varicosities (nodules) is noted in the experimental group. This denotes that the course of development may be slightly delayed but has rehabilitated since the severely affected neonatal stage. The neuropil appears much more dense at this age since dendritic extent is now signficantly reduced in the experimental group. While numbers of primary dendrites remain the same in both groups, there is a suggestion of increased branchedness in the experimental group.

The obvious differences of dendritic morphology in the experimental group reflect variations in the neuropil as well as reactions of the rehabilitated cell to its surround. The slight reduction in dendritic extent may indicate resistance to dendritic growth by increased glial proliferation in the experimental brainstem while an increase in branchedness illustrates a compensatory effort to sustain dendritic elaboration by the neuron.

278.11 CEREBELLAR HISTOGENESIS DURING GRAFT VERSUS HOST DISEASE. E.N. Crom*, G.W. Miller*, and W.S.T. Griffin (SPON: R.S.A. Tindall), The Univ. Texas Hlth. Sci. Ctr., Dallas, Texas 75235.

A variety of stresses have been shown to alter cerebellar histogenesis. Graft versus host disease (GVHD), caused by grafting mature lymphocytes into an immature host, induces easily assessable alterations in cerebellar histogenesis without comprimising structural integrity. We have previously reported alterations in both cerebellar DNA synthesis and total accumulation of DNA during the progression of this neonatally-induced immunological disease, and this study was an investigation of events which could lead to these findings. Utilizing short pulses of 3H-thymidine to label DNA for assessing the rate of DNA synthesis, autoradiography, to determine the mitotic index, histological analysis of the ratios of cells in mitosis per germinal cell and the stage of mitosis of each mitotic figure, we showed that upon comparison of diseased animals with littermate controls the rate of DNA synthesis was slowed in diseased animals and there were fewer cells with silver grains above them in spite of the fact that there were equal numbers of germinal cells in the external granular layer and an equal number of mitotic figures in diseased and control animals. However, an analysis of the stage of mitosis of each mitotic figure revealed that significantly more mitotic figures in cerebella from animals with GVHD were in metaphase and the subsequent stages of mitosis. Preliminary electronmicroscopic studies of mitotic figures have failed to reveal significant alterations in spindle formation or the chromasomes. These findings suggest that the lesser accumulation of DNA overtime in cerebella from animals with GVHD is not due to a decrease in available DNA precursor nor is it due to a decrease in the number of germinal cells in the external granular layer, but rather it is due to an inability of mitotically active cells to proceed from metaphase anaphase and telophase into G_0 or G_1. We believe that the cerebellar effects due to GVHD can be generalized and will provide a model system for the study of histogenesis in other areas of the CNS as well as in a variety of other organs, both lymphoid and nonlymphoid.

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278.12 SELECTIVE DEPLETION OF SPINAL NOREPINEPHRINE IN THE NEONATAL RAT BY SYSTEMIC AND INTRASPINAL 6-HYDROXYDOPAMINE: EFFECTS ON THE POST-DECAPITATION REFLEX. R. M. INGS* and B. A. PAPPAS, Department of Psychology, Carleton University, Ottawa, Ontario, Canada. K1S 5B6

Neonatal 6-hydroxydopamine (NS-6-OHDA, sc., days 1 & 2) did not alter the levels of dopamine (DA) or serotonin in the thoracic spinal cords of Wistar rats aged 5,10,15,20,30, or 60 days. Norepiniphrine (NE) levels were reduced at ages >15 days when a precipitous decline from the control levels was observed. In NS-6-OHDA groups the latency of the post-decapitation reflex (PDR, normally around 1-3 sec.) was infinite at 5 and 60 days but was only attenuated at 15 through 30 days. The number of bilateral clonic kicks in these groups, although markedly attenuated, followed the inverted u-shaped profile of vehicle groups including a plateau from 15 to 20 days of age. A possible α-NE mediation of the temporary return of the PDR at 15 through 30 days in 6-OHDA treated rats was rejected since chlorpromazine, phenoxybenzamine or yohimbine administered to 17 day old rats did not further attenuate the PDR. A serotonin mediation of the PDR at this age was contraindicated by the absence of any effect of p-chlorophenylalanine.

Injection of 6-OHDA (10 ug) directly into the spinal cords (IS-6-OHDA) on the first two days of life selectively decreased NE at 35 days to 7,14, and 13 % of control levels in cervical, thoracic, and lumbar spinal cord segments respectively. The cortex plus hippocampus and cerebellum showed no alteration while brainstem levels were increased to 163% of control. This may be due to localized sprouting of NE terminals from the locus coeruleous when the spinal projections from this nucleus are destroyed. Serotonin levels were elevated in all areas but significant only in the thoracic cord segment. The PDR was completely eliminated by IS-6-OHDA at this age.

Pretreatment with desmethylimipramine eliminated the NE reduction, and protected against the loss of the PDR, but surprisingly did not result in DA depletions in any area. Increased brainstem DA levels however, suggested a stimulated local DA arborization process in some ways analogous to that observed after IS-6-OHDA alone.

278.13 BEHAVIORAL DEFICIT INDUCED IN ADULT RATS FOLLOWING PRENATAL ADMINISTRATION OF ANTIBODIES TO GANGLIOSIDE. Rapport, M.M. and Karpiak, S.E., (SPON: H. Barden). Div. of Neuroscience, N. Y. State Psychiatric Institute and the Depts. of Psychiatry and Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N. Y. 10032.

In previous experiments (1,2) rats receiving an injection at 5 days of age of antiserum to brain ganglioside (into the cisterna magna) showed at maturity impaired performance on a complex learning task (DRL), as well as chemical and morphological alterations in the somatosensory cerebral cortex. The contents of ganglioside sialic acid, galactocerebroside and RNA were decreased (with no loss in DNA), and changes in the number and configuration of oblique dendritic spines were seen. The animals otherwise developed normally and demonstrated typical patterns of behavior. No changes were observed in rats injected either with antiserum which had been absorbed with pure G_{M1} ganglioside (to remove specific antibodies) or saline. In order to determine if an earlier insult would induce similar or more pronounced behavioral alterations, pregnant rats (Day 19 of gestation) were injected intravenously either with antiserum to brain ganglioside or with control antiserum (absorbed with pure G_{M1}ganglioside) or saline. All rat litters developed normally. At 70 days of age, rats were tested on the same DRL paradigm as had been used in the earlier experiments with animals injected on postnatal day 5. The test design was based on progressively more difficult DRL learning schedules: 5,7,10 and 15 sec. delay sequences over a 40 day period. Rats injected prenatally with antiserum to ganglioside showed a significant learning deficit of the DRL behavior only at the DRL-15 sec. level ($p<.025$). Since rats injected at Day 5 had shown deficits at both the DRL-10 ($p<.05$) and DRL-15 sec. ($p<.01$) levels, the postnatal 5th day appears to be a more critical developmental period than the 19th embryonic day for immunologic insult with antibodies to G_{M1} ganglioside. This result is consistent with the hypothesis that interference with dendritic arborization is the mechanism by which antiganglioside antibodies inhibit development.

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1. Rapport, M.M., Karpiak, S.E., Kasarskis, E.J., Bass, N.H. (1979) Trans. Am. Soc. Neurochem. 10, 234.
2. Kasarskis, E.J., Karpiak, S.E., Rapport, M.M., Bass, N.H. (1979) Trans. Am. Soc. Neurochem. 10, 233.

278.14 ENRICHED PROTEIN INTAKE DURING EARLY BRAIN GROWTH OF ARTIFICIALLY REARED RATS. Jaime Diaz, Colleen Stamper*, Elizabeth Moore*, Jack Schacher*, and Frances Petracca*. Dept. of Psychology, University of Washington, Seattle, WA 98115.

Low protein intake during development may produce deficits in brain growth, even though the available protein will be shunted preferentially to the developing brain.

The artificial rearing procedure first introduced by Messer, et al. (J. Nutr., 98, 1969) permits the direct examination of dietary variables upon brain development. The milk formula used by Messer, et al. contains 5.5 grams% protein, whereas rat milk contains approximately 9 grams% protein (Dymza, et al., J. Nutr., 84, 1964). The purpose of the present study was to examine the effects of two formulas - the "Messer" formula and a formula containing 8.5 grams% protein - on animals artificially reared.

Four day old female Long-Evans hooded rat pups were matched by weight and assigned to one of two groups: 1) animals artificially reared with a formula containing 5.5 grams% protein (the diet used by Messer) or 2) animals artificially reared with a formula containing 8.5 grams% protein (the Messer formula with 3 grams% of casein hydrolysate added). All the animals were infused with the same amount of formula at the same rate. The animals were weighed each day, reflex tested, and the occurrence of developmental milestones was noted. On day 18, all the animals were tested in an open field and then sacrificed. Their brains were removed, dissected and weighed as were their livers, kidneys, and spleens.

The results indicate that by day 18 there was a significant difference in body weight between the two groups. Animals receiving the protein enriched diet were 11% larger ($p<.05$). This difference was also reflected in peripheral organ weights. For all brain parameters there were no differences between the groups. The ontogeny of reflexes and the occurrence of developmental milestones followed the same pattern. Significant differences were found in open field behavior between the two groups.

The daily weights of the animals fed formula with less protein matched those of their normally reared siblings. Although the body weights of animals fed the higher protein formula were greater than those of animals fed the lower protein formula, the brain weights of the animals in the higher protein group matched those of the animals in the lower protein group. Nevertheless, the significant differences in the behavior of the two groups in the open field suggest that the added protein in the formula did exert an influence on brain development which may be too subtle to detect by gross tissue weights.

278.15 THE EFFECTS OF NERVE GROWTH FACTOR (NGF) AND OF ANTI NGF-ANTIBODIES ON THE DEVELOPMENT OF SUBSTANCE P-CONTAINING SENSORY NEURONS. U. Otten, M. Goedert*, N. Mayer* and F. Lembeck*. Department of Pharmacology, Biocentre of the University, Basel, Switzerland and Department of Experimental and Clinical Pharmacology, University of Graz, Austria.

The investigation of the physiological importance of NGF for the development of sensory neurons has been limited so far by the absence of specific biochemical marker substances for these neurons. The recent demonstration that the undecapeptide substance P is present in sensory neurons suggests that it might be such a marker. Substance P is synthesized in dorsal root ganglia and transported to the terminals of C-fibres located in the dorsal horn of the spinal cord and in the skin. In the present study the effects of NGF and of purified anti NGF-antibodies on the content of substance P in rat dorsal root ganglia and in their respective target organs, spinal cord and skin were examined. The effects on tyrosine hydroxylase activity in sympathetic superior cervical ganglia were included as a control for the effectiveness of both NGF and its antibody.

Repeated injections of NGF led to an increase in substance P in rat spinal ganglia (Th 10-12) from 131±20 to 220±14 pg substance P/ganglion. The administration of anti NGF-antibodies caused a gradual decrease in substance P up to 17 days after injection. This reduction was paralleled by a decrease in substance P content of both the spinal cord and the skin. Neither NGF, nor its antibody had any significant effect on the protein content of dorsal root ganglia.

The total tyrosine hydroxylase activity in superior cervical ganglia showed a 3-fold increase after repeated administration of NGF, whereas the injection of anti NGF-antibodies produced an almost complete disappearance of tyrosine hydroxylase activity, thus indicating optimal effects of NGF and of its antibody on sympathetic ganglia.

Our results indicate that postnatal sensory neurons are responsive to NGF and that they require NGF or a cross-reacting NGF-like molecule for their normal development as evidenced by the decrease in substance P content after the administration of anti NGF-antibodies to newborn animals.

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278.16 NEURONAL DEATH IN NORMAL AND NGF-TREATED SPINAL GANGLIA. V. Hamburger, J. Brunso-Bechtold and J. Yip. Dept. of Biology, Washington University, St. Louis, MO 63130.

We have established well-defined periods of neuronal death for the two populations of neurons in thoracic dorsal root ganglion 18 of the chick embryo, by counting clearly degenerating neurons in every other section. In the population of large, early differentiating ventro-lateral neurons (VL), the period extends from stages 25 to 33 (4½ - 7½ days), with a peak at stage 27. Degeneration in the small, late-differentiating dorso-medial neurons (DM) extends from stage 34 to stage 36 (8-10 days), with a peak at stages 34 and 35.

To test the effect of Nerve Growth Factor (NGF) on survival of neurons, we made daily injections of 6 µg of NGF into the yolk sac, from stage 21 (3½ days) to the day preceding sacrifice. Degeneration in the VL neuron population was reduced by at least 50%; degeneration in the DM neurons was prevented, except for a small amount of cell death toward the end of the degeneration period.

Preliminary data for brachial ganglion 15 indicates similar periods of degeneration for VL and DM populations, except that the degree of neuronal loss of LV cells is considerably lower than in ganglion 18. NGF injections, following the same protocol as above, had the same effects.

These results show that NGF has a trophic maintenance effect on neurons of embryonic spinal ganglia in vivo. This is the first demonstration that VL cells respond to NGF.

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278.17 ANTI-ISCHEMIC EFFECTS OF PENTOBARBITAL IN CATS-CEREBROPROTECTION AFTER 6 AND 72 HOUR ADMINISTRATIONS. J. P. Hansen* and W. J. Giardina. Department of Pharmacology, Abbott Laboratories, North Chicago, IL 60064.

The protective effects of pentobarbital against cerebral ischemia in animals have been widely reported. (Michenfelder, et al., Archives of Neurology, 33:345, 1976; Moseley, et al., Neurology, 25:870, 1975). For the purpose of studying peak time of pentobarbital's cerebroprotective action, regional cerebral ischemia was produced in cats. Cats were anesthetized with ketamine HCl (15 mg/kg/i.m.) and pentobarbital (5 mg/kg/i.v.). The left middle cerebral artery (LMCA) was occluded by electrocoagulation using a transorbital approach. In the first experiment, 3 sham operated cats and 4 cats with LMCA occlusion were sacrificed 72 hours after surgery. In the second experiment, pentobarbital (90 mg/kg) [3 cats] or saline (36 ml) [3 cats] was administered parenterally in divided injections over 72 hours. Administration began 30 min. after occlusion, and cats were sacrificed at 72 hours. In the third experiment, pentobarbital (20 mg/kg) [3 cats] or saline (8 ml) [3 cats] was administered parenterally in divided injections over 6 hours. Administration began 30 min. after occlusion of the LMCA, and cats were sacrificed at 48 hours. Neurological examinations were made at 1, 3, 24 and 48 hours after LMCA occlusion. Five min. before sacrifice, anesthetized cats were injected i.v. with sodium fluorescein (10% solution). Cats were then perfused with normal saline and buffered formalin. Brains were sectioned in situ at sterotaxic coordinates AP +19, +14, +9, +4 and -1. The anterior surface of each section was photographed under ultraviolet light. The area of induced fluorescence for each section was measured with a planimeter. Planimetry was also used to measure the increase in the size of the affected hemisphere caused by edema. Light microscopy was used to examine the anterior surface of each tissue section for ischemia-induced damage. Following LMCA occlusion, cats developed hemiplegia. They showed marked curvature of the torso, and when able to walk, circled to the left. Cats receiving pentobarbital for 6 or 72 hours showed less ischemia-induced injury to brain tissue than corresponding saline treated controls. Ischemia-induced injury to brain tissue in the 6 and 72 hour treatment groups was similar. These data suggest that the cerebroprotective effects of pentobarbital against ischemia-induced cellular insult occurred within the first few hours after its administration. Prolonged administration of large doses of pentobarbital may not be required to achieve a cerebroprotective action.

278.18 NEW GERBIL STROKE MODEL. Charles J. Hannan, Jr. Clinical Investigation Service, Eisenhower Army Medical Center, Fort Gordon, GA 30905.

Variability in occurrence and extent of cerebral infarction with the unilateral carotid occlusion version of the gerbil stroke model is a serious limitation of the method. It was hypothesized that unilateral infarction could be more consistently obtained by limiting the reactive hyperemia through the contralateral carotid artery. It was observed in gerbils, which had both carotids exposed, that unilateral occlusion invariably resulted in distension of the patent carotid artery. Presumably, the animals with sufficient anterior communicating artery capacity would avoid infarction and those with limited interhemispheric blood flow capacity would develop infarction and probably die within 3 days. In an attempt to control for this variability a modified Ligaclip® (Ethicon brand small tantalum ligating clip, LC-100) was used to restrict, but not prevent, blood flow in the patent right common carotid artery of gerbils that had their left common carotid artery completely occluded. To prevent complete closure of the ligating clip, an approximately 1 mm long segment of a 30 gauge needle was epoxied to the inner surface. Nineteen male retired breeder Mongolian gerbils (70-102 grams) were prepared as described above while under ketamine anesthesia (100 mg/kg,ip). The totally occluded left carotid artery was clamped with a standard small ligating clip and the artery cauterized distal to the occlusion.

Mortality data was compiled for five days after which survivors were perfusion-fixed with buffered formalin. Horizontal sections of brain were histologically examined. All modified ligation clips were removed and measured under a microscope. Results are summarized below:

		Modified Clip Width (mm)		Gerbil Weight (gms)	
Day 5	n	Mean ± SD	Range	Mean ± SD	Range
Died	9	.20±.03	.15-.22	80.9±8.9	70-89
Survived	10	.24±.03	.20-.28	85.4±10.7	72-102

One-way analysis of variance reveals a significant difference ($p<.01$) between the clip widths of animals which died and those which survived. There was no significant difference in weight between these groups before the occlusion was induced. The mortality (47%)was higher in these animals than in other retired breeders given only a unilateral occlusion (unreported data) and points to the usefulness of this model, however greater uniformity in the width of the flow-restricting clip must be attained.

279.1 TRANSFER OF MICROPEROXIDASE ACROSS CEREBRAL ARTERIOLES IN THE MOUSE UNDER NORMAL CONDITIONS. E. Westergaard. Anatomy Department C, University of Copenhagen, Copenhagen, Denmark.

From previous light microscopical and fluorescens microscopical studies it has been concluded that there is a blood-brain barrier (BBB) to intravenously injected Evans blue, trypan blue and Na-fluorescein. However, the possibility exists that small amounts, not visible because of the methods' limitations, in fact crossed the BBB. The mentioned dyestuffs bind to albumin in the blood. Later horseradish peroxidase (HRP) was used as tracer, and it was demonstrated by electron microscopical investigations, that a few, short segments of arterioles transferred HRP across the endothelium. HRP (MW: 40,000) does not bind to albumin, and it is likely that the mechanism underlying the passage is vesicular transfer. Other tracers have been used, e.g. microperoxidase (MP, MW: 1,900). It has been observed that MP, like HRP, is transported across the endothelium in short arteriolar segments (15-30 μ in diameter). No sign of interendothelial movement was obtained. The tight junctions might have functioned as a barrier to this small molecule. Some of the injected MP bind to albumin, but part of the MP circulated freely in the blood stream. It is also concluded for MP, that the tracer might have been transferred by vesicles across the endothelium in a few segments of small arterioles of the brain. However, it was not possible to determine whether or not the amount of MP, that crossed the endothelium was bound to albumin. There was no sign of intercellular movement.

279.2 MEASUREMENT OF BRAIN GLUCOSE UTILIZATION WITH THE CAROTID INJECTION TECHNIQUE. W.M. Pardridge, P.D. Crane*, L.D. Braun*, and W.H. Oldendorf*. UCLA School of Medicine, Los Angeles, Ca. 90024

The carotid injection technique, used previously to study the kinetics of blood-brain barrier glucose transport, may be modified to quantitate the rate of cerebral glucose utilization in individual rats. An approximately 200 uL bolus of Ringer's solution containing 2-^{14}C-glucose and ^{3}H-methylglucose (M) was rapidly injected via a common carotid artery in barbiturate-anesthetized rats. At various times up to 4 min after injection, circulation was terminated by microwave irradiation. Based on a 2-compartment model, the following relationships were derived,

$$\ln G_F/M = \ln G_F^o/M^o - k_3 t \quad (1)$$

$$G_T/M = \left(\frac{k_2}{k_2 + k_3}\right) e^{-k_3 t} + \left(\frac{k_3}{k_2 + k_3}\right) e^{k_2 t} \quad (2)$$

where k_2 = the rate constant of glucose or methylgluocse efflux from brain to blood, k_3 = the rate constant of glucose phosphorylation, t = the time after carotid injection, G_F/M = the ratio of ^{14}C/^{3}H dpm in the post-column neutral fraction divided by the ^{14}C/^{3}H dpm ratio in the injection solution (the superscript refers to zero time) and G_T/M = the ratio of ^{14}C/^{3}H dpm in brain without chromatographic separation divided by the same ratio in the injection solution. The use of model (2) is based on the assumption that no loss of brain ^{14}C-radioactivity occurs due to efflux of labeled metabolites of 2-^{14}C-glucose over short experimental periods. This latter assumption was tested by a third model,

$$G_T/G_F = \left(\frac{k_2}{k_2 + k_3}\right) + \left(\frac{k_3}{k_2 + k_3}\right) e^{(k_2 + k_3)t} \quad (3)$$

where G_T/G_F = the ratio of total homogenate ^{14}C dpm to ^{14}C dpm in the post-column neutral fraction. The G_T/G_F ratios at 0.25-4.0 min after carotid injection were fitted to equation (3); these results indicated k_2 = 0.14 ± 0.08 min^{-1} and k_3 = 0.21 ± 0.02 min^{-1} (mean ± SD). The plot of ln G_F/M vs time (equation 1) was linear (r = 0.99) with a slope, k_3 = 0.21 min^{-1}. The rate of brain glycolysis = (brain glucose concentration) x (k_3) = (2.6 umol/g) x (0.21 min^{-1}) = 0.55 umol/min/g for cortical tissue in the barbiturate-anesthetized rat. Conclusions: a) The identical estimates of k_3 as determined by either equation (1) or (3) supports the validity of the assumption of negligible loss of radioactivity from 2-^{14}C-glucose metabolites. b) Blood-brain barrier transport and brain glucose metabolism may be studied with the carotid injection technique using a ^{3}H-methylglucose internal reference.

279.3 SEX AND DEVELOPMENTAL EFFECTS ON GLUCOSE UPTAKE IN CEREBRAL MICROVESSELS. K. M. A. Welch. Dept. of Neurology, Baylor Coll. of Med., Houston, TX 77030.

Experimental studies of brain glucose uptake in vivo have shown clear developmental and sex differences (Daniel et al., J. Physiol.274:141-148,1978). The experiments reported here have examined the influence of development and sex on glucose uptake into the endothelial component of the blood-brain barrier.

Microvessels were isolated by albumin flotation and glass bead filtration (Goldstein et al., J. Neurochem.28:723-725,1977) from the brains of rats of 3 week (early development) and 9 month (adult) age. In vitro 2-deoxy-D-glucose (2-DG) uptake into the isolates was studied. 2-DG uptake was higher in 9-month old rats than in 3-week old rats. Further, in 3-week old rats, 2-DG uptake was higher in females than in males.

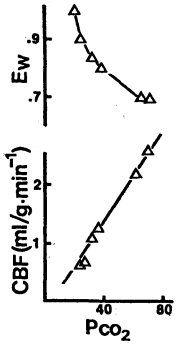
The increasing uptake of glucose with age is consistent with increasing cerebral energy demand as these animals reach their adult prime. Further, it is suggested that the significant difference occurring between the sexes for the 3-week old animals is an indication of the differential rate of maturing for males vs. females, that the blood-brain barrier reflects this differential rate, and that this barrier has not yet reached maturity in the males. There may be a "pivot-point" age for each sex after which the blood-brain barrier is complete.

(Supported by a grant from the Epilepsy Foundation of America.)

279.4 SIMULTANEOUS MEASUREMENT OF BRAIN PERMEABILITY AND BLOOD FLOW IN THE RAT. G. Irwin* and S. Preskorn (SPON: C. Hughes). Depts. of Psychiatry and Clinical Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103.

Tracer techniques have been used extensively to evaluate cerebral microcirculation. These techniques measure the uptake of a bolus of radionuclide-labeled test substance relative to uptake of a reference tracer during a single transit through brain vasculature. Results are routinely expressed as brain uptake index (BUI) or extraction fraction (E). Interpretation of such results is confounded by dependence of these measures on factors other than capillary permeability. As shown by Renkin (Am. J. Physiol., 1959), uptake during a single vascular transit (E) is related to the permeability coefficient (P), capillary surface area (S), and blood flow (F) as defined by the equation: ln (1-E) = -PS/F. Alteration in E may reflect changes in F as well as changes in PS. For this reason, simultaneous measurement of E and cerebral blood flow (CBF) as accomplished by the ^{15}O method of Raichle and co-workers (Amer. J. Physiol., 1976) is highly desirable. However, need for extensive support equipment (e.g., a linear accelerator) render this method impractical for most investigators. A simple small-animal procedure by which E and CBF might be measured is thus needed.

In the present study, Ew--the cerebral extraction fraction for water--was determined by administering i.v. a tracer solution of ^{3}H-water and ^{14}C-butanol as previously described (Preskorn et al., JPET, 1980). CBF was measured by comparing brain uptake of ^{14}C-butanol with the quantity of ^{14}C-butanol in an arterial blood sample withdrawn at a known, uniform rate. This measure of CBF was validated by comparing results with CBF measured using ^{141}Ce-labeled microspheres. The response of Ew and CBF to altered arterial P CO_2 is shown below. Our measures of Ew and CBF were also shown to be sensitive to amitriptyline, an antidepressant known to effect cerebral microcirculation (Preskorn and Hartman, Biol. Psych., 1979). (Supported by USPHS grants GM 15956 and MH 27201).



279.5 INCREASED LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) FOLLOWING OPENING OF THE BLOOD-BRAIN BARRIER BY HYPERTONIC ARABINOSE INFUSION AND BY ACUTE HYPERTENSION. S.I. Rapoport, W.R. Fredericks*, D. Dow-Edwards, E.D. London. Lab. of Neurosciences, National Inst. on Aging, GRC, Baltimore City Hosp., Baltimore, MD 21224.

The blood-brain barrier (BBB) at the continuous cerebrovascular endothelium can be made permeable by infusing a hypertonic solution of arabinose or mannitol into the carotid circulation (Rapoport et al., Am. J. Physiol., in press). Osmotic BBB opening transiently increases brain uptake of ^{14}C-2-deoxy-D-glucose (Spatz et al., Neuropath. Appl. Neurobiol. 2, 53, 1976) and increases local cerebral glucose utilization (LCGU) (Pappius et al., Ann. Neurol., 5, 211, 1979), as measured by the method of Sokoloff et al. (J. Neurochem. 28, 897, 1977). We wanted to know if the increased LCGU following BBB opening is influenced by the stimulation of central α- or β-adrenergic receptors, or if the increase is due to entry of K^+ (from a high plasma concentration to a low brain extracellular concentration) which could stimulate neuronal activity. We also wanted to know if LCGU rises following hypertensive BBB opening.

In conscious rats, the BBB was osmotically opened on the ipsilateral hemisphere by retrograde infusion of 1.8 molal arabinose for 30 sec. into the external carotid artery at a rate of 0.1 ml/sec. 0.35 ml of 2% Evans blue albumin was injected i.v. 5 min before arabinose, and served as an indicator of the degree of BBB opening. Each animal treated in this way was in one of 4 groups: control, propranolol (β-adrenergic blocker, 5 mg/kg i.v. 5 min before and 2.5 mg/kg 25 min after arabinose), phenoxybenzamine (α-adrenergic blocker, 40 mg/kg i.p. 15 min before arabinose), or maintained on a potassium-free diet to reduce plasma K^+ below brain extracellular K^+.

^{14}C-2-deoxy-D-glucose was injected i.v. 15 min after arabinose, and LCGU was estimated by comparing optical densities in autoradiographs of perfused and contralateral cerebral hemispheres. In other rats, the BBB in one cerebral hemisphere was opened by acute hypertension. Isotonic saline was infused into the common carotid artery for 10 sec at a rate of 0.4 ml/sec in a cephalad direction after ligating the external carotid artery (Rapoport, Exp. Neurol., 52, 467, 1976). LCGU was elevated not only in the ipsilateral hemisphere which was stained blue, but also in the contralateral hemisphere. The results indicate that BBB opening, whether by osmotic infusion or by hypertension, is followed by an increased LCGU. The increase is not mediated by α- or β-adrenergic receptors, and is not due to entry of K^+ from plasma to brain. At present, the cause of the increased LCGU is not known.

279.6 IMPAIRED CEREBROSPINAL FLUID ABSORPTION IN THE DEVELOPING RABBIT FOLLOWING EXPOSURE TO LEAD. J. D. Mann and S. L. Cookson*. Dept. Neurology, U. North Carolina, Chapel Hill, N.C. 27514.

Lead intoxication in developing animals is associated with pericapillary hemorrhages, brain edema and increased intracranial pressure. Since bulk absorption of cerebrospinal fluid (CSF) into the venous circulation is a critical part of intracranial pressure regulation, an increase in resistance to absorption would contribute significantly to the development of cerebral edema in association with exposure to lead. Transport of CSF through endothelial cells covering the arachnoid villi is the final process in CSF absorption. In the present study, we have tested the hypothesis that lead, an endothelial cell toxin, interferes with endothelial cell transport of CSF at the arachnoid villi, resulting in impaired CSF absorption.

Intracranial pressure/volume regulation and CSF dynamics were assessed under halothane anesthesia in groups of albino rabbits at 10, 22 and 35 days of age. Lead acetate was administered orally in doses of 3.5, 10.0 and 165.0 mg/day respectively for five consecutive days prior to testing. Controls were give sodium acetate in equimolar (acetate) amounts. Brain lead levels were 0.316 ± 0.024 ug/g (S.D.) for lead intoxicated animals compared to 0.023 ± 0.008 ug/g for controls. Assessment of intracranial fluid dynamics was carried out using a constant flow, manometric infusion technique with continuous monitoring of intracranial pressure (Mann et al., Ann. Neurol. 3:156-165, 1978). Data were analyzed for resistance to CSF absorption, rate of CSF formation, and intracranial compliance.

Changes reflecting maturation of the CSF bulk flow system were noted in the control animals. With development there was a progressive fall in resting intracranial pressure, a rise in CSF formation rate, and a marked improvement in CSF absorption at higher intracranial pressures. Resting intracranial pressure was significantly elevated in lead exposed animals compared to age-matched controls in all three groups ($p<.01$). Brain water content was not increased and CSF formation was unchanged compared to controls. Resistance to CSF absorption was increased in all lead exposed animals at intracranial pressures above 200 mm H_2O, reflecting a disturbance in high pressure, high volume CSF absorption. This finding was particularly noteable in the 10 day old animals where resistance to absorption was increased by more than 40% at pressures of 300 mm H_2O. Our results indicate that lead induced impairment of brain barrier systems includes mechanisms for bulk removal of CSF from the intracranial compartment, independent of the development of brain edema. Results also support the concept of lead as an endothelial cell toxin in the venous portion of the cerebrovascular tree, adversely affecting endothelial cell regulation of CSF absorption.

279.7 GRAFTED BRAIN TISSUE ELICITS BLOOD-BRAIN BARRIER CHARACTERISTICS IN MESENTERIC BLOOD VESSELS. P.A. Stewart and M.J. Wiley*. Dept. of Anatomy, Univ. of Toronto, Toronto, Ont. Canada M5S 1A8

Brain capillaries have structural and functional characteristics that constitute a regulatory interface or "barrier" between the blood and the brain. Because the endothelial cells of the brain capillaries are interconnected by tight junctions, blood-bourne solutes cannot diffuse between them, and so must pass through them to enter the brain. The endothelial cells contain specific transport systems that facilitate the passage of some molecules and degradative enzymes that inhibit or prevent the passage of others. Avian brain capillaries are characterized structurally by tight junctions, and histochemically by alkaline phosphatase and cholinesterase activity. We have found that the density of mitochondria is higher in avian brain capillaries than in capillaries elsewhere in the body, as has been described in other species.

We questioned whether these "barrier" characteristics develop only in brain capillaries or whether capillaries elsewhere in the body could form a barrier if they were made to vascularize grafted brain tissue. A major obstacle in this approach is that when embryonic tissues are transplanted to an ectopic site, their own blood vessels survive and form a part of the new vascular system. This has made the results of previous experiments difficult to interpret. We overcame this problem by transplanting fragments of early embryonic quail brain that had not yet been vascularized into the coelomic cavity of 3-day host chick embryos. The grafts grew and differentiated and were vascularized by the chick mesenteric vessels. The distinctive morphology of the quail nucleus allowed us to demonstrate unequivocally that the capillaries in these grafts are of host origin.

After 2½ weeks of growth the graft capillary endothelial cells were connected by tight junctions and contained a high density of mitochondria and few pinocytotic vesicles. Both alkaline phosphatase and cholinesterase activity could be demonstrated histochemically in the capillaries. A trypan blue solution injected intramuscularly into the host embryo was excluded from the graft tissue. All of these characteristics were absent in the capillaries of the adjacent gut and mesentery.

These results indicate that during normal embryogenesis the development of barrier characteristics in the in-growing capillaries may be elicited by the brain tissue.

279.8 RELATIVE PERMEABILITY OF THE BLOOD-BRAIN AND BLOOD-CSF BARRIERS TO ^{22}Na AND ^{36}Cl. Quentin R. Smith, Conrad E. Johanson, and Dixon M. Woodbury. Dept. Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah 84132.

Though radioactive ion movement across the brain capillaries (blood-brain barrier) has been investigated extensively in the past, few experiments have examined the role of the choroid plexus epithelium (blood-CSF barrier) in ion movement into the CNS. At present, the concept of a high resistance capillary endothelium in parallel with a low resistance choroidal epithelium has evolved. However, the net influx into the CNS would move largely across the blood-brain barrier due to the greater total surface area of the brain capillaries. To test this hypothesis, we conducted the following experiments.

Sprague-Dawley albino rats (90-110 g) were bilaterally nephrectomized under ether anesthesia 4 hr prior to sacrifice. At 1/12, 1/6, 1/4, 1/2, 1, 2, 4, 6, or 8 hr before death, each animal was injected with 0.1 μCi/g of ^{22}Na or ^{36}Cl (0.9% NaCl). Samples from the CNS (cerebral cortex, cerebellum, lateral ventricle choroid plexus (LVCP), fourth ventricle choroid plexus (4VCP), and cisternal CSF) and the periphery (plasma, blood, skeletal muscle, and submaxillary salivary gland) were assayed for radioactivity. Values were expressed as a space (%) = 100 x (dpm/g tissue or CSF)/(dpm/g extracellular fluid H_2O). Uptake curves for the CNS regions were resolved into components by graphical analysis to obtain rate constants and volumes of distribution for the components. Lastly, rate constants were converted to permeability-surface area products (PA), which can be directly related to permeability when the corresponding surface-areas are known.

Cerebral cortex PA values for ^{22}Na and ^{36}Cl agree well with previously published estimates, $PA_{Na} = 0.11$ and $PA_{Cl} = 0.09$ g extracellular fluid/hr/g tissue. For the cerebellum, $PA_{Na} = 0.16$ and $PA_{Cl} = 0.14$, 50% greater than for the cerebral cortex. As predicted by the hypothesis, PA values for the choroid plexuses were up to 3 times larger than those for the brain; LVCP $PA_{Na} = 0.10$ and $PA_{Cl} = 0.31$, 4VCP $PA_{Na} = 0.25$ and $PA_{Cl} = 0.23$. Literature estimates of surface area/g tissue for the capillaries of the cerebral cortex (100-240 cm^2/g) and for the basolateral membranes of the choroid plexus (112-180 cm^2/g) are approximately equal. However, when the mass of the rat brain (1.2 g) is compared to that of the choroid plexuses (0.005 g), the net radioisotope influx into the CNS would be largely associated with movement across the blood-brain barrier. (This work was supported by NIH grants NS 13988, GM 07579, and AM 20935.)

279.9 CHANGES IN THE CONCENTRATION OF BICARBONATE AND HYDROGEN ION IN THE IN VITRO CHOROID PLEXUS EPITHELIUM IN RESPONSE TO ALTERATION IN CEREBROSPINAL FLUID BICARBONATE AND pH. Z. Parandoosh* and C.E. Johanson. Dept. Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah 84132.

There is considerable evidence that the choroid plexus (CP) has an active role in cerebrospinal fluid (CSF) pH regulation. However, the mechanisms of H/HCO_3 transport in the CP are not well understood. To quantify changes in choroid cell [H] and [HCO_3] in response to alterations in the CSF concentration of these ions, we utilized an in vitro CP preparation and the ^{14}C-DMO (dimethyloxazoladinedione) method to indicate cell pH.

Choroid plexuses from the lateral and fourth ventricles in adult Sprague-Dawley rats were pooled for incubation in a synthetic CSF medium. Each of the 28 incubations was carried out for 30 min at 37° C. CSF [HCO_3] was varied over a range of 10 to 52 mM/L by displacing NaCl in the medium with $NaHCO_3$; thus, for each incubation CSF [Na] and osmolarity were constant at 141 mM/L and 290 mOsm/L, respectively. Bath pCO_2 was held as constant as possible between 20 and 30 torr. After each experiment measurements were made of CP H_2O content and ^3H-inulin space (for extracellular fluid volume), and of CSF pH and pCO_2. CP pH was calculated from the steady-state distribution of ^{14}C-DMO between tissue and medium.

There was an inverse relationship between CSF [HCO_3] (range 10 to 52 mM/L) and both the CP H_2O content (range 82 to 78%) and the CP ^3H-inulin space (range 42 to 35%). At CSF HCO_3 concentrations of 10, 18 and 52, respectively, the choroid cell [HCO_3] was 2, 7 and 19 mM/L H_2O; corresponding values for cell pH were 6.51 + .02 (SEM), 6.98 + .04 and 7.42 + .03. Thus, the Δchoroid cell [HCO_3]/Δ CSF [HCO_3] was approximately 0.4, similar to the corresponding value of 0.35 for in vitro renal tubule cells (Struyvenberg et al., 1968). The relationship between internal and external acidity can be described by a straight line, $y = -56 + 4.4x$ ($r = 0.92$), where y and x are [H] in CP and CSF, respectively. Thus, the slope of Δchoroid cell [H]/Δ CSF [H] is approximately an order of magnitude greater than that for renal tubule cells. The substantial increase in choroid cell [H] from 39 to 309 nM/L as the medium (CSF) [H] is increased from 16 to 78 nM/L suggests that there is a sensitive homeostatic transport mechanism in CP for removing H ion from the CSF as this fluid becomes more acidic. (Supported by NINCDS grant #NS 13988.)

279.10 A NEW TECHNIQUE TO EVALUATE REGIONAL BLOOD-BRAIN BARRIER PERMEABILITY TO ZINC. Scott A. Burton* and C.E. Johanson. (SPON: S. Turkanis) Univ. of Utah Col. of Med., Salt Lake City, Utah 84132.

Zinc has been associated with a number of CNS disorders; high zinc serum levels with epilepsy and zinc deficiency with depression, confusion, and dementia. Zinc uptake was measured by a single-injection method. Isotonic NaCl (0.2 ml, pH 7.4) containing $^{65}ZnCl_2$ and ^{14}C-iodoantipyrine was injected (< 0.4 sec) into the inferior vena cava of etherized Sprague-Dawley rats. In contrast to the Oldendorf technique, this method permits uptake studies in infant rats and insures that all areas of the CNS are adequately perfused with the injectate. 30 sec after injection, animals were decapitated and the brains rapidly removed. The brain uptake ratio (BUR) of Zn was defined as:

$$BUR = \frac{DPM\ ^{65}Zn(tissue)/DPM\ ^{14}C\text{-iodoantipyrine(tissue)}}{DPM\ ^{65}Zn(injectate)/DPM\ ^{14}C\text{-iodoantipyrine(injectate)}}$$

Zn BUR was studied in the fourth ventricle choroid plexus (4CP), cerebellum (CER), medulla (MED), cerebral cortex (CC), olfactory bulb (OB), and hippocampus (HIPP) in adult rats (I), in 1-wk old rats (II), in adult rats using pentobarbital instead of ether (III), and in adult rats substituting antipyrine for iodoantipyrine (IV). BUI values (V) were obtained with adult rats using the Oldendorf technique. (VI) is the ratio of the 2-hr uptake (DPM ^{65}Zn/g tissue ÷ DPM ^{65}Zn/g plasma H_2O) after I.V. zinc injection in adult rats to the BUR values of group I. Means + SEM for N = 4 are given below:

	I	II	III	IV	V	VI
4CP	0.60(.04)	0.34(.08)	0.81(.08)	0.62(.04)	0.58(.07)	4.7
CER	0.14(.01)	0.14(.03)	0.14(.02)	0.17(.01)	0.09(.02)	2.4
MED	0.11(.01)	0.11(.03)	0.08(.01)	0.12(.01)	0.14(.01)	3.2
CC	0.12(.01)	0.09(.02)	0.12(.02)	0.13(.01)	0.05(.001)	2.9
OB	0.22(.01)	0.11(.02)	0.23(.01)	0.21(.02)	0.06(.005)	1.6
HIPP	0.08(.01)	0.09(.02)	0.08(.01)	0.11(.01)	0.04(.004)	3.0

In adult controls (I), Zn BUR was similar in all regions except in the 4CP and OB where it was 2-4X greater; a similar relationship among BUR values was found in infant animals (II). Except for 4CP, BUR values in pentobarbital-anesthetized animals were comparable to those in etherized rats. There was no considerable difference between BUR values obtained using either antipyrine or iodoantipyrine as the internal standard. A comparison of group V with group III reveals that BUI values were disproportionally higher than BUR values in the 4CP, CER, and MED; these differences occur since solutes injected into the carotid artery do not adequately perfuse these areas. The similarity among the ratio values in group VI shows that there is a constant correlation between Zn BUR and 2-hr Zn spaces in all CNS regions. (Supported by NIH grants NS 13988 and GM 07579.

279.11 QUANTITATION OF VASOGENIC CEREBRAL EDEMA BY NUCLEAR MAGNETIC RESONANCE ANALYSIS. Andrew M. Rudin*, J. S. Schneider, M. J. Glucksman* and David M. Kramer*. (SPON: N. Peress). Depts of Chemistry and Psychology, SUNY at Stony Brook, N. Y.

There exist measurable differences in proton nuclear magnetic resonance spin lattice relaxation times (T_1) of various tissues and between normal and diseased states of the same tissue. The T_1 differences appear, in general, to be related to differences in water content. NMR zeugmatographic imaging, a non-invasive technique, may be used to measure all these differences and generate two or three dimensional images which may be useful for detecting various disease states. Since vasogenic cerebral edema results in localized increases in tissue water content, we have chosen to assess the potential of water proton NMR analysis to identify regions of cerebral edema.

Vasogenic edema was produced cryogenically in 8 cats. After a suitable incubation period, brains were rapidly excised and sectioned. The water proton spin lattice relaxation times (T_1) were measured using a 180°-τ-90° pulse sequence at 4 megahertz.

T_1's are significantly higher for edematous tissue and these increases are correlated with water content increases in the tissue, as shown in Table 1.

Table 1

	Mean T_1 (Msec.)	Mean % H_2O
Control	381.7 ± 56.4	74.9
Edema	492.0 ± 36.3	78.8

The findings presented here indicate that the NMR relaxation time, T_1, is sufficiently sensitive to detect small changes in water content in cerebral tissue. The ability to accurately detect regional cerebral edema is of obvious clinical importance. These findings are of particular interest in light of recent developments in proton NMR zeugmatographic techniques which have been used to obtain true three-dimensional images of human subjects. Examples of three-dimensional zeugmatographic images will be presented.

279.12 SERUM OSMOLARITY AND GLUTAMATE-INDUCED BRAIN DAMAGE. M.T. Price, J.W. Olney, O.H. Lowry,* & S. Buchsbaum* Departments of Psychiatry & Pharmacology, Wash. Univ. Med. Sch., St. Louis, MO 63110.

Glutamate (Glu) and aspartate (Asp) are excitatory neurotoxins which selectively destroy neurons in circumventricular organ (CVO) regions of brain following systemic administration, presumably because CVO lack blood brain barriers (BBB). Since both Glu and Asp are common food additives, their neurotoxicity invites controversy; e.g., R.J. Wurtman argues that CVO do have BBB that protect CVO unless intake doses of Glu or Asp are high enough to create "hyperosmolar opening" of BBB. He reasons that Glu and Asp are safe because an 80 mosmolar increase in serum osmolarity is required to "open" BBB and neither man nor animal would ever voluntarily ingest enough Glu or Asp to induce such an extreme osmolar disturbance in blood. Here we report our recent evidence relevant to this argument.

Administering Glu or Asp or both (2 g/kg) to immature mice and measuring Glu or Asp concentrations in microdissected samples of CVO and non-CVO brain tissue at intervals from 15-180 min after treatment, reveals striking increases (3-8 X control values) of either amino acid in CVO and no increase in non-CVO regions (the expected result if CVO lack BBB).

When infant rats are given a CVO-damaging dose of Glu sc (0.5 g/kg) and serum osmolarity is measured at 15 min intervals by freezing point osmometry, no significant increase is detected at any post-treatment interval up to 1 hr; thus, acute toxic degeneration of CVO neurons occurs in the Glu-treated infant rat in the absence of any change in serum osmolarity.

When mannitol or sucrose are administered in high dosage (3 g/kg) to immature mice in combination with a CVO-damaging-dose of Glu (0.5 g/kg), the severity of CVO lesions is not increased compared to control mice receiving only 0.5 g/kg Glu. Hyperosmolarity does not augment Glu neurotoxicity.

When weanling mice are deprived of fluids overnight, then offered aqueous solutions of Glu or Glu + Asp (10% Glu, 5% Glu, or Glu 2.5% + Asp 2.5%), they *voluntarily* ingest enough Glu or Glu + Asp to sustain conspicuous CVO damage.

It is also known that the more potent excitotoxins (e.g., N-methyl aspartate)induce CVO brain damage at doses as low as 10 mg/kg sc and Inouye has shown autoradiographically that tracer amounts of C^{14} labelled Glu pass readily from blood into CVO but not into other regions of brain. The evidence suggests, contrary to Wurtman's thesis, that CVO lack BBB and serum osmolarity lacks relevance to the determination of Glu or Asp safety. Supported by USPHS grants NS-09156, NS-08862, DA-00259, RSA MH-38894 (JWO) and American Cancer Society BC-Q4.

279.13 EFFECT OF pH ON BLOOD-BRAIN BARRIER TRANSPORT OF AMINO ACIDS. Lester A. Wade, Helen M. Brady* and Darnell M. Barbay*. Dept. Physiology, Tulane Univ. Sch. Med., New Orleans, La. 70112.

Previous studies using Ehrlich ascites cells have shown that the rate of amino acid uptake by the leucine (L) neutral amino acid transport system is dependent upon extracellular pH. Garcia-Sancho (1977) also reported that the portion of glutamic acid transported by the L neutral amino acid transport is dependent upon the pH of the incubating solution. In the present experiments, we determined the effect of pH on amino acid transport at the blood-brain barrier using the intracarotid single pass bolus injection techniques in the rat. A mixture of a radioactively-labeled amino acid and ^{3}H-H_2O over a pH range was injected into the carotid artery, the rat was decapitated at 5 seconds, and the brain dissected into 5 regions before counting. The injectant also included one of the following buffers (10mM): Bicine, Hepes, Pipes, Mes and ε-aminocaproate. We measured the uptake of the synthetic amino acid (^{14}C)2-aminonorbornane-2-carboxylic acid (BCH) (0.25mM) over a pH range of 4.5 to 8.0. BCH is specific for the L neutral amino acid transport system. The greatest rate of BCH uptake was at a pH 7.0 (BUI = 19.1), with a lower uptake at pH 7.5 and 6.5 *(BUI of 15.4 and 16.3 respectively). There were no further* changes in BCH uptake at a more extreme pH. When non-radioactive glutamic acid (10mM) was added to the injectant as a potential inhibitor of BCH uptake, blood-brain barrier transport of BCH was unaffected at pH 7.5, 6.5 and 5.5. At an injected pH of 4.5, glutamic acid decreased BCH uptake from 16.0 ± 0.07 to 11.2 ± 0.4. These results indicate a pH optimum of 7.0 for the L neutral amino acid transport system at the blood-brain barrier. Unexpectedly, the rate of BCH uptake at a strongly acidic pH was similar to that observed at pH 7.5. Glutamic acid interacted with the blood-brain barrier L neutral amino acid transport system only when the pH was below 5.5.

Supported by NS-13914.

279.14 6-HYDROXYDOPAMINE ABLATION OF AMITRIPTYLINE-INDUCED ALTERATIONS IN BLOOD:BRAIN BARRIER. S. Preskorn, B. Hartman, C. Hughes, H. Clark* and G. Irwin*. Depts. of Psychiatry, Kansas U., Kansas City, KS 66103 and Washington U., St. Louis, MO 63110.

The central adrenergic system (CAS) has been postulated to have central vasoregulatory functions. Supporting this hypothesis, all tricyclic antidepressants--which act as indirect adrenergic agonists by blocking norepinephrine re-uptake--increase the diffusibility of water across blood:brain barrier, E_w Preskorn and Hartman, Biol. Psychiat., 1979). Their potency is directly related to their affinity for α-adrenergic receptors (Preskorn et al., JPET, 1980).

To further investigate this hypothesis, the effect of CAS ablation on the drug-induced alteration in E_w was tested. Following stereotaxic cannula placement, 6-hydroxydopamine (6-OHDA, 125µg/10µl) was administered intraventricularly in artificial cerebrospinal fluid (CSF) over a 10 minute period. Controls received only the drug-free CSF. For both groups, E_w was measured in half of the animals 6 weeks later without *further treatment. The other half received amitriptyline* (AMI, 62.5µmols/kg intraperitoneally) 15 minutes prior to the measurement of E_w. This dose and time was based on the studies cited above. The results are shown in the table below.

Total Forebrain E_w
(Mean ± Standard Error)

Groups	Sham	6-OHDA	Sham +AMI	6-OHDA+AMI
E_w	0.71±.07	0.75±.08	0.88±.07*	0.74±0.10

*$p < 0.001$

6-OHDA treatment did not by itself alter E_w but did abolish the AMI-induced increase in E_w. The sham treatment, however, did not influence the AMI effect. CAS ablation was confirmed by immunohistochemistry and direct electrochemical quantification of biogenic amines using the contralateral forebrain for each animal. Histology revealed no ventricular or primary lesions.

These studies support the hypothesis that antidepressants alter BBB through their effects on the CAS and also the concept that one function of this system is central vasoregulation. Results from ongoing studies of adrenergic and serotoninergic blockers will also be discussed.

(Supported by USPHS grants GM-1596 and MH 27201.)

280.1 COMPARATIVE THREE-DIMENSIONAL MORPHOLOGY OF THE MAMMALIAN SUPRACHIASMATIC NUCLEI. R. Lydic, H.E. Albers*, B. Tepper*, M.C. Moore-Ede. Dept. Physiol., Harvard Med. School, Boston, MA 02115.

Total lesions of the suprachiasmatic nuclei (SCN) disrupt circadian (≃24 hr) oscillations normally displayed by many mammalian physiological variables. Partial lesions involving SCN, *pars preopticus suprachiasmaticus* (POSC), or retrochiasmatic area (RCA), however, suggest that anterior (A) and posterior (P) SCN may not be functionally identical (Fuller, C.A. *et al., Physiologist 22:* 41, 1979; Arendash, G.W. and Gallo, R.V., *Neuroendocrin. 28:* 349, 1979; Rusak, B., *J. Comp. Physiol. 118:* 145, 1977). We report structural differences along SCN AP axis by morphological analyses conducted at the gross nuclear level. Using brains from hamster, rat, cat, squirrel monkey (*Saimiri sciureus*) and rhesus monkey (*Macaca mulatta*), we have combined histological techniques with computer graphics to describe the three-dimensional (3-D) morphology of SCN. In each species SCN appear to have their own unique 3-D structure and geometrical relationship to third ventricle (III) and optic chiasm (CHO). In primates A- poles expand along the medio-lateral (ML) axis, but the P- poles extend dorsally, rotated 90° from the ML plane. SCN of rhesus are smaller (AP length ≃0.8mm, ML width of A- pole ≃0.7mm) and more diffusely organized than squirrel monkey (AP length ≃1.0mm, ML width of A-pole ≃0.8mm). In cat SCN are triangular in shape throughout their ≃1.0mm AP expanse. The A- pole has ≃0.6 to 0.8mm ML width which tapers to ≃0.4mm posteriorly. POSC in cat extends for ≃1.0mm along the AP axis. In hamster (AP ≃0.5mm, ML ≃0.2mm) and rat (AP ≃1.0mm, ML ≃0.5) SCN are easily visualized. Rodent SCN are cylindrically shaped along the AP plane and middle portions are deeply embedded in CHO, more so in rat than hamster. In hamster SCN P-poles are ventral rather than lateral to III and appear to merge at midline. SCN characteristics at the cytoarchitectonic and connectional level have not been systematically integrated with 3-D analyses at the nuclear level. The pattern of afferent or efferent fiber systems involving SCN, RCA or POSC may correlate with AP differences in SCN morphology. Retrograde transport studies suggest that the retinohypothalamic tract (RHT) of squirrel monkey innervates the caudal SCN. Destruction of RHT/SCN input may account for some of the specific effects associated with partial SCN lesions in squirrel monkeys.

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280.2 Effects of SCN Lesions and Feed/Starve Entrainment Conditions on Activity and Retention Performance Rhythms. F. A. Holloway, D. C. Bird*, J. Devenport*, & W. N. Tapp. Dept. Psychiat. & Behav. Sci., Univ. Okla. Health Sci. Ctr., Oklahoma City, OK 73190.

We previously reported (Holloway & Wansley, Behav Biol. 9:1 1973) that retention performance fluctuated periodically as a function of time since training and suggested that these oscillations may represent some state-dependent phenomenon based on internal biorhythms. Indeed, later research (Wansley & Holloway, Neurosci. Abst., 1:810, 1975) indicated that lesions of the suprachiasmatic nucleus (SCN) altered both circadian activity rhythms and retention oscillations. Recently (Tapp, Bird & Holloway, Neurosci. Abst., 4:181, 1978) we showed that the disruption in circadian rhythms after SCN lesions could be reversed in part by the imposition of a 24-hr Feed/Starve (FS) schedule. We now report that a 24-hr FS condition may result in the re-establishment of retention performance fluctuations in rats with SCN lesions.

In Experiment 1, independent groups of male albino rats (n=6) were defined by surgical condition (unoperated, sham-operated, bilateral SCN lesion) and by active avoidance retention test intervals (6, 12, 18, 24, 30, 36 hr). All animals were maintained under 12:12 LD conditions with ad lib food and water. Prior to avoidance training each animal's activity was monitored continuously for several days. Both unoperated and sham groups displayed clear circadian activity rhythms and major fluctuations in avoidance retention performance, (poor at test intervals of 6, 18 or 30 hrs and relatively good at 24 hr). The SCN animals as a rule displayed a marked attenuation of circadian activity and drinking rhythms. The SCN groups displayed no sign of fluctuations in retention performance across testing intervals. Experiment 2 utilized a similar protocol except that for half the animals food was available for only 2 hr each day beginning 1 hr after lights-off (12:12 LD). Active avoidance training was given 10-14 days after imposition of the FS-schedule. Subgroups of controls and SCN rats were given retention tests 6, 24, or 30 hrs post training. A separate set of control and SCN animals received identical treatment except no FS-schedule was imposed. Retention performance of the non-FS groups replicated their counterparts in Experiment 1. The control-FS group displayed poor retention at both 6 and 30 hr while the SCN-FS group showed poor retention only at the 6 hr interval.

These results suggest that the multiple fluctuations in retention performance may depend on the presence of some undetermined constellation of biorhythmic phenomena but not perhaps on the integrity of SCN. The 24 hr FS plus the 12:12 LD schedule appeared to have produced some change in circadian rhythmicity sufficient for the retention fluctuation to reemerge in the SCN animals.

280.3 LIMITS OF ENTRAINMENT TO RESTRICTED FEEDING SCHEDULES IN RATS WITH SUPRACHIASMATIC LESIONS. Friedrich K. Stephan, Dept. of Psychology, Florida State University, Tallahassee, FL. 32306.

Although rats with lesions of the suprachiasmatic nuclei lose their circadian rhythms in activity and drinking when food and water are ad lib., we have shown that these animals can anticipate periodic access to food when food is made available at 23 or 24 hr intervals, but not at 18 hr intervals (Stephan, Swann, & Sisk, 1979, Behavioral and Neural Biology 25, 346-363 and 25, 545-554). Thus, anticipatory activity may be analagous to entrainment of a circadian rhythm. The present experiment was designed to define the limits of entrainment of anticipatory wheel running in rats with suprachiasmatic lesions. One group of 8 rats was sequentially exposed to 24, 21, 20, and 23 hr, and the other group to 24, 27, 29, 31, and 33 hr feeding schedules. In the former group, all 8 rats entrained to the 24 hr schedule within 2-5 days. Only 4 rats entrained to 21 hr cycle and none to the 20 hr cycle. In the latter group, all 8 rats entrained to the 24, 27, 29, and 31 hr schedules. Entrainment to the 23 and 33 hr schedules is still under investigation. These results indicate that the lower limit of entrainment is near 21 hrs while the upper limit is in excess of 31 hrs. The asymmetry of these entrainment limits may provide an important clue to the functional properties of the mechanisms which mediate entrainment to periodic food availability.

280.4 SUPRACHIASMATIC NUCLEI LESIONS AND SENSORY REACTIVITY. Z. M. Wenzel*** and W. A. Bengelloun. Dept. Psychology, State University of New York, Binghamton, NY 13901 and Dept. Biologie, Université Mohammed V, Rabat, Morocco.

The profound arrhythmia produced by lesions of the suprachiasmatic nucleus of the hypothalamus suggests that this structure is involved in the generation and/or integration of circadian rhythmicity. It is not known,however, whether the generation of the rhythm itself is abolished, or the integration of internal and external periodic events. This study investigated whether SCN lesions alter the responsiveness to general environmental stimuli, a number of which might serve as synchronizing agents.

Adult, albino, male rats were individually housed under a light-dark cycle and were subjected to either SCN or sham lesions. Ten days following surgery, animals were tested for light reactivity in an open field, one week later reactivity to quinine and sucrose solutions, and three weeks later reactivity to noise in a shuttlebox. All testing was completed during the light phase of the cycle. Histological verification revealed complete SCN lesions.

In general, SCN-lesioned rats were more, rather than less, sensitive to environmental stimulus change. SCN animals were more reactive to a noise stimulus than controls and inhibition of fluid intake in response to quinine was enhanced ($F=5.46$, $df=1/38$, $p<.025$). Although activity levels were similar to controls in the presence of the flashing white light stimulus, decreased baseline activity under red light again suggests increased responsiveness in SCN rats ($F=8.59$, $df=2/76$, $p<.001$).

These results indicate that rats with SCN lesions are not lacking external stimulation or synchronizers, but, in fact, are more reactive than intact animals. These data suggest that the SCN may have an inhibitory influence on response to external stimuli, and perhaps on synchronizer input to the circadian system.

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280.5 DIURNAL VARIATION IN THE RESPONSIVENESS OF HIPPOCAMPAL PYRAMIDAL NEURONS TO SEROTONIN: MICROIONTOPHORETIC EVIDENCE. S. Brunel* and C. de Montigny (SPON: Laurent Descarries). Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada H3C 3T8.

Chronic treatments with tricyclic antidepressant drugs or electro-convulsive shocks have been shown to induce a selective increase of the effect of iontophoretically-applied serotonin (5-HT) on rat hippocampal pyramidal neurons (de Montigny and Aghajanian, 1978; Gallager and Bunney, 1979; de Montigny, 1980). The time course of this sensitization to 5-HT is consistent with that of the therapeutic response to these treatments. Most of the patients suffering from a major affective disorder exhibit diurnal variation of mood during their depressive phase, usually with worsening in the morning and alleviation in the evening. In the present study, the responsiveness of hippocampal pyramidal neurons to iontophoretically-applied 5-HT, norepinephrine (NE) and γ-aminobutyric acid (GABA) was examined at two periods of the day.

Male Sprague-Dawley rats (200-275 gm) were used. Morning experiments were carried out between 8:00 and 12:00 hrs and evening experiments between 20:00 and 24:00 hrs. Unitary recordings of pyramidal cells were obtained from the CA_1 and CA_3 regions of the hippocampus under urethane (1.25 g/kg, i.p.) or chloral hydrate (400 mg/kg, i.p.) anesthesia. The following solutions were used for iontophoresis: 5-HT creatinine sulfate (0.002 M in 0.2 M NaCl, pH: 3.9) (Regis); GABA (0.05 M in 0.05 M) NaCl, pH: 4) (Calbiochem); NE bitartrate (0.1 M, pH: 4) (Regis); acetylcholine (Ach) chloride (0.02 M in 0.2 M NaCl, pH: 4) (Calbiochem). Ach was used to activate silent or slowly discharging units. The responsiveness of hippocampal neurons was estimated from the charge $I \cdot T_{50}$ required to obtain a 50% decrease from the baseline firing rate {charge $I \cdot T_{50}$ = current (nA) x time(s)}. To control the micropipette efficacy variable, the same micropipette was used for two consecutive experiments.

In a first series of experiments carried out under urethane anesthesia, the responsiveness of pyramidal cells to 5-HT was about 4 times greater in rats tested in the evening than those tested in the morning. The effect of NE was not markedly changed. In a second series of experiments in chloral hydrate-anesthetized rats, the sensitivity to 5-HT was enhanced 6 folds in the evening whereas that to GABA tended to be decreased.

These data suggest that the postsynaptic 5-HT receptors of rat hippocampal neurons undergo marked diurnal variations in their responsiveness. Such changes in 5-HT receptor sensitivity, should they occur in the human brain, could be related to the diurnal variation of mood occuring during the depressive phase of major affective disorders. (Supported by M.R.C. grant).

280.6 **SEROTONIN MIMICS THE ACTION OF OPTIC EFFERENTS IN RESETTING THE PHASE OF THE CIRCADIAN OSCILLATOR IN THE APLYSIA EYE.** **J. Nadakavukaren* and M. E. Lickey (SPON: D. P. Kimble). Depts. of Biology and Psychology, Univ. of Ore., Eugene, OR 97403.**

The two eyes of Aplysia are independent circadian oscillators. They are connected to the brain via optic nerves. As demonstrated by Prichard, neural signals from the brain can initiate phase resetting of the eye rhythm via efferent activity in the optic nerve. The present experiments were undertaken to see if chemical synaptic transmission is involved in neural resetting and if so what putative transmitters might duplicate the action of the optic efferents. Using Prichard's paradigm, Aplysia were entrained to LD 12:12 for at least 3 LD cycles. Following the last dawn, animals were exposed to continuous light (LL) for 12 to 24 hours. At the end of LL the eyes, together with the brain, were removed from the animal and placed in a culture dish. Compound action potentials (CAPs) were recorded from the optic nerves with suction electrodes in constant darkness (DD). Just before the onset of DD one of the eyes was detached from the brain by severing the optic nerve. The frequency (number of spikes/half hour) of CAPs plotted against time is a rhythmic function. The data were analyzed by comparing the phase of the rhythm of an attached eye to that of a detached eye. High Mg^{++} low Ca^{++} solutions, when bath applied separately either to the eye or the brain, blocked phase-resetting by the optic efferents. Serotonin bath applied to the detached eye mimicked the important characteristics of neural resetting. As described by Prichard, these characteristics include: (1) The phase of the 5-HT treated (or attached) eye is different than its untreated (or detached) paired control and the magnitude of the difference is a precise function of the duration of LL pretreatment. (2) There is a restricted "critical period" during which 5-HT (and efferent activity) can reset the ocular phase. The critical period recurs during the second cycle in vitro. The fact that bath application of 5-HT can mimic the action of optic efferent activity indicates that the information specifying the magnitude of phase resetting is stored in the eye, not the brain. Activity in the optic efferents appears to be an "enabling" signal rather than an "informative" signal. 5-HT is effective in a dose range of 10^{-6}M to 10^{-4}M. At 2 X 10^{-3}M 5-HT blocks neural resetting when applied to either the eye or the brain. This is perhaps a case of receptor desensitization at saturating doses of 5-HT. Other putative neurotransmitters and transmitter analogs were tried and found to be inactive in phase resetting. Corrent, McAdoo and Eskin, using a different paradigm, also found that only 5-HT among various putative transmitters can reset the eye rhythm. 5-HT may be a phase resetting transmitter in the Aplysia circadian system. **(NSF 77-28251)**

280.7 **A cAMP ANALOG WITH A PHOSPHODIESTERASE INHIBITOR RESETS THE PHASE OF THE CIRCADIAN OSCILLATOR IN THE APLYSIA EYE.** **W. P. Jordan, S. O. Hiaasen*, and M. E. Lickey. Dept. of Psychology, Univ. of Ore., Eugene, OR 97403.**

Each eye of Aplysia contains at least one circadian oscillator which can be entrained to environmental light cycles. In vitro, a circadian rhythm of compound action potentials (CAPs) may be recorded from the optic nerve of either a detached eye or an eye that remains attached to the cerebral ganglion. Prichard has shown that, following LD 12:12 entrainment, extension of the final light time to 15-27 hr in vivo causes eyes that remain attached to the cerebral ganglion to be reset in vitro due to efferent activity in the optic nerve. The resetting occurs during a critical period in the early subjective day. Using Prichard's paradigm, Nadakavukaren and Lickey, in a companion abstract, report that bath applied serotonin (5-HT) will reset a detached eye in vitro in a manner that mimics the action of the optic efferents.

The present in vitro experiments demonstrate that bathing a detached eye with a medium containing a cAMP analog (dibutyryl-cAMP, 10^{-3}M) and a phosphodiesterase inhibitor (3-isobutyl-1-methyl-xanthine (IBMX), 10^{-3}M) also phase shifts the ocular oscillator. A 5.5 hr application during the critical period for an attached or a 5-HT treated detached eye caused large (9-11 hr) phase shifts in the CAP rhythm. Separate application of either drug at 10^{-3}M failed to phase shift the CAP rhythm. However, addition of 5-HT at 10^{-7}M to a 10^{-3}M IBMX medium produced large phase shifts. Nadakavukaren and Lickey found the threshold for 5-HT application alone to be about 10^{-6}M. The synergism between 5-HT and IBMX, as well as that between db-cAMP and IBMX, suggests that cAMP acts as a second messenger for 5-HT within the eye. cAMP has been implicated in the action of 5-HT at several other sites in Aplysia.

Two-hour pulses of db-cAMP at 10^{-3}M with IBMX at 10^{-3}M during the critical period produced large phase shifts in detached eyes similar to those produced by the 5.5 h drug pulses. The 2 hour pulses have been applied at various phases of the circadian cycle. The phase shift due to the drugs varies with the phase of treatment. Further experiments are under way to see if there is a critical period for cAMP analogs and phosphodiesterase inhibitors actions as there is for 5-HT and neural activity. **(Supported by NIH 1 F32 NS06425 and NSF 77-28251)**

280.8 EFFECTS OF CONSTANT DARKNESS AND RE-EXPOSURE TO CONSTANT LIGHT ON THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY AFTER "SPLITTING" HAS OCCURRED. D. Earnest* and F. Turek (SPON: A. Eskin). Dept. of Biol. Sci., Northwestern University, Evanston, IL 60201.

Exposure of golden hamsters to constant light (LL) often results in the splitting of the circadian rhythm of activity into two distinct components which become stably coupled to each other at about 180^{o} out of phase. Since splitting has never been observed to develop in hamsters maintained in constant darkness (DD), we sought to determine what effect transfer to DD would have on hamsters whose activity rhythms had already split into two components during exposure to LL. In addition, we investigated what effect re-exposure to LL would have on these animals.

Adult male hamsters whose activity rhythm had split into two distinct components during exposure to LL were transferred into DD on 13 different occasions. In all cases, splitting was abolished within 1-3 days of exposure to DD and a normal free-running rhythm with a bimodal distribution of activity was observed. After 15-30 days in DD, the hamsters were transferred back into LL at various circadian times. The effect of the transfer from DD into LL appeared to depend on the phase relationship between the transition into LL and the activity rhythm. In 4 of 6 hamsters transferred into LL 4-5 hours after the onset of activity, splitting of the activity rhythm into two distinct components was evident within 2-4 days. In the other two animals, a very erratic pattern of activity was observed initially which eventually led to the split condition. In contrast, transfer of 7 hamsters into LL at other circadian times simply resulted in the activity rhythm continuing to free-run in a normal fashion.

These studies suggest that transfer of split hamsters from LL to DD results in the rapid re-establishment of the normal phase relationship between the two circadian oscillators which underlie the two components of activity during splitting. This coupling, however, appears to be very labile as demonstrated by the rapid desynchronization of the components of the activity rhythm upon transfer back into LL. The rapid induction of splitting when hamsters are transferred into LL 4-5 hours after the onset of activity may be related to the phase response curve of this species. This region of the phase response curve represents a transition point. If hamsters maintained in DD are exposed to a brief light pulse 4-5 hours after the onset of activity, the rhythm may be phase delayed or phase advanced. Perhaps the rapid onset of splitting observed in hamsters transferred from DD into LL at this circadian time is due to one oscillator being phase advanced while the other is phase delayed by the abrupt onset of light. (Supported by USPHS Grants HD-12622, HD-09885, and HD-00249.)

281.1 DIFFERENTIAL DISTRIBUTION OF THE Ca BUFFERING IN APLYSIA NEURONS. D. Tillotson and A.L.F. Gorman. Boston University School of Medicine, Boston, MA 02118.

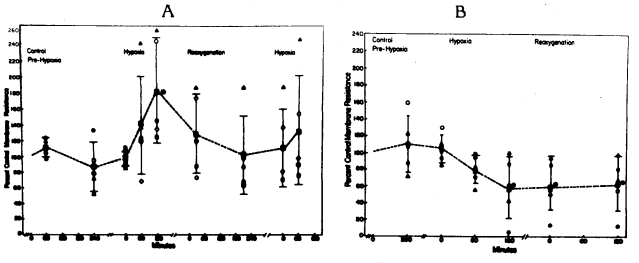
The intracellular concentration of Ca^{2+} ions in nerve cells is known to be in the submicromolar range. Mechanisms for maintaining this resting level and handling increases in Ca^{2+} during membrane activity continues to be the subject of study. Experiments reported here were performed to investigate the distribution of the Ca^{2+} buffering machinery in the interior of nerve somas. Selected neurons in the abdominal ganglion of Aplysia were voltage-clamped and injected, to a known concentration, with the Ca^{2+} sensitive dye arsenazo III. The differential light absorbance change associated with a standard iontophoretic Ca^{2+} injection (20 nA, 5 sec) was used as a monitor of Ca^{2+} buffering in a region near the tip of the Ca^{2+} microelectrode. Absorbance measurements were made at various points in the soma as the Ca^{2+} electrode was advanced into or withdrawn from the cell. It was reasoned that changes in the measured peak absorbance corresponded to changes in buffering such that the largest signals would be obtained in regions of lowest buffering. We found the smallest absorbance signals in a cytoplasmic layer near the plasma membrane and largest signals near the center of the cell. To control for the possibility that this result was a consequence of the measurement procedure rather than the buffer distribution, a large iontophoretic injection of the Ca^{2+} chelator EGTA (100 nA, 5 min) was made. A substantial concentration of internal EGTA should render the whole of the interior of a cell equivalent with respect to Ca buffering. After EGTA injection the previous standard injection (20 nA, 5 sec) produced no measurable absorbance change. The localization experiment was repeated using a 10X greater Ca^{2+} injection intensity (200 nA, 5 sec) and the predicted uniform distribution was obtained. Our results suggest that the Ca^{2+} buffering machinery is differentially distributed in the molluscan nerve soma being greatest in a cytoplasmic layer near the plasma membrane. This study supported by NIH grant NS11429.

281.2 THE SELECTIVITY OF THE Ca^{2+} ACTIVATED K^+ CHANNEL FOR MONOVALENT CATIONS. J.C. Woolum and A.L.F. Gorman. Boston University School of Medicine, Boston, MA 02118.

The calcium activated potassium current is of considerable importance for the function of mammalian as well as of invertebrate neurons. We studied the Ca^{2+} activated K^+ current of the molluscan neuron soma membrane to investigate whether the selectivity of this channel for various monovalent cations is similar to that for the delayed rectifying K^+ channels or to that for the leakage channels (or neither). The relative permeabilities of the Ca^{2+} activated K^+ channel for a number of monovalent cations were measured using the reversal potential method described by B. Hille (J. Gen. Phy. 1973, 61, 669). Cells in the abdominal ganglion of Aplysia were voltage clamped (two electrode voltage clamp) and a third electrode was used to inject Ca^{2+} ions by iontophoresis into the soma. We find that the Ca^{2+} activated K^+ channel is quite selective. The relative order for the selectivity of the channel is $P_K > P_{Rb} > P_{NH4} > P_{Cs} > P_{Na}$, P_{Li}, P_{TMA} (the Na^+, Li^+ and tetramethylammonium permeabilities are too small to measure with our technique) and is similar to that found for the delayed rectifier K^+ channel of myelinated axon. For the molluscan neuron Ca^{2+} activated K^+ channel the permeability ratio $P_{Cs}/P_K = 0.04$ whereas for the delayed rectifier K^+ channel of myelinated axon $P_{Cs}/P_K < 0.077$. Our results suggest that the mechanisms responsible for channel selectivity (channel size, etc) are similar for the Ca^{2+} activated K^+ and the delayed rectifier K^+ channels, but differ from those for the leakage channels. This study supported by NIH grant NS11429.

281.3 DETERMINATIONS OF MEMBRANE RESISTANCE, I-V RELATIONSHIPS, AND INTRACELLULAR K^+ ACTIVITY IN HYPOXIC AND NORMAL APLYSIA CALIFORNICA NEURONS. P.E. Coyer. Laboratory of Neurophysiology and Cerebrovascular Research, Dept. of Neurology, Univ. of Alabama in Birmingham, The Medical Center, Birmingham, Alabama 35294.

Recently I have found two responses of Aplysia californica neurons to hypoxia (see figures below). While one group (A) of neurons in the abdominal ganglion was observed to be resistant to hypoxia, another group (B) was found to be non-resistant during exposure to hypoxic bath conditions lasting 2 hours. A period of reoxygenation lasting up to 4 hours followed hypoxia during which recovery of the neurons' membrane potentials and resistances were observed. During hypoxia the resistant neurons (N=7) had membrane resistances that increased significantly above ($P < 0.05$); paired t-test) control measurements. Non-resistant neurons (N=5) depolarized, and their membrane resistances decreased significantly below ($P < 0.05$) control measurements following 2 hours' exposure to hypoxia. For the non-resistant neurons, these changes were irreversible, and the electrophysiological and/or anatomical bases accounting for these differences existing between resistant and non-resistant neurons is being investigated. I-V curves were constructed with an X-Y plotter before, during, and after hypoxic bath conditions monitored with PO_2 microelectrodes. Hypoxia was achieved by nitrogen bubbling and is defined as a PO_2 of less than 20 torr in the suffusate. The non-resistant, characterized by linear I-V curves, showed a decrease in membrane resistance in the depolarizing direction. The resistant group showed rotation (i.e. a change of slope measured as a line tangent to the 0 current point) of non-linear I-V curves. These changes were reversible. Intracellular K^+ activity was determined in both groups (Coyer, Neurosci. Abs. 5, 1979), and I-V curves were constructed during pump inhibition (low K^+_o in the bathing solution). The effects of pump inhibition achieved in this manner were reversible for both resistant and non-resistant neurons. However, the effects of hypoxia as described above were not. Supported in part by NINCDS Grants NS 07123 and NS 08802.



281.4 PRELIMINARY EVIDENCE OF ABNORMAL ELECTRICAL MEMBRANE PROPERTIES FOR DOWN'S DRG NEURONS IN CELL CULTURE. B.S. Scott, T.L. Petit, L.E. Becker*, B.A.V. Edwards*. Surrey Place Centre, Toronto, Canada M5S 2C2.

Cell cultures were prepared from normal and Down's syndrome dorsal root ganglia (DRG). Both pre- and post-natal specimens were utilized; seven normal and four Down's. Cultures were maintained in medium with normal (4mM) and elevated (20mM) potassium (K) since the latter was found to enhance neuron survival. After various periods of incubation, cultures were transferred to normal K medium and their electrical membrane properties (EMP) determined.

A qualitative comparison of the EMP of the normal and Down's DRG neurons revealed no differences. Therefore we carried out a quantitative investigation of various EMP including resting membrane potential (Vm), specific membrane resistance (Rm), and capacitance (Cm), membrane time constant (τ), rheobasic current (I_{RH}), rheobasic voltage depolarization (ΔV), action potential duration (Δt), overshoot (OS), after hyperpolarization (AHP) and absolute refractory period (ARP).

As indicated in the Table, Down's neurons had significantly reduced OS, AHP and ΔV compared to normal neurons for both K media. Also Down's neurons had reduced Rm in 4K, but increased Rm in 20K suggesting K had a differential effect on Rm of normal and Down's neurons. Statistically significant correlations were found between certain EMP and various factors including developmental stage, culture duration, type of action potential (i.e. monophasic or biphasic falling phase) and cell size. However, the abnormal Down's EMP mentioned above were also indicated when these factors were taken into consideration by using samples matched for developmental stage, type of action potential, etc. These preliminary results suggest that Down's neurons may have subtle abnormalities in their EMP which may be the neurobiological basis of the mental retardation observed in this syndrome.

	4K				20K			
	Normal	n	Down's	n	Normal	n	Down's	n
Vm (mV)	49.5	204	54.7*	464	55.2	337	56.1	274
Rm (Ωcm^2)	997	148	909	392	937	238	1136*	259
τ (msec)	3.76	164	3.56	380	2.86	247	3.80*	258
Cm ($\mu F/cm^2$)	4.19	134	4.04	363	3.32	211	3.49	244
Δt (msec)	3.49	141	2.80*	394	2.27	213	2.31	265
ARP (msec)	3.80	134	3.29	360	2.69	186	2.88	255
OS (mV)	26.9	97	20.7*	431	21.1	130	18.7*	263
AHP (mV)	7.69	78	6.04*	413	7.08	156	4.41*	263
ΔV (mV)	11.7	134	9.2*	369	13.1	221	9.4*	245
I_{RH} (mA)	.559	165	.514	386	.824	259	.477*	259

*$p < 0.05$, t-test

281.5 SPECIFIC MEMBRANE CAPACITANCE AND RESISTANCE OF MOUSE DORSAL ROOT GANGLION NEURONS. Donald H. Perkel, Thomas H. Brown, Jane C. Norris*, and John H. Peacock. Departments of Biological Sciences (DHP), Neurobiology (JCN), and Neurology (JHP), Stanford University, Stanford, CA 94305, and Division of Neurosciences (THB), City of Hope Research Institute, Duarte, CA 91010.

Because of their simple morphology, sensory ganglion neurons lend themselves well to studies of the specific membrane properties of a vertebrate neuron. Previous estimates of the specific electrical constants in these neurons failed to take into account the electrotonic structure of the cells; that is, the actual distribution of current flow through the membrane of the soma as well as that of the process. Failure to take the electrotonic structure into account can cause large errors in the estimates of specific membrane capacitance (C_m) and resistance (R_m). The present study examined the electrotonic structure and specific membrane properties of mouse dorsal root ganglion neurons in mature dispersed cell cultures.

Electrophysiological recordings were made from cultures bathed in defined salt solutions. The soma of each cell was penetrated with two independent microelectrodes, using one for passing current and the other for recording membrane potential. Voltage transients were induced within the ohmic range of the membrane by imposing a hyperpolarizing current step. The slope of the resultant charging curve was measured and analyzed to estimate cable-theory parameters of the neuron: the electrotonic length (L) of the process and the ratio (ρ) of input conductance of the process to that of the soma. Three different analytical methods were used to estimate the cable parameters. The results of the three methods were in substantial agreement. Their adequacy was further investigated by using the parameter values in computer simulations which furnished theoretical charging functions which were then compared with the corresponding experimentally derived curves. In every case, the two sets of curves were within experimental error.

In 14 cells, the mean (±SE) values of ρ and L were , respectively, 1.3±0.3 and 1.1±0.1. The values of C_m and R_m were determined from the electrotonic structure combined with soma area measurements. The mean (±SE) values of R_m and C_m were, respectively, 6900±1000 Ωcm^2 and 0.90±0.1 $\mu F/cm^2$. This value of C_m agrees with that of other carefully studied biological membranes. Discrepancies with some earlier studies of sensory ganglion neurons can be attributed to the effects of electrotonic structure. This implies that C_m is nearly constant in a wide variety of cells. [This research was supported by NIH grants NS-09744 (DHP) and NS-12151 (JHP).]

281.6 EFFECTS OF LOCAL ANESTHETIC QX-314 ON MEMBRANE PROPERTIES OF HIPPOCAMPAL NEURONS. B.W. Connors and D.A. Prince. Dept. of Neurology, Stanford University Medical School, Stanford, CA 94305.

Local anesthetics are known to block peripheral nerve conduction by suppressing voltage-sensitive sodium and potassium conductances. We have used the quaternary lidocaine derivative QX-314 to characterize local anesthetic actions on a mammalian CNS neuron. This agent is permanently charged and exerts its effects most potently when applied to the inside of the membrane. Studies were performed on CA1 cells of the transverse hippocampal slice maintained in vitro. Intracellular electrodes were filled with 4 M K acetate or QX-314 (0.1-0.3 M) + 4 M K acetate.

Immediately following impalement with a QX-314-containing electrode, fast, TTX-sensitive action potentials could be evoked synaptically or by direct current injection. Over several minutes, the amplitudes of these Na^+ spikes gradually decreased and thresholds increased. During the period of partial action potential suppression, spike amplitudes rapidly attenuated during repetitive stimulation at 5-50 Hz. Complete block occurred within 5-10 min following impalement, at which time it was usually possible to restore spike generating capacity by delivering a hyperpolarizing pulse before stimulation.

Often, as fast Na^+ spikes became depressed, long duration (10-20 msec), regenerative depolarizing events could be evoked at low thresholds. These potentials were insensitive to focal application of TTX (10^{-5} M), but were abolished by the application of Mn^{++} (10 mM) and were presumably mediated by Ca^{++}. It may be that the anesthetic depression of voltage-sensitive K^+ conductance allowed the generation of Ca^{++} spikes at thresholds lower than normal. When Ba^{++} (0.25-1.0 mM) was added to the bathing medium, broad, TTX-resistant spikes or depolarizing plateaus occurred spontaneously or could be triggered by brief current pulses. Ba^{++}-induced events persisted even after an hour of recording with a QX-314-containing electrode.

Intracellular QX-314 abolished depolarizing anomalous rectification, which is mediated in part by inward Na^+ current and known to be depressed by TTX. During prolonged recordings there were no systematic changes in resting membrane potential or excitatory and inhibitory synaptic potentials. Some delayed rectification always remained.

These results are consistent with the hypothesis that internal QX-314 inhibits voltage-dependent sodium conductances in CNS neurons without altering calcium or synaptic conductances. This agent is a useful pharmacological tool for assessing CNS membrane properties.

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281.7 VOLTAGE CONTROL OF SPONTANEOUS BURSTING IN HIPPOCAMPAL CA3 NEURONS J. J. Hablitz and D. Johnston, Sect. of Neurophysiol., Dept. of Neurol., Baylor Col. of Med., Houston, TX 77030.

At rest, in the absence of externally applied currents, CA3 pyramidal neurons usually burst spontaneously at a low rate. These bursts consist of clusters of APs triggered by an underlying depolarization. Displacement of the resting membrane potential by applied DC currents markedly affect both the rate and configuration of of the spontaneous bursts (SB). Hyperpolarization by 5-10mV increases both the duration and period between SBs; another 5-10mV of hyperpolarization leads to suppression of bursting. Depolarization initially evokes an increase in the frequency and decrease in duration of SBs while further depolarization results in a shift to repetitive firing. Local application of 5mM Ba^{2+} produces an increase in the frequency and duration of SBs and leads to development of plateau bursts 30-50mV in amplitude and 500-800mS in duration. Subsequent application of 10mM Co^{2+} suppressed SBs. This evidence suggests that SBs result from voltage dependent intrinsic membrane events. They do not appear to be synaptically mediated since no underlying depolarization was uncovered during hyperpolarization and SBs persisted when all evoked synaptic activity was abolished by high Mg^{2+}, low Ca^{2+} perfusion.

SBs were recorded also in neurons where all regenerative Na^+ activity was blocked by perfusion with TTX. Bursting in the TTX was readily apparent following either Ba^{2+} application or caesium (Cs^+) injection. SBs consisted of one or more regenerative events, presumably Ca^{2+} spikes, triggered by a slow membrane depolarization. This slow oscillation could result in a plateau depolarization lasting many seconds; these plateaus could end spontaneously or be terminated by hyperpolarizing currents. Small applied currents caused the membrane potential to move rapidly between resting and plateau levels; maintained hyperpolarizing currents prevented the further occurrence of SBs.

Under voltage clamp conditions we have previously described a persistent inward current in CA3 neurons. Under unclamped conditions, after Cs^+ injection, the persistent inward current exerts almost complete control over the membrane potential; small applied currents moving the membrane potential rapidly into and out of the region of negative slope resistance (approx. -50 to -10 mV) seen under clamp. This inward current in CA3 neurons resembles the slowly inactivating inward current responsible for endogenous bursting in invertebrate and spinal neurons suggesting that the burst tendancies of CA3 neurons may arise from similar mechanisms. (Supported by NIH Grants NS-15772 and NS-11535.)

281.8 In the presence of tetraethylammonium, a late delayed depolarization follows the action potential of rat sympathetic neurons grown in cell culture. Trisha Suppes* (Spon: P.H. O'Lague). Dept. Anatomy, UCLA, Los Angeles, CA 90024.

Intracellular microelectrodes were used to study the electrophysiological properties of sympathetic neurons dissociated from the superior cervical ganglia of newborn rats and grown in cell culture. Neurons tested (n=52) had resting potentials of -55 to -70 mV and action potential amplitudes of 70 to 100 mV recorded during continuous perfusion of the cultures (see O'Lague et al., DEV BIOL 67:384, 1978, for methods and perfusion media). When cultures were exposed to a modified perfusion media containing tetraethylammonium ions (60 mM, TEA+) the action potential duration increased (range 150 to 520 mS, measured at half peak amplitude) and the resting potential depolarized (range 5 to 12 mV, n=8). In TEA+ perfusion media the action potential was followed, after a delay of 1-2 seconds, by a substantial depolarization. This late delayed depolarization (LDD) had peak amplitudes, range from 4 to 26 mV, and its duration ranged from 18 to 70 seconds (n=12). The values obtained for the amplitude and duration of the LDD were constant and reproducible in a given cell, and the LDD was present regardless of the age of the culture studied (20 to 90 days). The LDD was accompanied by a conductance increase (10 to 76%, n=5). In preliminary experiments to test the ionic dependence of the LDD, no effect on the LDD was seen with extracellular Na+ completely replaced by tetramethylammonium (n=3) or with the addition of tetrodotoxin (a blocker of voltage-sensitive Na+ channels, 3 micromolar, n=10) to the TEA+ perfusion media. In contrast, the LDD was reversibly abolished by conventional Ca++ channel blockers Mn++ (2.8 mM, n=5) or Cd++ (0.75 mM, n=5), or by reducing extracellular Ca++ (to less than 0.2 mM) in the TEA+ perfusion media. Further details of the ionic basis and properties of the LDD will be presented.

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281.9 QUINUCLIDINYL BENZILATE (QNB) INTERACTION WITH IONIC CHANNELS OF THE NERVE TERMINAL AND NICOTINIC RECEPTOR. G. G. Schofield*, E. X. Albuquerque and J. E. Warnick. Dept. of Pharmacol. & Exptl. Therap., Univ. of Maryland, Sch. of Medicine, Baltimore, MD 21201.

The effects of the muscarinic antagonist QNB were studied on frog sciatic-sartorius preparations using conventional microelectrode techniques. These studies revealed sensitive sites at both pre- and postjunctional regions of the nicotinic synapse. QNB produced a concentration dependent decrease in miniature endplate potential (MEPP) amplitude such that at 10 and 100 μM, MEPP amplitude was 77% and 24% of control, respectively, while MEPP frequency and resting membrane potential were unaffected. In curarized sartorius muscles, QNB (60 μM) produced a stimulation dependent increase in the latency of both the nerve terminal action potential and the endplate potential (EPP). However, conduction velocity in the isolated sciatic nerve of the frog was unaffected by this concentration of the agent. In Mg^{2+}-treated preparations QNB produced a concentration dependent decrease in the quantal content of EPPs. QNB (60 μM) also decreased the rate of rise and increased the half decay time of directly elicited action potentials in sartorius muscles and reduced the probability of secondary (repetitive) action potentials without affecting the process of delayed rectification. Repetitive stimulation in these muscles enhanced the effect of QNB on the rate of rise and half decay time of action potentials. Under voltage clamp conditions QNB produced concentration dependent decreases in the peak amplitude and time course of endplate currents (EPCs). Concentrations of QNB greater than 30 μM produced non-linearity in the current-voltage relationship and a reduction of the voltage sensitivity of the rate of decay of the EPCs. Similar effects were noted on miniature EPCs. Noise analysis of EPC fluctuations in response to microiontophoretically applied acetylcholine in the presence of QNB revealed no effect on the single channel conductance but a shortening of the channel lifetime. At lower temperatures (10 °C) when acetylcholine modulated channels remain open for longer periods of time, QNB produced a greater decrease in the ionic channel lifetime although no change in the channel conductance was observed when QNB was compared to control. Thus in addition to its marked effect on the muscarinic receptors it significantly blocks sodium conductance and the ionic channel of the acetylcholine receptor. The locus of the latter effect is primarily the open conformation of the channel. The effect of QNB on the electrical properties of nerve and muscle may explain some of the hallucinatory properties of the agent. (Supported in part by USPHS Grant NS-12063.)

281.10 ELUCIDATION OF THE SITE OF ACTION OF OPIATE ANTAGONISTS WITH THE IONIC CHANNEL OF THE NICOTINIC ACETYLCHOLINE RECEPTOR. L. de Oliveira* and E. X. Albuquerque. (SPON: L. Guth). Dept. of Pharmacol. & Exp. Therap., Univ. of MD School of Medicine, Baltimore, MD 21201.

The interaction of naltrexone and levallorphan with the ionic channels at synapses of the frog sartorius muscle has been investigated with conventional techniques for measurement of endplate or miniature endplate currents (EPC and MEPC) and acetylcholine noise analysis. Naltrexone, a pure opiate antagonist shortens the time constant of decay (τ) of both EPC and MEPC. A semi-logarithmic plot of τ vs. membrane potential reveals a curvilinear relationship prominent at negative to positive potentials whose onset of inflection seen at negative potentials is a function of the drug concentration. At 60 and 300 μM, naltrexone accelerated the single exponential decay of the EPC, at –150 mV, from 3.3 to 0.8 msec and from 3.3 to 0.3 msec, respectively. This pattern shows a nonlinear function when the inverse of τ was plotted against the drug concentration at the hyperpolarized region. At drug concentrations of 300 μM, EPC peak amplitude was decreased to values less than 10% of the corresponding control condition. The onset of the nonlinearity in the current/voltage relationship was also dependent on the drug concentration. The ability of naltrexone to interact with the open conformation of the ionic channel was further evidenced by an increase in drug efficacy at a lower temperature (i.e., 10 °C rather than 22 °C). Acetylcholine noise analysis experiments show that channel lifetime and single channel conductance parameters also decreased in a voltage dependent manner and as a function of drug concentration. At a membrane potential of –75 mV and drug concentration of 40 μM, the values for channel lifetime and single channel conductance were 0.6 msec and 16 pS, respectively vs. 1.2 msec and 26 pS for control conditions. Further investigations on naltrexone suggest that the action of this agent is on the closed and open conformations of the channel. Levallorphan, a mixed antagonist of morphine also reveals a similar depressant action on both the peak amplitude and τ of the EPCs although some striking differences were noticed. Levallorphan has a stronger depressant effect on EPC amplitude than naltrexone. Indeed, levallorphan (100 μM) markedly depressed the peak amplitude of the EPC such that at –150 mV, the peak amplitude of the EPC was reduced to about 10% of control values and such an effect was highly voltage dependent. The ability of these agents to react with ionic channels of the acetylcholine receptor at concentrations similar to those used for opiate antagonism suggests that in addition to reacting with the opiate receptor, these agents display similar potencies at other types of receptor-ionic channel complexes. (Supported in part by USPHS grant NS-12063.)

281.11 REACTION OF HISTRIONICOTOXIN AND ITS ANALOGS WITH THE IONIC CHANNEL DURING AGONIST ACTIVATION OF THE ACETYLCHOLINE RECEPTOR AT THE NEUROMUSCULAR JUNCTION. M. A. Maleque*, C. E. Spivak*, M. Adler*, L. Masukawa*, J. E. Warnick, J. W. Daly*[1] & E. X. Albuquerque. Dept. of Pharmacol. & Exp. Ther., Univ. of MD Sch. of Medicine and LBC[1], NIH, Bethesda, MD 20205.

Natural and semisynthetic analogs of the saturated alkaloid histrionicotoxin (HTX; 2pR, 6S, 7pS, 8aS)-7-(cis-1-buten-3-ynyl)-8-hydroxy-2-(cis-2-penten-4-ynyl)-1-azaspiro [5.5] undecane) are: H_4–iso, H_8– and H_{12}–HTX. Both HTX and H_{12}HTX have been examined for their ability to block K conductance and to interact with the ionic channel of the ACh receptor. These studies did not include either a dose-response relationship or any of the analogs of HTX. The present study used voltage clamp techniques to analyze the endplate currents (EPCs) in frog sartorius muscles. Both HTX and H_{12}HTX depressed the peak amplitude of the EPC at all membrane potentials in a concentration dependent manner. However, the time constant of decay (τ) of the EPC exhibited saturation of the response. For example, at –90 mV, with 10, 20, 30 and 40 μM HTX, the peak EPC amplitude was reduced by 56, 86, 90 and 92% while the corresponding reduction in τ was 38, 47, 47 and 49%. With 10, 20, 30 and 40 μM H_{12}HTX, the peak EPC amplitude was reduced by 61, 72, 84 and 89%, while the corresponding reduction in τ was 36, 40, 43 and 44%, respectively. The depression of peak EPC amplitude is concentration dependent up to at least 40 μM of the toxins while τ exhibits saturation at 10–20 μM. At 4 μM H_4–iso-HTX and 25 μM H_8–HTX, peak EPC amplitude was reduced by 76 and 65% and τ was shortened by 31 and 34%, respectively, values similar to those seen with HTX and H_{12}HTX. H_4isoHTX appears to be the most potent compound. Under control conditions, the amplitude of the EPC increased initially during tetanic stimulation (25 Hz for 1 sec) and then returned to control level at the end of the train. Within 10 sec the tetanic train could be repeated 2-3 times without any difference. In the presence of HTX (35 μM) or H_{12}HTX (30 μM), there was a slight potentiation of the first 2-3 EPCs and then a rapid rundown of the EPC amplitude. At the end of the train the EPC amplitude was depressed by 57%. With 10 sec rest intervals (or less) between tetanic stimulation, subsequent trains of EPCs could be elicited in which the initial EPC in the second and third trains were 65 and 57% of the initial EPC in the first train, respectively. Although the peak amplitude of the EPC decreased significantly during the trains of stimuli, τ was unaltered during the entire tetanus (first, second or third trains). At 35 μM HTX, the mean value of τ of the first, fifth and last EPC during any tetanus was 65, 67 and 69% of control, respectively. With H_{12}HTX (30 μM) the first, fifth and last EPCs were 60, 67 and 70% of control, respectively. Thus, the complete block of peak amplitude without further shortening of τ during activation produced by the agonist in presence of HTX or H_{12}HTX suggest that the agent reacts with the channel in resting or closed conformation, open and at least one intermediate nonconducting stage. (Supported by USPHS grant NS-12063.)

281.12 INTERNAL AND EXTERNAL SITES OF ACTION OF QUATERNARY COMPOUNDS AT THE NICOTINIC ACETYLCHOLINE RECEPTOR-CHANNEL COMPLEX. L. G. Aguayo* and E. X. Albuquerque. (SPON: R. A. Sjodin) Dept. of Pharmacol. & Exp. Therap., Univ. of MD Sch. of Med., Baltimore, MD 21201.

The effects of several quaternary molecules were studied on the endplate region of the frog sartorius muscle using bath application or intracellular injection. Tetraethylammonium (TEA), atropine methyl bromide, phencyclidine methiodide (PCP MeI) and piperocaine MeI were injected into the sarcoplasm just beneath the postjunctional membrane from microelectrodes filled with 2 M, 2 M, 0.01 M and 0.001 M solutions, respectively. Taking advantage of the ability of these quaternary agents to block K conductance, the agents were released inside the cell and the prolongation of the muscle action potential was monitored. While external application of TEA (50-1000 μM) decreased the peak amplitude of the endplate current (EPC) and its time constant of decay (τ) with a simultaneous shortening of channel lifetime, this agent and atropine methyl bromide were ineffective when injected internally. However, PCP MeI (3–30 μM) had a potent action on EPCs and miniature EPCs (MEPC) when applied to either the inside or outside of the membrane. PCP MeI caused nonlinearity of the peak amplitude and a shortened channel lifetime while it sensed only 7% of the membrane potential at its binding site. Internal injection of the agent caused significant depression of the EPC and MEPC peak amplitude, the effect being more evident initially on the peak amplitude than on τ. However, as the internal concentration of the agent increased inside the cell, the effect on τ became more evident at less negative membrane potentials, somewhat different to that observed when it was applied externally. The peak amplitude of the EPC recorded at –90 mV was reduced to 43 and 46% of control with external application of PCP MeI (10 μM) and with internal application, respectively. PCP MeI had no effect on the reversal potential of the EPC which implies that the selectivity of endplate channels for Na and K ions remained unchanged. The power spectra of MEPCs were then measured in voltage-clamped fibers before and after application of drug. The half power frequencies at –60 mV were 88 Hz (τ = 1.8 msec) and 118 Hz (τ = 1.35 msec) in control and in the presence of internally applied PCP MeI, respectively. At –90 mV the corresponding values were 79 Hz (τ=2.0 msec) and 72 Hz (τ = 2.2 msec). Preliminary experiments with piperocaine MeI have shown similar effects when applied to both sides of the membrane. The ability of PCP MeI and piperocaine MeI to block the ionic channel of the acetylcholine receptor at both the outside and inside of the membrane in contrast to TEA and atropine methyl bromide, shows that active sites for binding drug may explain some of the mechanism related to voltage and time dependent effects of agents and a possible assymetry of the ionic channel. (A portion of these initial studies with PCP MeI were presented earlier as part of a New York Academy of Sciences Symposium on "Carriers and Membranes", February, 4-7, 1980.) (Supported in part by USPHS grant NS-12063.)

281.13 SWELLING OF NERVE FIBERS DURING ACTION POTENTIAL. K. Iwasa* and I. Tasaki. Lab. of Neurobiology, National Institute of Mental Health, Bethesda, MD 20205 and Marine Biological Lab., Woods Hole, MA 02543.

Concurrent with production of an action potential, a small rapid swelling of nerve fibers was observed in a variety of invertebrate nerves. The material used includes claw nerves of the crab, walking leg and claw nerves of the lobster, claw nerves of the crayfish, and squid giant axons. A piezo-ceramic bender (Gulton Industries, Inc.), pressing the surface of a nerve via a bristle with a small disc attached to the tip, was employed to demonstrate a pressure increase of about 1 dyne/cm^2 in squid giant axons and 5 dyne/cm^2 in crab nerves. A fiber optic device, Fotonic sensor (Mechanical Technology, Inc.), was used for recording a displacement of the nerve surface, and showed outward displacement of 0.5-0.7 nm in squid giant axons and 5-20 nm in crab nerves. The swelling, measured in terms of both pressure increase and surface displacement, was shown to start simultaneously with the action potential at the site of mechanical recording. In squid giant axons the rapid swelling phase is followed by a slower contraction phase. A small decrease in the length was also observed in both squid axons and crab nerves (I. Tasaki and K. Iwasa, Biochem. Biophys. Res. Commun., in press). In frog sciatic nerves no mechanical responses could be demonstrated.

Application of long pulses of electric currents was shown to generate mechanical changes in the nerve fiber. At the site of cathodal polarization, there was a smooth rise in the swelling pressure. This smooth rise was interrupted by a large abrupt rise associated with generation of a propagated action potential. At the site of anodal polarization, a large mechanical change representing a contraction of the fiber diameter was observed.

The sum of the volume of the surrounding seawater and that of nerve fibers was found to remain roughly unaltered when the fibers were excited. From this suggestive evidence it seems probable that the observed swelling is produced by invasion of water into the fibers. These observations opens up the possibility of offering new interpretations of various optical and biochemical changes that are known to occur when the nerve fibers are excited electrically.

281.14 MEMBRANE CURRENTS OF SINGLE, FRESHLY ISOLATED SMOOTH MUSCLE CELLS STUDIED WITH VOLTAGE CLAMP. Joshua J. Singer and John V. Walsh, Jr. Dept. of Physiology, Univ. of Massachusetts Medical School, Worcester, MA 01605

Single, smooth muscle cells, enzymatically dissociated from the stomach muscularis of the toad, Bufo marinus, were used to study membrane currents with the voltage clamp technique. By using single cells, we avoided the difficulties associated with syncytial tissue preparations,i.e., narrow intercellular spaces which constitute a substantial series resistance preventing uniformity of membrane potential and allow local ionic accumulation or depletion; intercellular coupling via resistances of unknown quantity and characteristics; and neural elements which may indirectly alter the membrane permeabilities of the muscle. The isolated cells were impaled with two micropipettes (30-60 MΩ), each of which was connected to a high input impedance amplifier with current injection capability. One pipette was used exclusively for recording membrane potential; the second pipette was used for current injection, but over a low range of currents where it was "in balance" the second pipette could also be used to monitor membrane potential. Thus at low current levels the membrane potential could be recorded at two separate points in the cell. Each electrode was shielded over most of its length. Since in previous studies of ours it was shown that Ca^{++} but not Na^+ was a carrier of the inward current of the action potential and that the rate of repolarization of the action potential was decreased by TEA, studies were carried out at elevated $[Ca^{++}]_o$ and in the presence of TEA (45 mM). In current clamp mode, action potentials elicited on anode break and recorded simultaneously in both electrodes were superimposable (within 2%) indicating that the cell was essentially isopotential during the action potential. In voltage-clamp mode, a positive command step gave rise to a net inward current followed by a net outward current. The net outward current reached a peak value after several hundred milliseconds and then declined over a period of seconds. The initial inward current displayed a region of negative slope resistance. In "two pulse experiments" the net inward current was reduced by more than 50% during the second pulse, requiring more than a second for full recovery.

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281.15 CONDUCTION BLOCK IN PERIPHERAL NERVES: A STRUCTURE-ACTIVITY STUDY OF A SERIES OF TRIAZINONE COMPOUNDS. G.A. Condouris, D.M. Havelin*, W.L. Studt* and G.H. Douglas*, Dept. Pharmacol., N.J. Med. Sch., CMDNJ, Newark, N.J. 07103 and W. H. Rorer, Inc., Fort Washington, Pa. 19034.

A series of triazinone compounds was examined on isolated myelinated fibers of the desheathed frog sciatic-peroneal trunks to compare conduction block potency with oil/water partition coefficient and the degree of ionization. Conduction block potency was established for both a simple block of low stimulation frequency (0.5 Hz) and for a block of trains with high frequency impulses (200 Hz). Because of the low water solubility of some compounds, all were first dissolved in a small quantity of dimethylsulfoxide (DMSO). Although DMSO had no effect on nerve excitability, recovery of conduction block was always accomplished with drug-free Ringer solution containing the same concentration of DMSO as in the drug solutions. One member, WHR 1489, was selected as the reference compound for comparisons. Chemically, it is 1-(2'6'-diethylphenyl)-4-methylamino-1,2-dihydro-1,3,5-triazin-2-one-hydrochloride. WHR-1489 proved to have weak simple blocking activity, but substantial ability to block conduction at high frequencies, i.e., it causes high frequency failure (HFF). Simple local anesthetic activity is thus dissociable from HFF activity. Other members of this series had varying proportion of these two pharmacologic actions. They differed chemically by having either diethyl or dimethyl substitutions on the 2,6 positions of the phenyl ring; and also by having one of the following substitutions on the 4-amino position of the triazin: hydrogen, methyl, ethyl, propyl, or butyl.

All compounds have pKa values less than 3, therefore are uncharged at physiologic pH. Oil/water partition coefficients (P) ranged from 1.5 to more than 10,000. Only compounds with P between 10 and 65 had HFF activity without any simple blocking activity. Compounds with P >100 showed both kinds of blocking activity. Three members of the dimethyl series were inactive as blocking agents. Simple anesthetic potency was generally greater for the diethyl series, with the higher N-alkyl derivates showing significant activity; HFF potency resided mainly in the diethyl series.

The results show that uncharged amines can have nerve depressant activity and that HFF activity can reside with a molecule that has low simple local anesthetic potency.

282.1 KAINIC ACID NEUROTOXICITY IN THE RAT LATERAL GENICULATE NUCLEUS. W.R. Woodward and B.M. Coull*. Depts. of Neurol. and Biochem., Univ. of Oregon Health Sci. Ctr., Portland, OR 97201

Local injections of kainic acid (KA) into the nervous system produce destruction of neuronal perikarya while sparing axons and terminals of neurons projecting into the injection site. As a prelude to neurotransmitter studies we have examined the effects of KA injections into the dorsal lateral geniculate nucleus (dLGN) on geniculocortical and corticogeniculate neurons in Sprague Dawley rats.

Projections of dLGN neurons to visual cortex were visualized by autoradiography of radiolabelled, axonally transported phospholipids. Twenty-four hours following ^{3}H-choline injections (30 nl, 100 µCi) into dLGN, silver grains were found over a band of fibers in the outer third of the callosal radiation, immediately subadjacent to cortex, extending from dLGN to layer IV in area 17.

We made slow (5 minute) injections of KA (30 nl of 20 mM KA in buffered saline, pH 7.1) into one dLGN and buffered saline into the contralateral, control dLGN followed by survival times of 10 days. Extensive neuronal degeneration in dLGN, optic radiation, and layer IV of area 17 on the KA injected side was confirmed by a modified Fink-Heimer stain. The distribution of neuronal degeneration products coincided with the choline labelled dLGN fibers described above. On the contralateral, control side, no degeneration was observed.

The functional effects of KA injections into dLGN were assessed by comparing physiological recordings and orthograde axonal transport between injected and control sides. Ten days after a KA injection, microelectrodes containing ^{3}H-proline and ^{3}H-fucose (30 nl, 50 µCi each in saline) were used to record single unit activity from cells in area 17 and then to inject the labelled substances (Woodward & Lindström, Brain Res. 137:37, 1977). In contrast to the many units with definable receptive fields found in cortex on the control side, only spontaneous units with no definable receptive fields were found in cortex on the KA injected side. Following 24 hour survivals, no differences in labelling between control and lesioned sides were found in autoradiographs of labelled material transported from visual cortex to dLGN and superior colliculus.

In conclusion, the neuronal degeneration and alteration in physiological activity in the visual cortex confirm that KA injected into dLGN destroys geniculocortical neurons, and orthograde axonal transport of labelled proteins in corticogeniculate neurons suggests that these neurons are functionally spared.

This work has been supported by the Oregon Medical Research Foundation and by NEI grant 02456.

282.2 SYNAPTIC TERMINALS IN DORSAL LATERAL GENICULATE N. FROM NEURONS OF THE THALAMIC RETICULAR N.: AN ELECTRON MICROSCOPE AUTORADIOGRAPHIC STUDY. V.M. Montero and G.L. Scott*. Dept. Neurophysiology, Univ. Wisconsin, Madison, WI 53706.

Although the projection of TRN on dLGN and on other dorsal thalamic nuclei has been described (Cajal, '11; Jones, JCN 162: 285, '75), its synaptic terminals in these nuclei are still unknown. Also, there is no knowledge about the origin of terminals with flat vesicles in dLGN; however, they are known to survive retinal or cortical lesions. To address these questions, the visual segment of TRN of gray rats was injected with H3 proline to study its projection and synaptic terminals in dLGN, using light and electron microscopy autoradiography (LMAR, EMAR). A short survival time of 6-8 hr was used to avoid transneuronal transport of the label. The LMAR material showed that connections of TRN on dLGN are distributed along projection lines, indicating a retinotopic arrangement comparable to that of striate cortex to TRN projection (Montero et al., Brain Res. 138:407, '77). For the EMAR material, ultrathin sections of dLGN were covered with a layer of Ilford L4 emulsion, exposed for 8-12 wks and developed with D19. The identification of labeled TRN terminals in dLGN was done by quantitative analysis (Ogren and Hendrickson, JCN 188:179, '79) and by examination of labeled profiles in serial sections. Both types of analyses showed that TNR labeled terminals in dLGN are characterized by tightly packed flat synaptic vesicles and dark mitochondria. These F terminals are presynaptic, with symmetrical synapses, to perikarya, and to proximal, intermediate and distal dendrites, frequently adjacent to RL (retinal) or RS (cortical) terminals synapsing the same dendrite. They do not take part in intraglomeruli serial synapses, as P profiles (with pleomorphic vesicles) do, and they are not postsynaptic to other terminals (RL, RS, F or P). These properties are similar to those described for F terminals in rat's dLGN by Lieberman and Webster (J. Neurocytol. 3:677, '74), with the exception of F to P synapses described by these authors. Since there is electrophysiological evidence of inhibitory influences of TRN on dLGN relay cells (Sumimoto et al., Exp. Neur. 51:110, '76; Yigling and Skinner, EEG Clin. Neur. 41:476, '76), these results provide yet another example of association of terminals with flat vesicles and inhibitory synapses (Uchizono, Nature 207:642, '65; Gottlieb and Cowan, Z. Zellf. 129:413, '72). TRN terminals are strategically positioned to block excitatory inputs from retina or visual cortex on dLGN relay cells. These results suggest, in addition, that TRN terminals in other thalamic relay nuclei may also correspond to F terminals that are present in these nuclei.

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282.3 THE NATURE OF THE SUPERIOR COLLICULUS INPUT TO THE LATERAL GENICULATE NUCLEUS. S. Molotchnikoff and P. Lachapelle*. Dept Sciences biologiques, Université de Montréal, Qué. Canada.

We have reported previously that the Superior Colliculus (CS) projects to the dorsal Lateral Geniculate Nucleus (CGL) (Exp. Brain Res. in press). The aim of the present investigation is to study the neurophysiological relationships between the CS and the CGL. This goal was achieved by simultaneously recording single unit activity evoked from both structures in anesthetized and paralysed rabbits. The experimental protocol consisted of three successive steps. In the initial step (first control: (C1) a moving spot was swept across the geniculate cell's receptive field. In the second step (test: T) the same stimulus was used but this time its presentation was triggered by the collicular cell's spontaneous activity; in this way the stimulus presentation was made to be time locked with the CS endogenous neural activity. In the third step, a second control (C2) was repeated. In all three steps the frequency of stimulus presentation was gated to be approximately the same. This sequence was adopted to test over 142 pairs of cells. The geniculate evoked responses thus obtained in each step were compared.

Analysis of the results showed that the CS exerts two separate effects on CGL units. First in 37 pairs (26%) conditioning the stimulus presentation to collicular firing produced a significant enhancement ($P < .005$) of geniculate discharges. Second, in 24 pairs (17%) the CS decreased geniculate evoked responses ($P < .005$). In 82 pairs (57%) no significant effects were observed. Increment effect was more pronounced if the distance (D) between the R.F. was short ($0^{o} < D < 40^{o}$) or if the collicular and geniculate R.F. were far apart ($120^{o} < D < 180^{o}$). On the other hand, the decrement effect was attenuated with the distance separating the two R.F. Furthermore, collicular cells which enhanced the geniculate responses were recorded in the superficial layer of the CS (upper part of SGS), whereas collicular cells leading to the opposite effect were mostly encountered close to the stratum opticum layer.

These results clearly point toward a collicular contribution to the processing of the visual information conveyed by the retino-geniculo-cortical pathway, thus bringing new insights on less known collicular functions.

Supp. DGES and CRSNG to S.M.

282.4 EVIDENCE FOR DIRECT LATERAL GENICULATE PROJECTIONS TO HIPPOCAMPAL FORMATION IN MAN. T. L. Babb, C. L. Wilson, E. Halgren and P. H. Crandall. Brain Research Institute, UCLA, Los Angeles, CA 90024.

Although it has long been known that the geniculostriate fibers pass over and lateral to the temporal horn in man (Meyer's loop), only recently has it been suggested that geniculate axons may actually terminate in the temporal lobe. MacLean and co-workers have demonstrated in the squirrel monkey that neurons in the posterior hippocampal gyrus give tonic on-responses to sustained light and that terminal degeneration may be followed into that gyrus following lesion of the lateral geniculate. Using fine wire microelectrode recordings in epileptic patients (Babb and Crandall, Electroenceph. clin. Neurophysiol. 1976, 40: 225-243) the following evidence has been found to support the notion of a direct geniculate projection to hippocampal formation in man: 1) Neurons give unimodal on or off responses to light (Babb et al., Neurosci. Abstr. 1976, 380). 2) The latencies are as short as 27 msec but may be longer than 100 msec, (Wilson et al., Neurosci. Abstr. 1979). 3) The neurons may respond as a geniculate X-cell by sustained firing to sustained illumination. 4) The rate of firing may be directly related to the light intensity. 5) The neurons may exhibit the Chang-effect. 6) Some neurons have discrete receptive fields similar to those plotted in animal striate cortex.

One of three neurons in one patient having receptive fields was recorded from the right posterior hippocampal gyrus and had the following characteristics: 1) The field was monocular and in the left upper quadrant, 30 degrees from the vertical meridian. 2) The size of the field was 1 degree in the shape of a circle with an on-response to a dim light in the mesopic range while dark adapted (approx. 0.3 ft-l ambient illumination). 3) In the photopic range of background illumination the location of the receptive field was the same but the size and shape decreased to a vertical ellipse 30 mins. by 15 mins. (i.e., a black bar covering that field caused cessation of firing that was sustained).

These results demonstrate that there are lateral geniculate axons that project into the hippocampal formation of man, representing a source of visual information to the limbic system significantly different than the cortico-cortical projections from peri-striate.

282.5 TECTAL AND PRETECTAL PROJECTIONS TO THE LATERAL THALAMUS OF THE RAT. Scott M. Thompson* and Richard T. Robertson (SPON: Earle A. Davis). Department of Anatomy, College of Medicine, University of California, Irvine, CA 92717.

Brainstem projections to the lateral thalamic complex were studied with retrograde transport of horseradish peroxidase (HRP). Small unilateral iontophoretic injections of HRP were made into the lateral dorsal (LD) and lateral posterior (LP) nuclei of the rat thalamus. Frozen sections were cut in the horizontal or transverse plane and the tissue was processed by conventional histochemical techniques using benzidine dihydrochloride as the chromagen.

Injections of HRP confined to LD resulted in retrogradely labelled cells in the pretectum, superior colliculus, ventral lateral geniculate nucleus, zona incerta, and thalamic reticular nucleus. Labelled pretectal cells were most prominant in the ipsilateral nucleus of the optic tract (NTO), olivary nucleus (NOL), posterior pretectal nucleus (NPP), and the anterior pretectal nucleus (NPA). A few labelled cells were seen bilaterally in the medial pretectal region. A few labelled cells were observed at the rostral tip of the superior colliculus in laminae III and IV. Retrosplenial and presubicular cortices were also labelled by anterograde and retrograde transport of HRP from LD injections.

Injections of HRP confined to LP resulted in retrogradely labelled cells in the pretectum, tectum, and the ventral lateral geniculate nucleus. Only a few labelled cells were observed in the pretectal nuclei and these were found ipsilaterally in NTO, NOL, NPP, and the dorsal part of NPA. Labelled cells were observed throughout the rostral-caudal extent of the superior colliculus in laminae II and III. Peristriate cortical areas were also labelled by anterograde and retrograde transport of HRP from LP injections.

Although the distributions of pretectal cells projecting to LD and LP are similar, the number of cells projecting to LD is much greater. Conversely, many more cells in the superior colliculus appear to project to LP than to LD. Further, these cells that project to LP are found over a much greater portion of the superior colliculus. Thus, it appears that parallel neural systems relay visual information to lateral thalamus and then to cerebral cortex. One system involves the pretectum, lateral dorsal nucleus, and limbic cortex; the other system involves the superior colliculus, lateral posterior nucleus and peristriate cortex.

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282.6 FUNCTIONAL PROPERTIES OF CELLS IN THE DORSAL VENTRICULAR RIDGE OF TURTLE (PSEUDEMYS). K.R. Dünser, J.E. Fulbrook, A.M. Granda and J.H. Maxwell. Institute for Neuroscience, University of Delaware, Newark, DE 19711.

The major afferent visual pathway in turtle leads from retina to the contralateral optic tectum, thence via thalamic nuclei (mainly nucleus rotundus) into the dorsal ventricular ridge (DVR). The DVR is characterized anatomically by two types of cells according to Balaban, C.D. (J. Morph., 158: 291, 1978): large spiny neurons with overlapping dendritic fields of more than 300 μm; and aspiny neurons with few primary dendrites.

Extracellular unit recordings were taken from steel and tungsten microelectrodes at various depths in the DVR. Recording sites were identified by electrolytic lesions according to the Prussian-blue staining technique. Receptive fields (RF) were analyzed for size and driving characteristics.

Multicomponent responses were isolated from units apparently corresponding to the large spiny neurons of Balaban. The majority of cells responded to a host of spatial, temporal and chromatic stimuli over most of the monocular visual field. Cells responded well to slight movements anywhere within a RF larger than 140° in diameter but exhibited heightened responsiveness to stimuli falling in the region of visual space corresponding to the linear area centralis. Units habituated quickly to repetitive moving and stationary stimuli. All cells responded to broad chromatic spectra in both light and dark adaptation.

The large RF at later stages of the afferent visual pathway corresponds to similar findings in pigeon, where very large visual fields subtending angles of 150° have been reported in ectostriatum as well as nucleus rotundus (Revzin, A.M. and Karten, H.J., Brain Res., 3: 264, 1966/1967).

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282.7 EFFECTS OF NUCLEUS ROTUNDUS LESIONS ON VISUAL INTENSITY DIFFERENCE THRESHOLDS IN TURTLES. A.S. Powers and R. Frank*. Department of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

Preoperative intensity difference thresholds were assessed in seven eastern painted turtles (Chrysemys picta picta). The method used was to train the animals to discriminate between a very bright and a very dim stimulus. The stimuli were presented on two keys in a standard turtle chamber by a Kodak Carousel projector. The animals were required to make a simultaneous discrimination. After reaching criterion on the training pair (.1 vs. 2.0 log units of optical density) the turtles were trained on a procedure in which 28 trials were given per day, eight with the training discrimination and four with each of five more difficult discriminations. The animals were always reinforced (with beef baby food) for responding to the brighter stimulus (.1 log units of optical density). Since relatively little data could be collected per day, each threshold determination used data from four consecutive days. To determine the threshold, performance on each of the problems was plotted, and the threshold was taken as the point where the turtle responded correctly 75% of the time (50% being chance). Preoperative criterion was reached when the threshold did not vary more than .04 log units of optical density in two consecutive four-day blocks.

Following achievement of preoperative criterion, each animal was given bilateral electrolytic lesions aimed at nucleus rotundus thalami. After a week's recovery the threshold was measured again. Postoperatively, performance on the training stimuli continued to be above 90% correct, but thresholds were elevated in those animals with damage to nucleus rotundus. Two turtles with severe damage to nucleus rotundus (greater than 70% bilateral destruction) had elevations of .20 and .40 log units of optical density respectively and showed no recovery in three to five months of postoperative retraining. Two turtles with slight damage to nucleus rotundus (less than 40% bilateral damage) showed respectively threshold elevations of .15 and .12 log units optical density and recovered to preoperative levels after about two months of postoperative training. Three control animals, two with lesions of the thalamus dorsal to nucleus rotundus and one with a unilateral telencephalic lesion, showed little or no elevation of threshold.

These data suggest that damage to nucleus rotundus in turtles produces a deficit specific to vision. The subjects with lesions of nucleus rotundus showed no apparent impairment in their ability to press response keys, to eat, and to do easy discriminations. Their impairment was limited to the difficult intensity problems.

282.8 VISUOMOTOR CORRELATES OF THE ANURAN ACCESSORY OPTIC SYSTEM. K. Fite, N. Montgomery*, C. Wojcicki* & L. Bengston*. Psychol. Dept., UMass, Amherst, Mass. 01003.

The major component of the accessory optic system in anurans is the nucleus of the basal optic root(nBOR) which receives direct, bilateral input from a variety of retinal ganglion cells via the basal optic root(BOR) (Montgomery, Fite & Bengston, Neurosci. Abstr., 1979). Our recent studies indicate that nBOR also receives input from several cell groups in anterior thalamus which are post-synaptic to the optic neuropils. Both ganglionic and elongate neurons within nBOR project directly to the rostral portion of n. Profundus, which is located below the optic pretectal nucleus and also receives input from the optic tectum. (Based on connectivity and topographical position, n. Profundus appears to be the homologue of n. lentiformis mesencephali) Neurons in the peri-nBOR region, oculomotor complex and anterior-ventral cell groups also receive input from nBOR. The latter give rise to projections to the vestibular nuclei.

Previous studies have suggested that the accessory optic system mediates at least some aspects of visuomotor function, in particular, OKN in frog (Lazar, 1973). However, our recent results indicate that transection of BOR does not consistently eliminate horizontal OKN in Rana pipiens, although changes in the OKN frequency-velocity function may occur. Either direct damage to nBOR or elimination of both BOR and thalamic inputs to nBOR appear to result in a complete loss of OKN. Since large lesions of the pretectal region also are reported to reduce or eliminate OKN (Lazar, 1973; Ingle, pers. comm,), we hypothesize that n. Profundus may be a crucial element in the neural circuitry underlying OKN in anurans.

Monocular enucleation does not eliminate bidirectional OKN, although temporal-to-nasal stimulation is more effective than nasal-to-temporal for the remaining eye. Ipsilateral visual input to nBOR may sustain OKN for the nasal-to-temporal direction in monocular frogs. Preliminary analysis of prey-catching behaviors in BOR-transected frogs revealed an increase in the average latencies of orientations and strikes at live prey throughout the visual field. The accessory optic system of anurans may thus be involved in a variety of visually guided behaviors.

282.9 METABOLIC MAPPING OF AVIAN BRAIN AREAS RESPONSIVE TO RETINAL SLIP. Olivia McKenna and Josh Wallman. Dept. of Biology, City College of the City University of New York, New York, N.Y. 10031

Stabilizing eye movements require detection of retinal slip, i.e. movement of the visual world across the retina. The retinal signal is thought to be transmitted along anatomically distinct visual pathways to sites in the accessory optic system (AOS) and the pretectum (PT). In the AOS, electrophysiological studies have demonstrated that in the mammalian brain cells of the medial terminal nucleus and in the avian brain, cells in two subdivisions of the nucleus of the basal optic root (nBOR), the nBOR proper and the nBORd, are responsive to slow whole-field vertical movement. In the PT, the cells of the nucleus of the optic tract (NOT) in mammals respond to slow whole-field horizontal movement; structures in the avian brain with similar function have not been reported. To identify these areas and to confirm the earlier studies of the nBOR, we undertook a metabolic mapping study in chicks using the 2-deoxyglucose (2DG) method of Sokoloff.

Chicks with heads restrained were placed in a striped drum rotating at 2-4°/sec. After a 10 min pretrial, animals were injected with ^{14}C-2DG (0.16 μCi/g) and returned to the experimental apparatus for 45 min. Alternate brain sections were stained or placed against x-ray film to produce autoradiograms. Since visual pathways of chicks are crossed, differential stimulation of the two eyes provides differential stimulation of the two sides of the brain. In chicks tested with one eye stimulated by vertical moving stripes and the other eye either covered or viewing stationary stripes, the nBOR proper and nBORd contralateral to the stimulated eye became heavily labeled compared to the ipsilateral eye. This confirms the electrophysiological evidence that nBOR proper and nBORd process vertical retinal slip signals. Chicks tested with both eyes viewing horizontal moving stripes developed strongly asymmetric labeling; the lentiform nucleus of the mesencephalon (LM) and the lateral subdivision of the nBOR (nBORl) contralateral to the eye viewing temporal to nasal movement became densely labeled while the LM and nBORl contralateral to the eye viewing nasal to temporal movement showed little or no discernible label. The demonstration that the LM is responsive to horizontal stimulation supplements previous embryological evidence that LM is homologous with NOT. The similar labeling of the nBORl suggests that, although anatomically related to the nBOR, it is functionally related to the LM. The finding that only the side of the brain presented with temporal to nasal movement is heavily labeled argues that, as in the rabbit, the stronger optokinetic nystagmus shown to this direction of stimulation in the bird reflects differences in the sensitivity of the afferent pathways. (Supported by NIH grant EY-2937.)

282.10 NEURONAL MORPHOLOGY IN THE CHICKEN ECTOMAMILLARY NUCLEUS: A GOLGI STUDY. J. D. Peduzzi* and W. J. Crossland (SPON: H. Goshgarian). Dept. of Anatomy, Wayne State Univ., Detroit, MI 48201.

The cellular morphology of the ectomamillary nucleus (EMN) or nucleus of the basal optic root was investigated using the Golgi method. Ten-day old White Leghorn chicks were perfused with 3% glutaraldehyde and 3% paraformaldehyde, processed by Valverde's modification of the rapid Golgi technique, embedded in soft Epon and sectioned at 120 μm. Four types of neurons were found: 1) Multipolar neurons with large cell bodies (> 250 μm^2) which had rapidly tapering, frequently branched dendrites sometimes possessing moderate numbers of spines on the larger dendritic trunks. 2) Neurons with medium to large cell bodies (> 150 μm^2) which had dendrites issuing at opposite poles of the soma. The dendrites had varying numbers of spinous appendages on the larger trunks and resembled the large multipolar neurons. 3) Neurons with medium-sized cell bodies (150-250 μm^2) which had two dendrites leaving the soma at opposite poles or (infrequently) at right angles. These dendrites were long, lacked spines, tapered gradually and made relatively few branches. 4) Neurons with small round cell bodies (50-150 μm^2) which also had dendrites leaving the soma at opposite poles and which lacked spines. These dendrites tapered rapidly and branched more frequently than those in category (3). In general, most of the large and medium sized neurons had large dendritic arborizations extending across one-half to two-thirds of the mediolateral extent of the EMN. Nearly all categories of neurons exhibited relatively small diameter processes with conspicuous dilations along their length.

Although we are unaware of any ultrastructural studies on the chick EMN, as yet, it will be of great interest to determine the relationship of the retinal afferent synaptic terminals to these four cell types and especially to the spine bearing dendrite and varicose processes.

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282.11 ELECTROPHYSIOLOGICAL MAPPING OF THE EXTRAGENICULATE VISUAL THALAMUS IN THE CAT: RETINOTOPIC ORGANIZATION. Denis Raczkowski and Alan C. Rosenquist. Dept. Anat., Sch. of Med., Univ. Pensylvania, Phila., Pa. 19104.

In order to determine the retinotopic organization of the extrageniculate visual thalmus, we have been using single and multiple unit recording techniques to map the lateral posterior-pulvinar (LP-P) complex in lightly anesthetized (N_2O/O_2), paralyzed cats. Thirty minutes prior to recording, each animal was administered chlorolose (40 mg/kg) in a single intravenous injection. Electrode tracks were reconstructed anatomically. These findings were derived from experiments in eight cats. We have evidence for at least three separate representations of all or nearly all of the contralateral visual hemifield in the LP-P complex, which roughly corresponded to the parcellation scheme of this complex based on cortical efferent projections (Updyke, J. Comp. Neur.,173: 81-122, 1977). The representation of central vision was located along the lateral border of P and, again, on either side of the common border formed by LPl and LPi. The visual periphery was representated on either side of the common border formed by P and LPl, and also, along the medial edge of LPi. The mapping data indicted that there was litle or no expansion of central vision in any of these representations. The representation of the horizontal meridian (HM) can be visualized as a broad band oriented orthogonal to the three slab-like zones. Upper visual fields were located dorsal to the HM and lower visual fields were found ventral to the HM. With the exception of both the rostral and caudal portions of the LP-P complex, where the retinotopic organization was more complex, nearly every coronal level throughout the LP-P complex contained a representation of both the upper and lower visual fields. Thus, when viewed in three dimensions, the LP-P complex appears to contain a 'point to line' representation of the visual field, with any point in the visual field represented as a rostro-caudal linear array of points in the extrageniculate thalamus. Supported by EY-05342 and EY-02654.

282.12 LATERAL GENICULATE, LIMBIC AND MIDBRAIN AFFERENTS TO THE SUPRACHIASMATIC NUCLEUS AND PREOPTIC AREA. S. Anschel and M. Shivers*. Lab. of Neurophysiology, Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322.

We previously reported a major convergence of afferents to the medial hypothalamus from the preoptic, lateral septal, and midbrain areas (Anschel, S., Alexander, M., and Perachio, A. A., Neurosci. Abst. 4: 215, 1978, and in preparation). Neurons receiving these diverse inputs were also found to project to extra-hypothalamic areas. Such reciprocal connections are indicative of neurons that might subserve integrative roles in regulating physiological, endocrine and behavioral functions attributed to the medial hypothalamus. In the present study we concentrated on the medial preoptic area (MPO), preoptic area (POA), and suprachiasmatic nucleus (SCN). The type and origin of input that produced short latency responses in single neurons were determined. The efferent projection sites were identified by testing for antidromic activation of the neuron.

A transpharyngeal approach through the surgically exposed optic chiasm was used in urethane anesthetized male rats. Bipolar stimulating electrodes were previously implanted in limbic and midbrain sites. A lateral geniculate bipolar stimulating electrode was positioned at a height where a maximal visual flash evoked potential was recorded at the time of recording. An additional bipolar stimulating electrode was positioned on the surface of the median eminence.

Approximately 300 neurons were tested. Histological verification is complete on 71 neurons (MPO=35, POA=30, SCN=11) responsive to single pulse (0.1 msec, 0.2-2.0 mA) electrical stimulation. The major source of input to this region was from the septal area (both medial and lateral septum). Efferents from the septum were found to converge with inputs from the lateral geniculate nucleus, reticular formation, periventricular grey, region of the mammillary body, amygdala, and recurrent collateral axons of tuberoinfundibular neurons. Additional convergence on an SCN neuron was from the lateral geniculate nucleus and periventricular grey.

Based on tests for antidromic activation of these neurons, it was determined that MPO, POA, and SCN neurons reciprocally innervate limbic areas and midbrain. Reciprocal connections were also found between MPO and lateral geniculate. These findings illustrate the neural substructure whereby secondary sensory information may be processed to regulate the physiological and behavioral functions attributed to this area.

(Supported by NIH Grant #RR 00165)

282.13 AREA 17 AND ITS PROJECTIONS TO THE SUPERIOR COLLICULUS AND LATERAL GENICULATE NUCLEUS IN THE GOLDEN HAMSTERS. K.-F. SO* and L.S. Jen* (SPON: J.C. Hwang). Dept. of Anatomy, University of Hong Kong, Hong Kong.

Area 17 was delineated in the hamsters based on the distribution pattern of projections from the contralateral cortex and from the ipsilateral dorsal nucleus of the lateral geniculate body (LGd). The callosal projections were studied in the brains of 4 hamsters with corpus callosum transection 3 days before sacrifice(Fink-Heimer stain). The geniculocortical projections were obtained in 2 hamsters using the transneuronal method. A mixture of 50 µCi of ^{3}H-Proline and 50 µCi of ^{3}H-Fucose was injected into one eye of each animal. One animal was sacrificed 10 days and the other 20 days later and the brains were processed with the autoradiographic method. It is shown by the degeneration method that area 17 is completely surrounded by callosal projections arising from the contralateral cortex and the result also shows that the caudal part of area 17 continues and passes underneath the caudal pole of the cortex forming a cap-like structure. The distribution pattern of projections from LGd agrees with that of the callosal indicating that LGd projects exclusively to area 17 in the hamsters.

The corticotectal and corticogeniculate projections were studied by placing small iontophoretic injections of tritiated amino acids into various parts of area 17 in 4 hamsters. The animals were perfused one day later and the brains were processed with the autoradiographic method. Corticotectal projections resulted from such an injection were found as a discrete patch of grains located mainly in the lower half of the superficial gray (SG) of superior colliculus although sparse grains could also be detected in the upper half of SG. The distribution pattern of cortical fibers in LGd was in the form of a column running in a rostromedial to caudolateral direction. The cortical projections to the ventral nucleus of the lateral geniculate body (LGv) seems to be more restricted and appears as a small patch of grains. For each of these cortical projections to SC, LGd and LGv, a retinotopic organization was demonstrated.

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282.14 A COMPUTER-BASED PLOTTING AND ANALYSIS SYSTEM FOR BOTH LIGHT AND ELECTRON MICROSCOPES. R. Ranney Mize and John V. Harrell*. Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163

Analyzing the spatial distribution of organelles within biological structures is often essential to understanding their function. We have designed a computer-assisted microscope plotting system which maps the location of organelles within tissues prepared for either light or electron microscopy and analyzes their distribution. The system consists of three components: 1) Optical incremental shaft encoders; these encoders are attached to the stage drives of the microscopes and translate stage drive rotation to electrical impulses. The effective encoder resolution is 0.39 µm for the electron microscope and 14.7 µm for the light microscope. 2) A microprocessor-based conversion/control unit; this device converts the shaft encoder impulses to 16-bit parallel binary code for computer input and provides an LED x-y coordinate readout of stage position. 3) A Hewlett-Packard 9845S 62K byte graphics minicomputer; this computer is used for input-output control during data acquisition as well as for storage, analysis, and graphic representation of data. The software which operates the plotting system is written in enhanced BASIC and includes routines for set-up, tracing, plotting, and analysis. In set-up mode, the operator initializes the system and inputs identifying information about the experiment. In tracing mode, an outline of the biological structure is obtained. To accomplish this the operator moves the stage drives so that the contours of the structure pass under a cross-hair in the microscope eyepiece. This movement produces a digital trace of the structure which is stored by the computer. In plotting mode, the positions of different types of profile within the structure are recorded automatically by pressing a single special function key on the computer keypad. Thirty special function keys are available to encode separate types of profile. In analysis mode, the computer compares trace and plot data and calculates profile depth and medial-lateral distance of each plotted profile from the appropriate contours of the structure. The analysis routine also sorts profile types, calculates profile densities, and graphically represents the plots. Hewlett-Packard utility software statistically analyzes and graphs this data. The system is currently being used to map the spatial distribution of synapses in the visual system with the electron microscope and to plot the position of cytochemically labeled neurons using the light microscope. The system should prove useful in other areas of neurobiological research as well. (Supported by NIH Grant EY-02973 and New Faculty Research Grant H-00025.)

283.1 VIP INCREASES QUANTAL CONTENT AT THE NEUROMUSCULAR JUNCTION. Michael R. Gold and A. R. Martin. Dept. Physiol., Univ. of Colorado Med. School, Denver CO 80262.

Vasoactive Intestinal Polypeptide (VIP) is one of a number of peptidergic hormones that recently have been localized to the nervous system. Previous anatomical and physiological studies suggest a close association between VIP and cholinergic systems (e.g., Phillis et al., Can. J. Physiol. Pharmacol. 56: 337, 1978; Lundberg et al., PNAS 77: 1651, 1980). To assess if there might be direct interactions between these two systems, the effects of VIP on neuromuscular transmission were studied. All experiments were done on neuromuscular junctions in the sartorius muscle of the frog, using conventional electrophysiological and analytical techniques.

In Mg^{++}-blocked preparations (1.5 mM Mg^{++}, 0.3 mM Ca^{++}) bath application of VIP produced a dose-dependent (10^{-8}-10^{-7} M) increase in quantal content of the end-plate potential (e.p.p.) while having no significant effect on quantal size (i.e.miniature e.p.p. amplitude) or resting membrane potential. At a concentration of 10^{-7} M, quantal content was increased by 16 ± 3% (mean ± SEM, n=10). In preparations blocked with curare (2.6 µM) a similar increase (16 ± 5%, n=6) was observed in e.p.p. amplitude, suggesting that the effect of VIP was independent of the level of transmitter release.

In 5 cells the effects of ionophoretic application of VIP onto Mg^{++}-blocked end-plates were studied. Long ionophoretic current pulses (1.8 sec) of 130-220nA, from electrodes containing 0.3 mM VIP, 0.1 M NaCl, 1 mM MES (pH 5.5), produced no change in membrane potential or input resistance in the muscle fibers. However, during such pulses, the quantal content of e.p.p. produced by nerve stimulation was again increased (by 28 ± 5%), while quantal size was unaffected.

These results indicate that VIP modulates neuromuscular transmission by a presynaptic mechanism which increases quantal content. (Supported by NIH grants NS06283 and NS09660)

283.2 TRANSMITTER-LIKE EFFECTS OF ANGIOTENSIN II ON CULTURED SPINAL NEURONS. P. Legendre*, G. Simonnet*, B. Dupouy* and J.D.Vincent. Unité de Neurobiologie des Comportements, U.176,INSERM, rue C. Saint-Saëns,33077 BORDEAUX-CEDEX (France).

Evidence exists that angiotensin II (AII) may play a role in neuronal function (Phillips I. et al., Fed. Proc., 38 : 0008, 1979). The high density of AII immunoreactive nerve terminals found in the substantia gelatinosa and the lateral column suggests, moreover, that AII may act at the spinal cord level (Fuxe, K., et al., Neurosci. Lett., 2 : 229, 1976). The present investigation has studied the effects of AII on the membrane properties of cultured spinal neurons.

Neurons were dissociated from spinal cords of 13 day-old fetal mice and grown in tissue culture according to methods previously described (Barker, J.L. and Ransom, B.R., J. Physiol., 280 : 331, 1978). Intracellular recordings were made on the modified stage of an inverted phase microscope at 30°C using micropipettes filled with 3M KCl.Ten mM Mg^{2+} was added to the bathing medium to block ongoing synaptic activity and thus allow clearer examination of the membrane events. AII (Bachem.) was applied by pressure from an extracellular pipette containing 100 µM (dissolved in the bathing medium) placed close to the cell surface. GABA (Sigma) was iontophoresed from a 0.5 M solution brought to pH 3.5.

Application of AII caused an hyperpolarisation associated with a decrease in membrane conductance in 46 of 109 cells tested. The response was rapid in onset and in offset and no desensitization was observed with sustained application or closely repeated pulses of the peptide. The inversion potential of the hyperpolarizing response to AII (-20 mV) was identical to that of the depolarizing response to GABA recorded from the same cell when using KCl pipette.This suggests that the conductance inactivated by AII is the same conductance activated by GABA i.e. conductance to Cl^-. Furthermore,AII depressed and suppressed in a dose-dependent reversible manner the response to GABA when applied simultaneously. We conclude that AII may have an excitatory role at the level of spinal cord neurons.
(supported by INSERM, ATP 80.79.12. N°12).

283.3 EFFECTS OF MORPHINE AND MET-ENKEPHALIN ON THE NEURONAL RESPONSE TO SUBSTANCE P IN THE MESENCEPHALIC RETICULAR FORMATION OF THE RAT. (D.D. Spring* and H.J. Haigler, Dept. of Pharmacology, Emory Univ., Atlanta, GA 30322)

The mesencephalic reticular formation (MRF) is an area where a nociceptive stimulus (Haigler, Life Sci. 19, 841, 1976) and substance P (SP), a putative nociceptive neurotransmitter (Walker, et al., Experientia 32, 214, 1976) can produce an increase in neuronal firing. In the MRF, morphine (MS) and met-enkephalin (ME), administered microiontophoretically, blocked the excitation produced by a nociceptive stimulus (Hosford and Haigler, J. Pharmacol. Exp. Ther., in press, 1980). SP was administered microiontophoretically in the MRF to determine 1) if there was any correlation between SP and the neuronal response to a nociceptive stimulus (i.e., foot pinch); and 2) if iontophoretically administered MS or ME would block the neuronal response to SP.

Male Sprague-Dawley rats (220-350 gms) were anesthetized with chloral hydrate (400 mg/kg). A five-barrel micropipette was lowered into the brain through a hole drilled in the skull. The micropipette contained ME (10 mM), MS (50 mM) and SP (1 mM) in three side barrels; a fourth side barrel, filled with 4 M NaCl, was used as a current balance barrel; the central barrel, filled with 2 M NaCl and fast green, was used to record single unit activity. Most neurons tested with the nociceptive stimulus responded with a significant ($p < 0.05$:1-way ANOVA) increase in firing. SP, administered microiontophoretically, significantly increased ($p < 0.05$) the neuronal firing in 9 cells; significantly decreased the neuronal firing in 10 cells; and had no effect on neuronal firing in 23 cells. In 5 neurons, both SP and the nociceptive stimulus produced a significant increase in firing. In 1 cell, both SP and the nociceptive stimulus produced a significant decrease in firing. However, in some cases, SP and the nociceptive stimulus did not produce the same effect on neuronal firing. For instance, SP produced a significant decrease in firing in 4 cells in which the nociceptive stimulus produced a significant increase in firing. When tested in conjunction with SP, iontophoretic MS blocked the effects of SP on neuronal firing in 2 of 4 cells; iontophoretic ME, on the other hand, blocked the effects of SP in only 1 of 4 cells.

Because SP did not consistently mimic the response to the nociceptive stimulus (foot pinch) used in this study, SP may not be the neurotransmitter that mediates the type of pain associated with foot pinch. SP may mediate the increase in firing evoked by other types of nociceptive stimuli (e.g., radiant heat). However, SP may also mediate other behavioral effects (i.e., catatonia); MS and ME may also alter these behavioral effects of SP. (Supported in part by NIDA grant 1-R01-DA-01344-04.)

283.4 SOMATOSTATIN INHIBITS SUBSTANCE P-EXCITABLE DORSAL HORN NEURONS OF THE LUMBAR SPINAL CORD. M.F.Piercey and F.J.Einspahr*, The Upjohn Company, CNS Research, Kalamazoo, MI 49001.

According to Hökfelt et al. (Neurosci. 1:131-136, 1976), somatostatin (SRIF) and substance P (SP) exist in separate but comingling populations of small diameter sensory axons synapsing in the spinal cord dorsal horn. We have used multibarreled microelectrodes to iontophoretically eject these peptides onto lumbar dorsal horn neurons of unanesthetized decerebrate low (L_1) spinal cats. Center barrels of the microelectrode assemblies were used for recording nerve impulses extracellularly, while the outer barrels were filled with SRIF (1 mM in 165 mM NaCl), SP (1 mM in 165 mM NaCl), glutamate (500 mM, pH 8), 2M NaCl (for current controls and balancing) and pontamine sky blue (ejected at the end of the experiment to insure dorsal horn locations for recorded cells). As Randic' and Miletic' reported for the sacral cord (Brain Res. 152:196, 1978), we found that SRIF (100-200 nA) sometimes inhibited lumbar dorsal horn neurons. These inhibitions were typically weak (complete cessation of firing rarely occurred) and prolonged, the effects sometimes outlasting the ejection period by several minutes. Some of the cells inhibited by SRIF were excited by SP. Although SP-evoked responses usually had a slow onset, more rapid responses could sometimes be elicited with higher ejection currents. This suggests that slow onsets were due to the unusually poor ejection of SP from microelectrodes (shown in separate in vitro experiments). SP-excitable dorsal horn neurons were also excited by noxious (>45°C), but not by non-noxious cutaneous heat, supporting the hypothesis that SP may be the neurotransmitter for nociceptor afferents. Although it is not yet clear how the inhibitory responses to SRIF relate to sensory transmission, the fact that single dorsal horn neurons possess receptors for both SRIF and SP raises the possibility for complex processing of sensory information in the dorsal horn should the appropriate afferents converge on these cells.

283.5 RECEPTOR-MEDIATED RELEASE OF PLASMA MEMBRANE-ASSOCIATED CALCIUM AND STIMULATION OF CALCIUM UPTAKE VIA VOLTAGE-SENSITIVE CALCIUM CHANNELS BY THYROTROPIN-RELEASING HORMONE IN CULTURED PITUITARY CELLS. K.-N. Tan* and A. H. Tashjian, Jr.* (SPON: P. B. Dews) Dept. of Pharmacol., Harvard Med. Sch. and Lab. of Toxicol., Harvard Sch. Pub. Health, Boston, MA 02115.

GH_4C_1 cells are a clonal strain of rat pituitary cells which synthesize and secrete prolactin (PRL). They have specific functional receptors for the hypothalamic tripeptide thyrotropin-releasing hormone (TRH) which stimulates the acute release of PRL. GH-cells have recently been found to be electrically excitable, and TRH has been reported to enhance the generation of Ca-dependent action potentials (Ozawa, S., PNAS USA, 76:6017, 1979). By kinetic analysis of $^{45}Ca^{2+}$ uptake and treatment of the cells with trypsin-EDTA, we can distinguish calcium bound to the extracellular surface of plasma membrane from the intracellular pool. We report here that TRH has two distinctive effects on calcium fluxes in the cells. First, at 37°C, TRH (10^{-7} M) caused an acute release of calcium from cells into medium in a dose-dependent manner (ED_{50} = 0.8 nM; $t_{½}$ = 15 sec.). Calcium released was mobilized from a cellular compartment which was removed by treatment of intact cells for 10 min. at 24°C with trypsin(0.5%)-EDTA(0.02%) or viokase(0.1%), suggesting that the calcium was released from the outer surface of the cells. The second effect of TRH was to stimulate uptake of calcium into intracellular compartment. ED_{50} for stimulation was ~ 0.2 nM in good agreement with ED_{50} for stimulation of PRL release, 0.3 nM. TRH-enhanced calcium uptake was blocked by 200 ug/ml verapamil or 4 mM Co^{2+} suggesting the involvement of voltage-sensitive Ca-channels. The same concentrations of verapamil and Co^{2+} also inhibited TRH-stimulated PRL release. Calcium uptake, therefore, appears necessary for TRH-induced PRL release. On the other hand, TRH-mediated release of membrane calcium was not blocked by verapamil; calcium release alone was, therefore, insufficient to cause enhanced PRL release. Relative potencies of TRH and four TRH analogs for stimulation of calcium uptake and rapid release of membrane calcium correlated well with their potencies for stimulation of PRL release and affinities for the TRH receptor. TRH did not affect calcium fluxes in pituitary cells lacking TRH receptors. We conclude that two types of calcium movements in GH_4C_1 cells are regulated by the TRH receptor and that an increased uptake of calcium into the intracellular compartment via voltage-sensitive Ca-channels is required for TRH-enhanced PRL release.

283.6 NEUROTENSIN BLOCKS BEHAVIORS INDUCED BY INDIRECT, BUT NOT DIRECT DOPAMINE AGONISTS: EVIDENCE FOR MEDIATION AT A MESOLIMBIC DOPAMINE TERMINATION SITE, THE NUCLEUS ACCUMBENS. C. B. Nemeroff, D. Luttinger, A. J. Osbahr, III*, A. J. Prange, Jr.* Biol. Sci. Res. Ctr., Dept. Psychiatry, and the Neurobiology Program, University of North Carolina School of Medicine, Chapel Hill, NC 27514.

Neurotensin (NT), a neuropeptide, has been reported to produce a variety of effects after CNS administration in rodents including muscle relaxation, hypothermia, analgesia and potentiation of pentobarbital and ethanol-induced sedation (Brain Res. 128:485, 1977; PNAS 76:5368, 1979). Many of these properties of NT are shared by antipsychotic drugs. The purpose of the present study was two-fold: (1) we have previously shown that intracisternally (IC) administered NT blocks the increase in locomotor activity seen after d-amphetamine administration in mice. The effect of NT on the locomotor hyperactivity induced by another indirect-acting dopamine (DA) agonist, cocaine (20 mg/kg subcutaneously [SC]) and by two direct acting DA agonists: apomorphine (5 mg/kg, SC) and lergotrile (10 mg/kg, SC) were evaluated utilizing circular photocell cages. Such studies aid in discerning the locus of action (presynaptic vs postsynaptic) for the apparent "antidopaminergic" effects of NT. NT (1 µg IC) blocked the increase in locomotor activity induced by d-amphetamine and cocaine ($p < 0.05$) but did not alter apomorphine or lergotrile-induced hyperactivity. (2) The direct bilateral injection of NT or haloperidol into the nucleus accumbens (a termination site of the mesolimbic DA system) of rats significantly blocks the behavioral effects (↑ locomotor activity and rearing) of a low dose of d-amphetamine (2 mg/kg, IP) (Biol. Psychiat. 15:283, 1980). These results were extended by evaluating the effect of direct bilateral injection of NT or haloperidol into the caudate nucleus (a termination site of the nigrostriatal DA system) on stereotypies induced by a higher dose of d-amphetamine (5 mg/kg, IP). The incidence of certain behaviors was assessed by a trained observer ignorant of the treatment regimen (Brain Res. 132:507, 1977). NT injections (3 or 5 µg) into the caudate nucleus did not block d-amphetamine-induced stereotypy, whereas haloperidol (5 µg) did. Conclusions: (1) NT blocks the effects of indirect, but not direct DA agonists. (2) NT blocks d-amphetamine-induced behaviors thought to be mediated by the mesolimbic DA system but not those mediated by the nigrostriatal DA system. These findings, taken together with the current view of the importance of the mesolimbic DA system in the pathogenesis of schizophrenia, indicate that NT, which is found in high concentration in mesolimbic regions, may modulate dopaminergic function in regions thought to be involved in the pathogenesis of schizophrenia.

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283.7 INHIBITION OF CONDITIONED AVOIDANCE RESPONDING AND REWARDING ELECTRICAL SELF-STIMULATION OF THE BRAIN BY CENTRALLY ADMINISTERED NEUROTENSIN. D. Luttinger, R. Wiggins*, R. A. King, C. B. Nemeroff, A. J. Prange, Jr.* Biological Sciences Research Center, Depts. of Psychiatry & Psychology, and the Neurobiology Program, University of North Carolina, Chapel Hill, North Carolina 27514.

Neurotensin (NT), an endogenous tridecapeptide, produces several effects after CNS administration which are similar to effects produced by neuroleptics. These similarities include hypothermia, muscle relaxation, and antagonism of amphetamine-stimulated locomotor behavior. To extend the investigation of similarities of the effects of NT and neuroleptics, the actions of NT were assessed in adult male albino rats in two behavioral paradigms commonly used as screens for neuroleptic activity.

The effect of intracerebroventricular (ICV) NT on discrete trial avoidance responding in rats implanted with a lateral ventricular cannula was qualitatively similar to the effect of neuroleptics. Every thirty seconds an avoidance trial was initiated with the onset of a tone and light conditioned stimulus; if the rat did not move to the other side of the box within ten seconds, it was presented with the unconditioned stimulus--a 0.7 mA footshock for a maximum of five seconds. After a stable baseline criterion of greater than 90% avoidance responding was obtained, the rats were injected with NT (0.3-30 µg/10 µl) or 0.9% saline (10 µl), 30 min before behavioral testing. NT (⩾ 1 µg) decreased avoidance responses by approximately 30%; escape responses were unaffected. ICV administration of ß-endorphin and bombesin, two peptides previously shown to produce similar effects to NT, did so in this paradigm as well. Several neuropeptides did not affect avoidance responding after ICV administration: TRH, LHRH, MIF-I, bradykinin, and substance P.

In the self-stimulation paradigm, a unipolar electrode was placed in the A_{10} region and a cannula was implanted in the ipsilateral nucleus accumbens. Every lever press produced brain stimulation and the current (45 to 85 µA) was adjusted for each animal so that stable rates of responding were obtained (6000 to 9000 responses/hour). Injections of NT (1-5 µg/2 µl 0.9% saline vehicle), like neuroleptics, produced a dose-dependent decrement in the rate of self-stimulation. The 5 µg dose resulted in a 30-50% decrement; the 2 µg dose 20-40%; 1 µg exerted no effect. The onset of the decreased rate of self-stimulation after NT was observed approximately 15 min post injection and remained depressed for at least 45 min.

Conclusion: In both paradigms, the effects of NT were similar to neuroleptics. The data, together with previous findings, suggest that neurotensin may exert neuroleptic-like effects.

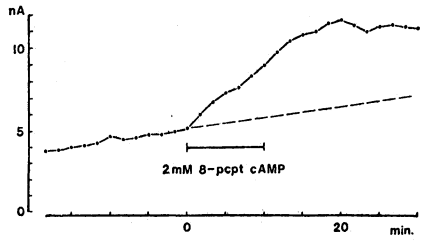
This work was supported by NIMH MH-32316, NH-22536, MH-33127 and NICHHD HD-03110.

283.8 HYPOTHERMIA AND INHIBITION OF FEVER INDUCED BY CENTRAL ADMINISTRATION OF ACTH AND α-MSH. J.R. Glyn* and J.M. Lipton. Depts. of Physiology and Neurology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Preliminary testing of the effects on body temperature of several peptides yielded two, ACTH(1-24) and α-MSH, which lowered body temperature (Lipton, J.M. & Glyn, J.R., Peptides, 1:15, 1980). In the present experiments, this response was dependent on the ambient temperature (T_a) in that both ACTH and α-MSH (1.25-5µg ICV) caused dose-related decreases in body temperature at T_a = 23°C, greater hypothermia at T_a = 10°C, but no temperature change at T_a = 30°C. Leukocytic pyrogen-induced fever and PGE_2-induced hyperthermia were also reduced by α-MSH and ACTH (5µg ICV). The reduction in temperature was associated with peripheral vasodilitation. The small peptide α-MSH is contained within ACTH (residues 1-13), and the shared sequence could be responsible for the effects on temperature. ACTH has been used clinically to reduce fever (Kass, E.H. & Finland, M., New Engl. J. Med., 243:693, 1950) and is released during endotoxin fever in man (Conn, J.W., et al., Recent Progress in Hormone Research, 10:471, 1954). ACTH and α-MSH are found in hypothalamic areas involved in temperature regulation (Kreiger, D.T., Liotta, A. & Brownstein, M.J., Brain Res. 128:575, 1977; O'Donohue, T.L., et al., Neuroendocrinology 27:281, 1979); therefore, these peptides may have a role in central thermoregulation in afebrile and febrile states. (Supported by National Institutes of Neurological and Communications Disorders and Stroke Grant NS 10046.)

284.1 Ca-ACTIVATED K CURRENT IS ENHANCED IN MOLLUSCAN NEURONS BY AGENTS THAT ELEVATE INTRACELLULAR CYCLIC AMP CONCENTRATION. D. Ewald & R. Eckert. Dept. of Biology, UCLA, Los Angeles, CA 90024.

Calcium ions were iontophoretically injected into somata of neurons of the Aplysia abdominal ganglion voltage-clamped at -30 to -40 mV in 1/10 K ASW. Ca pulses 1 to 2 sec long elicited outward current transients of 2 to 10 nA lasting 2 to 4 sec (measured at half peak amplitude) which reversed at the K equilibrium potential. Such current transients were produced with constant Ca injections delivered at 100-sec intervals for up to 3 hr. Agents known to increase intracellular cyclic AMP concentrations were superfused onto the ganglion in concentrations of up to 2 mM for periods of 10 min. Derivatives of cyclic AMP which resist hydrolysis by phosphodiesterase caused a significant increase in the amplitude of the K current. The magnitude of this increase was 30-50% (n=3) in response to 8-benzylthio cAMP and 40-120% (n=3) in response to 8-p-chlorophenylthio cAMP (Fig.). Simultaneous exposure to dibutyryl cAMP and either methylxanthine or imidazoladinone phosphodiesterase inhibitors caused an increase in amplitude of 30-40% (n=3) which was readily reversed at the end of exposure. The adenylate cyclase activator cholera toxin increased the K current amplitude 20-60% (n=4). Enhancement of the Ca-activated K current by these agents might come about by 1) an increased rise in intracellular levels of ionized Ca^{++} during Ca injections, due to reduced Ca-buffering capacity of the cytoplasm, or 2) an increase in the sensitivity of the K channel to Ca^{++}. (Supported by NIH grant NS08364.)



284.2 SEROTONIN STIMULATES PHOSPHORYLATION OF A 137,000 DALTON MEMBRANE PROTEIN IN THE ABDOMINAL GANGLION OF APLYSIA. C. Gregory Paris,* Eric R. Kandel, and James H. Schwartz. Div. of Neurobiol. & Behavior, Depts. of Physiol., Psychiat., and Neurol., Columbia Univ., P&S, New York, N. Y. 10032.

There is considerable evidence that a serotonin-stimulated adenylate cyclase initiates the synaptic events underlying sensitization of the gill withdrawal reflex in Aplysia. We therefore searched for proteins that might be phosphorylated, focusing on membrane components because they are likely to mediate the changes in Ca^{++} current implicated in the presynaptic facilitation of the reflex.

We have obtained reproducible SDS gel electrophoretic profiles of phosphorylated membrane proteins from excised abdominal ganglia incubated for 0.5 to 3 hours in $^{32}P_i$. We have identified a single protein (molecular weight 137 kD) whose extent of phosphorylation was increased dramatically in ganglia incubated in the presence of 10^{-4} M serotonin. This concentration was previously found to increase the content of cAMP in the ganglion maximally. The effects of serotonin were mimicked by dibutyryl-cAMP (10^{-4} M). Demonstration of ^{32}P-phosphorylation depended on the inclusion of a phosphodiesterase inhibitor: Ro-20 and IBMX, both at 10^{-4} M, were equally effective and much better than theophylline at 10^{-3} M.

From the physiological point of view, the phosphorylation was unexpectedly slow. The 137 kD protein was not visibly labeled after the ganglion had been exposed to serotonin for 0.5 hours; at one hour the protein was incompletely labeled; by two hours maximal labeling had occurred, and persisted for at least an additional hour. A partial explanation for these kinetics may be that exogenous $^{32}P_i$ only slowly becomes available to a critical pool of ATP in the ganglion. In support of this explanation, we found that $^{32}P_i$ was incorporated into ATP at a constant rate for as long as 24 hours.

We have found that the phosphoprotein is a membrane component. Membranes were prepared from the incubated ganglia, after removal of the bag cell clusters, and were exhaustively washed in a buffer of low ionic strength. In addition, the phosphorylated protein was not found in the cytoplasmic fraction of stimulated or unstimulated ganglia.

To localize the regions where phosphorylation takes place we isolated cell bodies and neuropil by dissection. Relatively small amounts of the protein were phosphorylated in cell bodies and neuropil from unstimulated ganglia. Exposure to serotonin greatly increased phosphorylation of the protein in both regions of the ganglion. A glial cell origin for this protein has not yet been ruled out. Experiments are now in progress to determine in single neurons where the protein is phosphorylated.

284.3 RELATIONSHIPS BETWEEN CYCLIC NUCLEOTIDES AND ELECTROPHYSIOLOGIC ACTIVITY IN HIPPOCAMPAL SLICES. J.A. Ferrendelli, A.C. Blank*, D.A. Kinscherf* and E.W. Lothman. Div. of Clin. Neuropharm. and Depts. of Neurol. and Pharm., Washington Univ. Med. Sch., St. Louis, MO. 63110.

Previous studies have indicated that cyclic AMP and cyclic GMP may be involved in pathophysiologic mechanisms of epilepsy. To better define the relationships between seizures and cyclic nucleotides we have examined the effects of [K+] on electrical activity and endogenous levels of cyclic AMP and cyclic GMP in incubated slices of hippocampus. In addition, the actions of exogenous cyclic nucleotide derivatives were assessed.

Tissue slices, 350 - 400 mμ thick, were prepared from freshly killed guinea pigs using conventional methods and incubated in an oxygenated physiologic buffer, pH 7.4, containing 127 mM NaCl, 2 mM KCl, 1.5 mM $MgSO_4$, 1.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.1 mM KH_2PO_4 and 10 mM glucose. Tissues were analyzed for cyclic nucleotide content by radioimmunoassay as described previously. Standard techniques for recording from CA_3 and stimulation of mossy fibers were used.

In control media the tissue slices exhibited random single unit discharges, and stimulation of mossy fibers produced complex evoked potentials of 10 - 20 msec duration in CA_3. Control levels of cyclic GMP and cyclic AMP were 0.92 ± 0.03 and 14.6 ± 0.7 pmoles/mg prot, respectively. Increasing the [K+] in the incubation media had a marked effect on both electrical activity and cyclic nucleotide levels. Cyclic GMP levels were increased 50 - 100%, in a dose-dependent manner by [K+] between 6 and 15 mM, and cyclic AMP levels were depressed 50%. At 6 - 9 mM K^+, evoked potentials were more complex and of longer duration, and spontaneous activity markedly increased. In most slices, 6 - 9 mM K^+ produced epileptiform activity. Higher [K+] progressively depressed activity, and with 15 mM K^+ there was electrical silence. At normal [K+] (3.1 mM), isobutylmethylxanthine (100 μM) elevated cyclic GMP levels, depressed cyclic AMP, and produced increased spontaneous and epileptiform activity in the slices. Addition of 8-Br-cyclic GMP (0.03 - 0.1 μm) also augmented evoked potentials, increased spontaneous activity, and, in some slices, produced epileptiform activity. In contrast, 8-Br-cyclic AMP (0.1 - 10 μm) always reduced activity.

The results of this study suggest electrophysiologic activity in hippocampal slices is positively correlated with cyclic GMP concentration and negatively correlated with cyclic AMP concentration. In addition, the results support the hypothesis that cyclic GMP is involved in seizure genesis and/or maintenance and cyclic AMP may have a role in seizure suppression.

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284.4 GUANOSINE 3';5'-MONOPHOSPHATE (cGMP) ACCUMULATION BY AND RELEASE FROM RAT SUPERIOR CERVICAL GANGLIA. R. L. Volle, B. A. Patterson* and L. F. Quenzer. Dept. of Pharmacol., Univ. of Connecticut, Farmington, CT 06032.

Ganglia treated with theophylline (1 to 10mM) accumulate cGMP when bathed in solutions containing elevated [K^+] (30 to 60mM) or sodium azide (NaN_3; 10^{-5} to 10^{-3}M). Intact and denervated ganglia contained 0.67 pmol cGMP·mg^{-1} protein. When $[K^+]_o$ was increased to 60mM in intact ganglia, cGMP content was raised to 30.8 pmol·mg^{-1} protein. K^+ had no effect on the cGMP content of denervated ganglia or on intact ganglia bathed in solutions lacking Ca^{++}. With intact ganglia, NaN_3 (10^{-4}M) increased cGMP content to 5.7 pmol·mg^{-1} protein. The response to NaN_3 was reduced by 40% in denervated ganglia and unaffected by low $[Ca^{++}]_o$. The loss of response to K^+ following preganglionic nerve section, paralleled the loss of ganglionic choline acetyltransferase activity and was complete within 24 hrs. The decreased response to NaN_3 was complete 48 hrs after nerve section. Cholinergic drugs (atropine, hexamethonium and eserine) had no effect on the cGMP response to K^+. Release of cGMP into the medium followed cGMP accumulation by the ganglia. K^+-induced release occurred only from intact ganglia and required Ca^{++}. The amount of cGMP in the medium was increased by 60mM K^+ from 0.02 pmol·ml^{-1}·ganglion to 0.20 pmol·ml^{-1}·ganglion after 10 min incubation. cGMP release produced by NaN_3 (10^{-3}M) was increased from 0.012 to 0.032 pmol·ml^{-1}·ganglion. When Ca^{++} was omitted from the medium, NaN_3 increased cGMP to 0.30 pmol·ml^{-1}·ganglion. These results suggest that cGMP accumulation occurs in pre- and postganglionic neurons, that depolarization per se cannot account for the responses to K^+ and that acetylcholine is not involved in the accumulation of cGMP caused by K^+. (Supported by NS 07540-13).

284.5 ELECTRICAL STIMULATION OF THE SUPERIOR CERVICAL GANGLIA INCREASES THE CONCENTRATION OF CYCLIC AMP OF RAT PINEAL GLAND. W.E. Heydorn, B. Weiss and A. Frazer. Depts. of Psychiat. and Pharmacol., Univ. of Penna., Dept. of Pharmacol., Med. Col. of Penna., and Vet. Adm. Hospital, Phila., Penna. 19104.

There is as yet no clear demonstration that electrical stimulation of discrete sympathetic nerves increases the concentration of cyclic AMP in a well-defined sympathetically innervated end organ. Recent results showing that desmethylimipramine (DMI) potentiates the effects of exogenously administered norepinephrine (NE) in elevating cyclic AMP levels in rat pineal gland suggested the means by which one might demonstrate a rise of cyclic AMP induced by electrically stimulating its sympathetic input. Rats were kept in constant light for 6 days prior to sacrifice. Animals were decapitated, both superior cervical ganglia (SCG) were rapidly isolated, attached to platinum bipolar electrodes and stimulated with square wave pulses. After stimulation, pineal glands were removed and frozen on dry ice within 20 sec. Cyclic AMP was measured by radioimmunoassay. In the absence of DMI, stimulation of the SCG produced small and inconsistant increases in cyclic AMP in pineal gland. However, in rats pretreated with DMI (40μmoles/kg, i.p., 1 hr before decapitation) electrical stimulation of the SCG produced a reproducible, marked (greater than 5-fold) and highly significant ($p<0.001$) elevation of cyclic AMP. In the DMI-treated animals, maximal elevations of cyclic AMP were found following 1 min of stimulation using 10V, 1 msec duration and a stimulus frequency of 10 Hz. Administration of reserpine, which depletes NE in sympathetic nerve terminals, blocked the rise of cyclic AMP caused by electrical stimulation of DMI-treated rats. Bretylium, which inhibits the release of NE caused by nerve stimulation, also reduced the elevation of cyclic AMP induced by SCG stimulation. Pretreating rats with the beta-adrenergic blocking agent, propranolol, prevented completely the increase of cyclic AMP caused by electrical stimulation whereas pretreating rats with the alpha-adrenergic antagonist, phentolamine, did not. These results demonstrate for the first time a pronounced elevation in cyclic AMP in a sympathetically innervated organ induced by electrically stimulating discrete sympathetic fibers innervating this organ. They indicate further that the increases in cyclic AMP are due to NE, released from presynaptic nerve terminals, stimulating β-adrenergic receptors located on the postsynaptic cell membrane. Supported by funds from the Vet. Adm., USPHS Grants GM 07302, MH 29094, MH 30096 and NS 16242.

284.6 EFFECT OF TREATMENT WITH CYCLIC AMP (cAMP) AND PARACHLOROPHENYLALANINE (pCPA) ON THE EEG AND BEHAVIORAL ACTIONS OF MORPHINE IN THE RAT. K. Sharifi Hossaini and M.S. Shahid Salles. Departments of Pharmacology and Physiology, Medical School, Shiraz University, Shiraz, Iran

Rats were prepared with permanent electrodes for recording EEG and EMG. Morphine injections of 10 mg/kg given s.c. were followed by the appearance of high voltage EEG slow bursts associated with stuporus behavior. This phase was suppressed by the appearance of behavioral arousal shown by EMG and behavior of the rat and afterwards behavioral sleep became apparent on the EEG. Administration of a 10 mg/kg dose of morphine to rats pretreated with pCPA (100 mg/kg/day for 3 days) was followed by an almost immediate EEG and behavioral arousal with only a few intervening episodes of behavioral stupor associated with slow bursts. Combined treatment with pCPA and cAMP (50 mg/kg, i.p., 24 hrs before morphine) does not affect the antagonistic effect of cAMP on morphine analgesia (Shahid Salles et al., Sleep Research 1 : 1979). The result of tailflick-tests in another series of animals pretreated with pCPA (100 mg/kg, i.p./day, for 3 days) or both pCPA and cAMP (50 mg/kg, i.p., 24 hrs before morphine) were in agreement with those obtained from our EEG recording. This may suggest that there is no interaction between cAMP and brain serotonin in the mediation of morphine's analgesic effect.

285.1 THE SPINAL CORD DORSAL HORN IN AN ANIMAL THAT LACKS UNMYELINATED AXONS IN THE DORSAL ROOT. Stewart C. Birse and Richard E. Coggeshall. Depts. of Anatomy and of Physiology and Biophysics and the Marine Biomedical Institute. The University of Texas Medical Branch, Galveston, Texas 77550.

The nervous system of the stingray, Dasyatis sabina, is remarkable because there are no unmyelinated axons (C fibers) in the dorsal roots and peripheral nerves. This presumably implies that the spinal cord does not receive a primary afferent C fiber input. In view of recent studies suggesting that a major input to the substantia gelatinosa in mammals consists of C fibers from the dorsal roots, the stingray offers an interesting opportunity for a comparative analysis of spinal cord organization.

The dorsal horn of the stingray is relatively large. Overlying the dorsal horn is a prominent layer of myelinated and unmyelinated axons which are in the same position as the primary afferent axons in the dorsal white columns of mammals. Under this fiber layer is an area that closely resembles the mammalian substantia gelatinosa. In the ray, this area is large, proportionately much larger than in the mammal or in a teleost, the goldfish. Myelinated fibers are almost completely absent and small, closely packed neurons form the major cellular component of the layer. Synaptic glomeruli, which consist of a central, relatively electron dense axonal terminal surrounded by numerous small dendrites, are common. These appear identical to the glomeruli which are regarded as the most characteristic features of mammalian substantia gelatinosa. Thus, in an animal that has a spinal cord that seems to lack a primary afferent C fiber input, the substantia gelatinosa is indistinguishable cytologically and proportionately larger from the substantia gelatinosa in mammals.

Subjacent to the gelatinosa is a region of gray matter which resembles the mammalian nucleus proprius. This area contains many myelinated axons. Dorsal root section leads to a wedge-shaped area of degenerating axons located in the lateral funiculus, substantia gelatinosa, and nucleus proprius in the segment of root entry. Rostral and caudal to the lesion, degeneration is prominent only in the nucleus proprius. Degeneration products can be traced for only one or two spinal cord segments and are never found in the dorsal fiber system.

These latter results suggest that primary afferents 1) synapse within one or two segments of their entry into the ray spinal cord, and 2) do not branch to form a dorsal column system as in the mammalian CNS. Therefore ascending sensory information is probably carried by second order myelinated neurons in the stingray. This work was supported by grants NS 06309, NS 10161, NS 11255, and NS 07377.

285.2 SEGMENTAL PATTERN OF THE EVENTS ASSOCIATED WITH THE EARLY DEVELOPMENT OF MARGINAL ZONES IN *XENOPUS* SPINAL CORD. Golda A. Kevetter and Raymond J. Lasek, Cept. of Anatomy, Case Western Reserve Univ., Cleveland, OH 44106

We have been studying the morphological transformations that result in longitudinal columns of axon pathways throughout the rhombencephalon and spinal cord of *Xenopus*. Previously we observed that the marginal zone developed in an orderly progression in both the rostrocaudal and dorsoventral axes (Anat. Rec., 1980, 196: 96). Early axons were located in fascicles on the ventrolateral border of the developing brainstem. The development of the marginal zone originated from foci located adjacent to differentiating cells throughout the rostral medulla. The pattern of marginal zone development reflected the addition of axons within fascicles and also the addition of fascicles of axons.

In order to analyze the early events which preceeded the longitudinal organization of the marginal zone, we examined 1 µm serial sections through the rhombencephalon and spinal cord of embryos stages 22-28 which had been embedded in epon. We investigated this rostrocaudal series for the segmental organization which preceeded the longitudinal formation of the marginal zone.

Axons were present before a distinct marginal zone could be identified. Longitudinally-directed axons were present at stage 22, shortly after the formation of the neural tube. The earliest axons passed lateral to differentiating cells and traveled along the periphery of the neural tube. The first longitudinally-directed axons appeared in the mid-medullary levels on the lateral aspects of the brainstem. Electron microscopy through the medulla at stage 22 verified that a small number (1-6) of axons were grouped in fascicles along the peripheral border. These axons were surrounded by ependymal processes.

In contrast, no longitudinal axon pathways were observed in the caudal rhombencephalon and rostral spinal cord at stage 22. However, rostral to the exit of the dorsal or ventral root, a small fascicle appeared which contained several broad cytoplasmic processes cut in cross section. Each bundle of processes could be followed caudally for short distances to the exit of the nerve root. Fascicles could not be identified immediately caudal to the exit of the ventral or dorsal root. This suggested that these processes exited with their associated nerve root. This segmental pattern of processes grouped into fascicles and involved with exiting nerve roots was repeated throughout the rhombencephalon and spinal cord.

Our results suggest that development of the segmental organization seen in *Xenopus* preceeds development of the longitudinal pattern. The segmental development is likely to be associated with the longitudinal pattern of marginal zone development.

285.3 MORPHOLOGICAL DEVELOPMENT OF SENSORY AXONS AND MOTORNEURONS IN THE BRACHIAL SPINAL CORD OF BULLFROG TADPOLES (RANA CATESBIANA) Sonal Jhaveri and Eric Frank. (SPON. P, Baccaglini) Dept. of Neurobiology, Harvard Med. Sch., Boston, MA. 02115.

The ingrowth of dorsal root (DR) axons and the development of motor neuron dendrites was studied for the 2nd. nerve, which innervates the foreleg, in tadpoles (stage 4 and older) and in adult frogs. By stage 13, sensory axons form a ventral neuropil region into which motorneuron dendrites enter, scantily at first, but densely by stage 17, when an adult-like pattern of distribution is observed.

A pellet of horseradish peroxidase was applied to the central stump of the 2nd. nerve, cut peripheral to the DR ganglion. After 15-24 hours, the animal was fixed and the spinal cord reacted with diaminobenzidine for the visualization of the transported enzyme. Diffuse filling of motorneurons and DR axons was observed.

In the adult frog, thick sensory axons course longitudinally in the dorsal cord. Finer axons enter the grey matter of the cord, forming two distinct neuropil regions: dorsomedially and ventrolaterally. Motorneuron dendrites are oriented ventromedially, dorsolaterally and dorsomedially. The dorsomedial dendrites enter the ventral neuropil, where they overlap extensively with the sensory axons.

In stage 4 tadpoles, longitudinally coursing DR axons are evident, but ventrally coursing fibers are scant, thin and are tipped by growth cones. The number of these thinner fibers increases throughout development until, by stage 10, the two neuropil regions are clearly distinguished.

Motorneuron dendrites develop concomitantly with the differentiating sensory axons which will contribute to their input. In stage 4 tadpoles, motorneurons bear dendrites oriented ventromedially and dorsolaterally. However, the dorsomedial dendritic field is scant; few motorneuron dendrites are observed close to the ingrowing sensory axons. Although all three dendritic fields increase in complexity throughout development, the most striking is the growth of the dorsomedially directed dendrites. Thus, as the differentiation of the ventral neuropil region of the sensory axons occurs, more and more motorneuron dendrites enter into it and lie in juxtaposition to the incoming axons.

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285.4 DEVELOPMENT OF THE MONOSYNAPTIC REFLEX IN THE FROG'S SPINAL CORD M. Westerfield* and E. Frank. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Development of stretch reflexes of the triceps muscles in the forelimb of bullfrogs was studied by electrical recordings from motoneurons in isolated spinal cords. In adults, there is a high degree of synaptic specificity: a) sensory afferents from triceps muscles have monosynaptic connections only with triceps motoneurons; b) polysynaptic and inhibitory responses from triceps sensory axons are seldom observed either in triceps or other brachial motoneurons. In contrast, during development triceps sensory afferents evoke polysynaptic responses in brachial motoneurons before most monosynaptic connections have appeared. When triceps afferents do form monosynaptic inputs onto motoneurons, these synapses are made specifically onto triceps motoneurons.

At stage 14, when touching the skin evokes reflex movements, triceps sensory afferents generate synaptic potentials in motoneurons within the triceps motor nucleus. These potentials are probably polysynaptic because they occur at latencies greater than 20 ms, show large latency fluctuations and fatigue at frequencies of 1 Hz. Recordings from the cut ventral root show a similar long latency signal indicating that the intracellular recordings did not miss a population of motoneurons with monosynaptic inputs from triceps afferents. This pattern persists until stage 17 when such potentials can still be recorded in most brachial motoneurons. At this time, however, short latency potentials are produced in some brachial motoneurons by stimulation of the ulnaris nerve, which innervates muscle and skin distal to the triceps muscles. This suggests that some sensory fibers innervating more distal parts of the arm form monosynaptic reflexes before triceps sensory axons, unlike the normal proximal-distal gradient of development. Near the end of stage 17, many motoneurons can first be identified by antidromic activation, and short latency, 5-10 ms, synaptic potentials are evoked by triceps sensory afferents in triceps motoneurons. In addition, these afferents still produce excitatory and inhibitory polysynaptic potentials in many motoneurons. By stage 19, one stage before the arms emerge through the body wall, synaptic input from triceps afferents has the same latency, 3-4 ms, and specificity seen in adults, and the polysynaptic potentials are reduced.

These results indicate that a monosynaptic reflex arc develops only after polysynaptic connections are formed. The monosynaptic pathway is highly specific from the time it first appears.

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285.5 DEVELOPMENT OF THE ANURAN SPINAL CORD: ASCENDING AND DESCENDING SYSTEMS. Cynthia J. Forehand and Paul B. Farel. Neurobiol. Prog. and Dept. Physiol., Sch. Med., Univ. N. Carolina, Chapel Hill, NC 27514.

Anurans present a unique opportunity to study the ontogeny of neuronal connections subserving tetrapod locomotion and somatic sensibility. Transition from the aquatic, limbless tadpole to juvenile frog (R. catesbeiana) occurs over a protracted period of time during which the animal is accessible for experimental observation. Using horseradish peroxidase (HRP), we have found that developmental changes in sources of descending fibers consist primarily of increases in size and number of retrogradely labeled supraspinal neurons. In contrast, differences in the sources, as well as the extent, of ascending projections were found in comparisons of tadpole and mature frog.

In both limb bud tadpole and adult frog, HRP injections into the lumbar enlargement retrogradely labeled neurons bilaterally in the paraventricular area of the hypothalamus, midbrain tegmentum, ventral vestibular nucleus, and reticular formation. In early tadpole stages the tegmental projection arises only from cells in the caudal midbrain. By Stage XIII, however, lumbar projections of rostral tegmental neurons are identified. In all stages the bilateral vestibulospinal projection is asymmetrical, arising from neurons in the caudal half of the contralateral ventral vestibular nucleus and the entire extent of the ipsilateral ventral nucleus.

More striking developmental differences are seen in ascending systems. Lumbar spinovestibular cells, while numerous in the frog, are rarely observed before tadpole Stage XV. Similarly, projections to vestibular nuclei by lumbar primary afferent fibers are present in frog but not in tadpoles as late as Stage XVII. Thalamic HRP injections in the adult label neurons dorsal and lateral to the solitary tract in the region of the bulbospinal junction, presumably representing a dorsal column nucleus. In contrast, although primary afferent fibers project to this area as early as Stage V, these presumptive dorsal column nuclei cells do not project to the thalamus before Stage XVII.

Developmental differences between ascending and descending fiber systems provide an intriguing comparison with the situation following spinal transection. Juvenile frogs whose spinal cords were transected as larvae have neurons from all normal supraspinal regions projecting to lumbar spinal cord (Forehand and Farel, Neurosci. Abstr.,5:677, 1979). However, ascending fiber systems originating below the level of the transection have not been demonstrated. The difference in regenerative properties of ascending and descending fiber systems may lie in their different degrees of maturity at the time of transection.

285.6 REFLEX DEVELOPMENT IN ANURAN LARVAE: ELECTROPHYSIOLOGICAL AND ANATOMICAL CORRELATES. Paul B. Farel. Dept. Physiol, Univ. N. Carolina Sch. Med., Chapel Hill, NC 27514

Motoneurons of the lumbar lateral motor column (LMC), which innervate the hindlimb, proliferate and differentiate during the larval (tadpole) period in anurans. In R. catesbeiana, the larvae are large enough to permit the use of conventional electrophysiological and anatomical techniques to monitor the developmental course of these motoneurons. In the present study, electrophysiological responses of dorsal and ventral roots were compared in tadpoles as early as St. IV (Taylor and Kollros) to those found in juvenile frogs. These data were correlated with anatomical results obtained by applying HRP to dorsal or ventral roots.

The lumbar enlargement of tadpoles, unlike that of frogs, contains large ventromedially situated motoneurons innervating axial musculature (primary motoneurons). These primary motoneurons, which mediate swimming, send their axons out the ventral root in a fascicle separate from those of LMC motoneurons. These fascicles were dissected apart in order to record simultaneously activity in the axons of primary and LMC motoneurons. All electrophysiological experiments were performed using an in vitro spinal cord preparation.

The only qualitative electrophysiological difference found between St. IV tadpoles and juvenile frogs was the appearance at St. VII/VIII of primary afferent depolarization elicited by antidromic activation of LMC motoneurons (VR-DRP). Prior to St. VII/VIII, primary afferent fibers scarcely impinge on spinal cord gray matter, although direct projections to more rostral spinal cord can be seen. Coincident with the appearance of fine bundles of sensory fibers invading the gray matter, the VR-DRP elicited by stimulation of LMC motoneuron axons could be demonstrated.

The DRP evoked by antidromic activation of primary motoneurons or by stimulation of descending fibers was present at all stages examined. Dorsal root-ventral root reflexes and reflexes evoked by stimulation of descending fibers, although of long latency (20-30 msec.), were present at the earliest stages examined in LMC motoneurons.

Reflexes involving primary motoneurons showed no marked tendency toward change during the larval period.

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285.7 CHANGES IN CUTANEOUS INPUT TO DORSAL HORN NEURONS FOLLOWING SPINAL CORD HEMISECTION IN CATS. G. L. Brenowitz and L. M. Pubols. Dept. Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

The responses of L7 dorsal horn neurons to electrical stimulation of the sural nerves and to tactile stimulation of the skin were studied in methoxyflurane anesthetized normal and acutely (6h-5d survival) or chronically (3-6 mo. survival) hemisected cats (right T_{13} hemisection sparing the dorsal columns). The somatotopic organization and sizes of light touch (1 gm von Frey hair) receptive fields were similar in normal and acutely hemisected groups. In chronic cats receptive fields in the lateral part of the dorsal horn ipsilateral to the lesion (proximal hindlimb fields) showed a significant ($p = 0.031$ in a binomial test) increase in size, when compared to fields on the contralateral side, and when compared to fields in normal and acute animals. Split fields with two separate components on the body surface were found infrequently in normal cats but were common in acute and chronic cats.

Recording sites yielding short latency ($\leq$ 5msec) reliable responses to electrical stimulation of the sural nerve were more numerous in caudal L7 than in rostral L7. In normal and acute cats, responsive sites were symmetrically distributed on both sides of the cord. In chronic cats, there were more responsive loci ipsilateral to the hemisection than contralateral to it (asymmetrical distribution). These physiological changes are correlated with recovery of function and collateral sprouting of primary afferents known to follow spinal cord hemisections in cats (Murray and Goldberger, J. Comp. Neurol. 158, 1974).

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286.1 INCREASED TYROSINE HYDROXYLASE ACTIVITY IN THE RAT SUPERIOR CERVICAL GANGLION FOLLOWING PREGANGLIONIC NERVE STIMULATION IN VITRO. N.Y. Ip and R.E. Zigmond. Department of Pharmacology, Harvard Medical School, Boston, MA 02115

Previous experiments have demonstrated that direct electrical stimulation of the preganglionic cervical sympathetic trunk produces an increase in tyrosine hydroxylase (TH) activity in the rat superior cervical ganglion in vivo (Zigmond and Chalazonitis, Brain Res., 164: 137, 1979). We now report that a similar elevation of TH activity occurs following preganglionic nerve stimulation in vitro.

Superior cervical ganglia from male Long-Evans rats were desheathed, superfused with oxygenated Kreb's solution and stimulated via the preganglionic trunk for periods of 30-90 min at 37°C. The effectiveness of stimulation was monitored by recording the elicited compound action potentials from the postganglionic internal carotid nerve. The contralateral ganglia from the same animals served as unstimulated controls. At the end of the stimulation period, "stimulated" and "contralateral control" ganglia were cultured for 48 h in BGJ_b medium supplemented with 10% newborn calf serum. TH activity was then assayed in these ganglia as well as in non-cultured ganglia taken from control animals ("in vivo control"). TH activity per mg protein was elevated in stimulated compared to contralateral control ganglia by 18, 31 and 47% following 30, 60 and 90 min of stimulation at 5 Hz. Stimulation at 10 Hz for 30, 60 and 90 min produced a 34, 50 and 49% increase in TH activity respectively. These increases in TH activity were not accompanied by changes in total ganglion protein. TH activity of cultured "contralateral control" ganglia was similar to that of "in vivo control" ganglia. The magnitude of the increase in TH activity was not affected by substituting 10% bovine serum albumin for newborn calf serum in the culture medium.

A study of the time course of the increase in TH activity in organ culture following 30 min of stimulation at 10 Hz showed that no change in TH activity occurred during the first 12 h in culture. After 24 h, TH activity was elevated by 32% and this increased activity was maintained for at least 48 h. Addition of the nicotinic antagonist, hexamethonium, at a concentration (1 mM) which completely blocked the compound action potentials normally recorded during preganglionic nerve stimulation, prevented the increase in TH activity in the stimulated ganglia.

These data demonstrated that preganglionic nerve stimulation in vitro produces a delayed increase in TH activity in the superior cervical ganglion comparable to that observed in vivo. This in vitro system should prove useful for further studies on the mechanism of the regulation of TH by preganglionic nerve activity.

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286.2 IN VITRO TRANSLATION OF TYROSINE HYDROXYLASE AND PHENYLETHANOLAMINE N-METHYLTRANSFERASE BY mRNA FROM BOVINE ADRENAL MEDULLA AND RAT PHEOCHROMOCYTOMA (PC12) CELLS. E. Baetge, T. Joh, B. Kaplan, E. Ross, D. Park, and D. Reis, Laboratory of Neurobiology, Dept. of Neurology, Cornell Univ. Med. College, New York, NY, 10021.

Tyrosine hydroxylase (TH) and phenylethanolamine N-methyltransferase (PNMT) from bovine and rat adrenal glands differ with respect to electrophoretic mobility, stability, pH optima and immunocrossreactivity. We sought to determine whether these differences in molecular forms are due to genetic variation or posttranslational modification of enzymes by: (1) synthesizing these enzymes in vitro using poly (A) mRNA from bovine adrenal medulla and PC12 cells, a tumor line of rat adrenal origin; (2) investigating the immunocrossreactivity of the translated enzymes from rat with those of bovine origin so as to compare their basic protein structures. We also sought to determine the relative proportion of TH and PNMT in bovine adrenal medulla translated in vitro. Antibodies used were anti-rat or bovine adrenal TH (THAb), either raised in rabbits or from hybridoma cell culture (Ross et al., Fed. Proc., 1980), and anti-PNMT (PNMTAb) raised against the rat or bovine enzyme.

From 10^6 dpm of total protein translated from bovine mRNA, 8,500 and 8,700 dpm for TH were identified by SDS-PAGE after precipitating by rat THAb and bovine THAb, respectively, indicating full crossreactivity of these Abs. From the same amount of protein translated by PC12 mRNA, 4,000 and 3,800 dpm were precipitated by rat THAb and bovine THAb, suggesting that the basic protein structure of TH translated by mRNA from both species is similar.

Rat PNMTAb precipitated PNMT translated from mRNA of bovine adrenal medulla even though the Ab inhibits the enzyme activity of only rat PNMT. From 10^6 dpm of total protein translated from bovine mRNA, 21,000 and 22,000 dpm were precipitated by rat PNMTAb and bovine PNMTAb, respectively, indicating that PNMT from both species has a similar basic protein structure.

Comparing the proportions of bovine mRNA translated, approximately 0.9% and 2.2% of total proteins were TH and PNMT, respectively. Thus the amount of PNMT translation is three-fold greater than TH. Furthermore, only 0.4% of total protein translated by PC12 mRNA was TH in contrast to 0.8% for bovine mRNA.

We conclude that: (1) the basic protein structure of TH and PNMT from rat is similar to those of bovine origin, (2) the differences in the biochemical properties of the enzymes in different species may be due to a post translational modification of enzymes, and (3) in bovine adrenal medulla, the amount of PNMT translated was three-fold greater than that of TH.

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286.3 IDENTIFICATION, USING MONOCLONAL ANTIBODY, OF TYROSINE HYDROXYLASE TRANSLATED IN VITRO BY POLY(A)mRNA. E. Ross, T. Joh, E. Baetge, B. Kaplan, M. Brodsky* and D. Reis. Laboratory of Neurobiology, Dept. of Neurology, Cornell Univ. Med. Coll., New York, N.Y. 10021.

Monoclonal antibodies (Ab) to rat striatal TH were produced by fusion of mouse lymphocytes, sensitized to TH, with murine plasmacytoma (NS1) cells. Eight percent of hybridomas secreted TH antibody, as detected by an enzyme linked immunosorbant assay (ELISA). Four stable lines (CMTH 29, 31, 78, and 95) were established by limiting dilution cloning. Antibodies were highly specific, as demonstrated both on ELISA, using TH antigen purified to homogeneity, and on immunoelectrophoresis, producing a single immunoprecipitin arc against a crude striatal TH preparation. High titres of CMTH Ab were obtained from ascities fluid of a mouse injected i.p. with a single hybridoma clone. Alone, these Abs failed to inhibit or fully precipitate TH activity from solution. However, with addition of rabbit anti-mouse IgG (RAM), CMTH-TH complexes were precipitated and virtually 100% of TH activity was removed from solution. All 4 CMTH Abs were used for immunohistochemically localizing catecholamine neurons of rat brain by the peroxidase-antiperoxidase method. The monospecificity of these Abs afforded unusually clear definition of cell bodies, axons and fine terminals with virtually no background staining.

Monospecific antibody (CMTH 31) was used to isolate the TH gene product, translated in vitro by poly(A)mRNA. Polysomes were prepared from bovine adrenal medulla or PC12 cells and poly(A)mRNA was isolated by affinity chromatography on oligo(dT)-cellulose. Translation was carried out using a rabbit reticulocyte system in the presence of [^{35}S]methionine. Radiolabeled proteins from the translation were incubated with CMTH Ab at 10°C overnight. CMTH-TH complexes were then precipitated with RAM. The precipitate was subjected to SDS-PAGE and a fluorograph of the gel was obtained. In both instances, a single protein band of MW=62,000 dlt was present, indicating that the CMTH 31 Ab is directed against an antigenic site common to both rat and bovine adrenal TH.

Monospecific antibodies to rat striatal TH have been produced which are useful for both immunotitration and immunocytochemistry. The CMTH 31 Ab unequivocally identified TH of subunit MW=62,000 dlt, translated in vitro by poly(A)mRNA from both PC12 cells and bovine adrenal medulla. The antigenic site recognized by this monospecific antibody is common to both rat and bovine TH.

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286.4 SEPARATION OF TYROSINE HYDROXYLASE FORMS BY ISOELECTRIC FOCUSING, K. A. Markey, S. Kondo*, V. Burroughs*, L. Shenkman* and M. Goldstein. New York Univ. Med. Cntr. Dept. of Psychiatry, Neurochemistry Lab., New York, N. Y. 10016

We have recently purified and characterized tyrosine hydroxylase (TH) from a cultured rat pheochromocytoma (PC-12) cells, (Mol. Pharmacol. 17, 79-85 (1980). As a continuation of this study, we have examined the isoelectric focusing patterns of purified TH as well as those of crude enzyme from rat striatum and adrenal glands. Isoelectric focusing was performed with an LKB Multiphor on polyacrylamide or agarose gels over a pH range of 3.5-10. Purified TH exhibited an isoelectric point (pI) of 5.3. After phosphorylation by purified catalytic subunit of c-AMP dependent protein kinase, purified TH showed two isoelectric points -- one at 5.3 and the other at 6.9. The major portion of enzyme activity was found at pI 6.9. ^{32}P incorporated into TH from ATP-γ^{32}P also focused at pI 6.9.

The isoelectric focusing was performed on TH obtained from rat adrenal glands and striatum. Crude striatal TH revealed two isoelectric points of 5.3 and 6.9. Preincubation of the striatal enzyme with alkaline phosphatase diminished the TH activity focused at pI 6.9. Rat adrenal TH obtained following decapitation of non-anesthetized animals showed a major peak of enzyme activity at pI 6.9 and a minor peak at pI 5.3. However, TH from anesthetized rats showed a single peak of activity at pI 5.3. These results suggest that TH can be separated into two forms upon isoelectric focusing. The form with a pI at 6.9 appears to be the predominant form after phosphorylation.

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286.5 AN ACTIVE SITE MODEL OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE (PNMT). CONFORMATIONAL AND STEREOCHEMICAL REQUIREMENTS FOR THE BINDING OF AROMATIC AND NONAROMATIC SUBSTRATES AND INHIBITORS. G. L. Grunewald, M. F. Rafferty*, R. T. Borchardt and Polina Krass*. Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045.

PNMT catalyzes the methylation of norepinephrine to epinephrine utilizing S-adenosyl-L-methionine as the methyl donor. We have previously reported [Mol. Pharmacol., 11, 694 (1975)] that substitution of the aromatic ring of the substrate phenylethanolamine (PEA) with a fully saturated ring (most notably cyclooctyl) resulted in improved affinity for the active site and provided a potent alternate substrate inhibitor for PNMT. The high inhibitory activity reported for amphetamine and related compounds [J. Med. Chem., 14, 322 (1971)] prompted us to synthesize and evaluate some saturated ring analogs of amphetamine of the type $RCH_2CH(CH_3)NH_2$ where R is cyclohexyl, 3-cyclohexenyl and cyclooctyl. On the basis of reports which indicated a 3-fold stereochemical preference of the enzyme for both the amphetamines (S>R configuration) and substrate (R>S) [Biochem. Pharmacol., 14, 1896 (1965)], the above compounds were resolved. As in the PEA analogs, it was found that replacement of the aromatic portion of amphetamine with a fully saturated ring greatly improved activity (R=phenyl, K_i=760 μM, R=cyclooctyl, K_i=86 μM). The saturated amphetamine analogs exhibited a 10-fold difference in activity of the enantiomers (but now R>S). In contrast, virtually no stereochemical preference was seen in the fully saturated PEA analogs. We also examined a number of conformationally restricted amphetamine analogs as probes of the optimal conformation of amphetamine at the binding site. A distinct preference was observed (10-fold) for the extended (trans) as opposed to the folded (gauche) conformation. Details of the biochemical evaluation and an interpretation with respect to a model of the active site of PNMT will be presented.

286.6 MESOCORTICAL DOPAMINE NEURONS: ROLE FOR AUTORECEPTORS IN SYNTHESIS REGULATION? M. J. Bannon, R. L. Michaud* and R. H. Roth. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510

The regulation of dopamine (DA) synthesis in rat mesocortical DA neurons was studied and compared to DA synthesis in nigrostriatal DA neurons. The increase in striatal DA content seen 35 minutes after γ-butyrolactone (GBL) treatment was prevented by activation of nerve terminal DA autoreceptors by apomorphine. In contrast, the increase in prefrontal cortical DA seen 35 or 60 minutes after GBL was unaffected by the DA agonists apomorphine and bromocriptine. Using the 30 minute accumulation of dopa after the administration of the dopa decarboxylase inhibitor Ro4-4602 (800 mg/kg) as an index of in vivo tyrosine hydroxylation, it was demonstrated that the increase in striatal DA following GBL was most likely due to an acceleration of DA synthesis. In contrast, GBL did not increase cortical dopa accumulation. GBL pretreatment did, however, completely prevent the rapid decline of DA seen following α-methyltyrosine treatment (250 mg/kg, 55 minutes), indicating that DA turnover had been inhibited in the mesocortical neurons, as has been previously demonstrated with other DA neurons. The monoamine oxidase inhibitor pargyline (75 mg/kg, 180 minutes before sacrifice) raised both striatal and cortical DA levels, leading to greatly attenuated dopa accumulation in both regions. Finally, the DA antagonist haloperidol (1 mg/kg, 45 minutes prior to sacrifice) greatly accelerated striatal dopa accumulation, but affected only a modest increase in cortical dopa accumulation which was completely prevented by the GBL-induced inhibition of impulse flow. These results suggest that synthesis in the prefrontal cortical terminals of mesocortical DA neurons is subject to end product inhibition and is only moderately altered by changes in impulse flow. The synthesis of DA in these terminals does not seem to be modulated by terminal DA autoreceptors. (Supported in part by USPHS Grants MH-14092, NS-10174, MH-25642 and the State of Connecticut).

287.1 TWO FORMS OF AMNESIA IDENTIFIED THROUGH AN ANALYSIS OF FORGETTING
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The nature of forgetting has been of special interest in studies of memory pathology because of what it can reveal about the nature of the defect and about the structure of normal memory. The present study was designed to compare forgetting rates in two groups of amnesic patients, using a procedure (Huppert and Piercy, 1977) developed to minimize the problem of comparing forgetting rates between groups that already differ in level of acquisition. Amnesic and control patients saw 120 colored pictures and then were tested for retention at 10 min, 2 hr, and 32 hrs. after learning. By exposing pictures eight times longer to the amnesic patients than to controls, we were able to equate amnesic and control performance at 10 min. after learning. We then asked whether forgetting from 10 min. to 32 hrs. after learning was normal or abnormal.

Two types of amnesic patients were tested: patients receiving a prescribed course of bilateral ECT (N=9) and patients with alcoholic Korsakoff disease (N=7). ECT patients saw each of 120 pictures twice for 4 sec. each. These patients served as their own controls and were tested again four months after treatment when they had recovered from anterograde amnesia. In the control condition, patients saw each picture once for one second each. The Korsakoff patients saw each picture twice for 4 seconds each. Their controls were age and IQ-matched alcoholics, who saw each picture once for one second each.

Korsakoff patients exhibited a normal forgetting rate. ECT patients performed at the same level as Korsakoff patients at 10 min. after learning but exhibited abnormally rapid forgetting. The results indicate 1) amnesia is not a unitary disorder. The amnesia associated with ECT is different from the amnesia associated with Korsakoff's disease; 2) forgetting rate can be affected by memory pathology; thus forgetting rates are not inextricably coupled to or determined by strength of acquisition.

Based on evidence from the medial temporal patient H.M., on indirect evidence that ECT amnesia is related to temporal lobe dysfunction, and on the diencephalic distribution of lesions in the Korsakoff syndrome, it is suggested that bitemporal and diencephalic amnesia are distinct syndromes of memory dysfunction, that the "stage" of memory function disrupted is different in each syndrome, and that the medial temporal and diencephalic regions normally contribute in different ways to the formation of memory.

287.2 PRESERVED LEARNING OF RULES AND PROCEDURES IN AMNESIA
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The memory deficit in the amnesic syndrome is global, affecting both verbal and non-verbal material irrespective of modality. Yet there have been numerous reports that amnesic patients can learn and remember perceptual-motor skills such as rotary pursuit and mirror tracing across several days of testing, frequently at a rate comparable to that of control subjects. These findings have suggested that perceptual-motor skills are afforded a special neurologic status. In order to determine whether what is preserved in amnesia is limited to motor skills, or whether motor skills are part of a broader class of preserved learning ability, the present study examined learning and retention of a pattern-analyzing skill selected to minimize motor involvement.

Patients with alcoholic Korsakoff syndrome, patients receiving bilateral ECT, and the patient N.A., who has chronic amnesia for verbal material, were trained to read word triads presented by mirror reflection in a tachistoscope on each of three consecutive days and also on a fourth day approximately 13 weeks later. The amnesic patients were able to acquire the mirror-reading skill at a rate equivalent to that of matched control subjects and were able to retain it for more than three months, despite amnesia for the words that had been read and despite negligible recollection of having previously performed the task. These findings are consistent with the recent finding that amnesic patients can learn the application of new numerical rules despite amnesia for the specific features of the training situation.

It is suggested that the class of preserved learning skills is considerably broader than previously believed. Perceptual-motor skills and the present pattern-analyzing skill may belong to a class of rule-governed or procedural-based operations whose information-processing and memory characteristics differ from those operations that depend upon specific, declarative, data-based material. This distinction, which is reminiscent of the classical distinction between "knowing how" and "knowing that", has been discussed in the cognitive science and artificial intelligence literatures. The experimental findings described here provide evidence that such a distinction is honored by the nervous system.

287.3 EFFECT OF SUPERIOR PARIETAL LOBE LESIONS ON LATERALIZED REACHING IN HUMAN.
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Tests were designed to differentiate homonymous half field abilities and side of motor activity. Three patients had sustained left parietal lesions of which two were verified surgically for localization at the cortical lip of the superior parietal lobule. The third was localized by EEG, neurologic exam and echo encephalography to this area. A fourth case had right surgical lesion near the parieto-occipital junction superior to occipital horn; while a fifth case had a tomography verified right superior parietal lesion. Matched controls also performed all tests. At the time of testing, all patients had grossly intact and stable neurologic exam, including full visual fields and lack of deficit in motility or somasthesis.

Patients (1-5) in experiment 1a were asked to reach ballistically for targets in the visual field, on a horizontal, opaque surface, from underneath that surface. Two right lesion cases were moderately to severely impaired reaching with contralateral (CL) limb into CL field. Left sided cases were moderately to severely impaired reaching with CL limb into either right or left fields. In experiment 1b (cases 1-4) a clear surface was substituted to allow visual guidance of the limb. All left lesion cases were severely impaired reaching with right hand into left field, and moderately impaired for the opposite condition. Case 4 was severely impaired as in 1a. Experiment 2a involved reaching for a point of light on vertical surface in dark. Left lesion cases were impaired as in experiment 1a. In 2b, room was illuminated to allow limb guidance, and there was moderate to severe impairment in CL fields in four cases (1-4) tested.

Other tests were visual localization in a perimeter, finger manipulation in reaching, and forearm supination/pronation, to evaluate the combined contribution to misreaching. Cases 3 and 4 erred in all CL fields; cases 1 and 2 were normal. These data suggest a deficit related to abnormalities of both CL limb and CL field not reducible to elementary disturbance of motility, vision, or somasthesis. Also, deficits for coding movements of the CL limb into the ipsilateral (IL) half field exist in left lesions, suggesting that remaining right parietal cortex is unable to compensate for visuomotor command during IL motor acts, or that the lesioned left lobe had significant sensory control over IL field stimuli. Finally, visual feedback of limbs (1b and 2b) during CL field reaching caused increase in deficits in 3 out of 4 cases and revealed an additional component-a disordered percept of CL fields-but only extant during limb guidance.
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287.4 COMPLEMENTARY PROGNOSTIC VALUE OF EEG AND I.Q. DATA WITH RESPECT TO SURGICAL OUTCOME IN TEMPORAL LOBE EPILEPSY.
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Previous retrospective studies of a group of 52 medically refractory temporal lobe epileptics in whom pre-lobectomy surface and depth ictal and interictal EEG recordings were collected, and for whom long term post-lobectomy seizure relief ratings were available, demonstrated that certain ictal and interictal EEG characteristics are indicative of: 1) poor seizure relief; 2) an absence of pathology in the resected lobe, 3) lower pre-surgical I.Q. scores, and 4) a drop in I.Q. scores following lobectomy. Negative interictal EEG signs included long duration spikes, bilaterally synchronous spikes, and diffuse bilateral slowing in background activity. Negative ictal EEG signs included bilaterally synchronous seizure onsets, variability in seizure onset locus, and unilateral surface onsets. These studies also showed a correlation between reduced pre-surgical I.Q. scores and poor post-surgical seizure relief. In the present study patients were analyzed according to: a) whether or not they displayed any negative ictal or interictal EEG signs, b) whether or not they were above or below the group mean in full scale I.Q., and c) whether or not they demonstrated good or poor post-surgical seizure relief. The results demonstrated that patients who displayed both negative EEG signs and lower I.Q. scores were more likely to display poor post-surgical seizure relief (85.7%) than patients displaying either lower I.Q. scores (57.1%) or negative EEG signs (62.5%). These results indicate that EEG and I.Q. data provide non-redundant information for predicting seizure relief following surgery for temporal lobe epilepsy and that decisions regarding surgery should incorporate both EEG and psychological measures.

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287.5 INITIAL AND FOLLOWUP NEUROPSYCHOLOGICAL STUDIES OF CHILDREN WITH POSTERIOR FOSSA TUMORS.A.L. Campbell, Jr., Aaron Smith and Marion Walker* Dept. of Psych., Howard U., WA.,D.C.20059 Neuropsych. Lab., U.ofM., Ann Arbor, MI,48209, Dept. of Neurosurgery, Primary Children's Hosp., Salt Lake City, UT. 54117

Accumulating findings indicate that the most common site of intracranial tumors in childhood is the posterior cranial fossa. The majority of these infratentorial tumors, usually medulloblastomas or astrocytomas, invade the cerebellum and fourth ventricle. While the literature abundantly details surgical procedures radiation and chemical treatment regimens and neuropathological findings, few studies have reported changes of higher cortical functions associated with the presence of posterior fossa tumors and/or their surgical resection.

This report summarizes the salient findings in repeated (15 month interval) postoperative neuropsychological examinations of 7 children with posterior fossa tumors and one child tested before and after excision of a recurring infratentorial tumor. In addition to the differential sensitivity of the various measures of higher (cognitive) and lower (sensory and motor) level functions indicated in initial postoperative tests, the varying frequencies and magnitudes of gains in 15 month follow-up studies also indicated differential rates of recovery and/or development in the test-retest interval. The practical and theoretical implications of these findings will be discussed.

288.1 ^{3}H-LISURIDE BINDING TO SEROTONERGIC AND ADRENERGIC RECEPTORS. G. Battaglia and M. Titeler. (SPON: P. Muller). Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada.

Fujita et al. (1) showed that ^{3}H-lisuride labeled D_2-dopamine receptors in rat brain. In order to determine if ^{3}H-lisuride labels other receptors, the binding of ^{3}H-lisuride to bovine frontal cortex crude membrane homogenates was studied using the assay procedure of Titeler et al. (2). Competition experiments revealed that 35-40% of the ^{3}H-lisuride binding was inhibited by excess serotonin and that 35-40% of ^{3}H-lisuride binding was inhibited by excess clonidine. Excess clonidine and serotonin produced an additive effect inhibiting 70-80% of ^{3}H-lisuride binding. Blockade of the serotonergic or adrenergic components was accomplished using 200 nM serotonin or 200 nM clonidine respectively. Results obtained from competition experiments performed in the presence of each blocking drug indicates that ^{3}H-lisuride labels α_2 adrenergic and serotonin receptors in the frontal cortex (Table). Further results obtained using ^{3}H-lisuride to label receptors in other brain areas and species will be discussed.

	IC_{50} values (nM)	
^{3}H-Lisuride binding in the presence of →	200 nM Serotonin	200 nM Clonidine
Lisuride	0.7	0.5
Phentolamine	1.4	1,800
Clonidine	5.6	1,800
d-LSD	200	11
Prazosin	1,400	9,000
Serotonin	5,000	7.0
Bufotenin	14,000	50

1. Fujita, N., Saito, K., Yonehara, N., Wantanabe, Y. and Yosida, H., Life Sci. 25, 969-974 (1979).
2. Titeler, M., Weinreich, P. and Seeman, P., P.N.A.S. 74(9), 3750-3753 (1977).

288.2 ^{3}H-YOHIMBINE BINDING TO α_2-ADRENERGIC RECEPTORS IN RAT BRAIN AND HUMAN PLATELET MEMBRANES. D.C. U'Prichard, M. Daiguji* and D.J. Kahn. Dept. of Pharmacol., Northwestern Univ. Sch. Med., Chicago IL 60611, and Dept. of Psychiatry, Chicago Univ. Pritzger Sch. Med., Chicago, IL 60637.

α_2-Adrenergic receptors have been labeled in brain and periphery with ^{3}H-catecholamine (CA) and ^{3}H-imidazoline (IM) ligands. At these sites agonists have high (nM) affinity and it is not clear whether these ligands label the entire population of α_2-receptors, or merely a fraction in a high-affinity state for agonists. This question is important in the interpretation of recent reports concerning changes in CA and IM α_2-sites, and is best resolved by examination of binding of a selective α_2-antagonist ligand. While the antagonist ^{3}H-dihydroergocryptine (DHEC) does not show appropriate selectivity especially in brain, yohimbine is α_2-selective in central and peripheral pharmacological and binding studies. ^{3}H-Yohimbine (YOH, 80-90 Ci/mmole) interactions at platelet and brain α_2-receptors were examined. In human platelet membranes, NE-displaceable ^{3}H-YOH binding was 85-90% of total binding, linear with increasing protein and maximum at pH 7.0. Association and dissociation kinetics were rapid and linear at 25°C, and ^{3}H-YOH labeled a single order of sites with a K_D of 1.2-1.5 nM, compared to k_{-1}/k_1 of 0.45 nM. Platelet ^{3}H-YOH binding exhibited α_2-receptor characteristics, with yohimbine 700 times more potent than prazosin. Antagonist affinities for inhibition of ^{3}H-YOH binding were similar to affinities for inhibition of CA suppression of adenylate cyclase activity. Agonists were quite potent inhibitors of ^{3}H-YOH binding, with K_I values of 50, 230, 70 and 26,000 nM for (-)-EPI, (-)-NE, clonidine and (-)-isoproterenol respectively. Agonist interactions showed apparent negative cooperativity (n_H 0.6-0.8). Agonist K_I and n_H values were not influenced by 1.0 mM Mg^{2+}, which however decreased ^{3}H-YOH binding 20%. 5.0 mM Mg^{2+} decreased agonist K_I values 2-fold. 10 µM GTP increased K_I values for (-)-EPI and (-)-NE 2-3 fold and increased n_H values to 1.0-1.1. ^{3}H-YOH labeled brain α_2-receptors with lower affinity and, unlike platelets, exhibited non-linear saturation kinetics. However agonist K_I and n_H values were similar at brain and platelet ^{3}H-YOH sites. ^{3}H-YOH is thus a useful α_2-receptor antagonist probe which appears to label the entire population of low- and high-affinity α_2-receptors in brain and platelets.

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288.3 AGONIST AND PARTIAL AGONIST RADIOLIGAND INTERACTIONS AT NEURAL AND NON-NEURAL α_2-RECEPTORS. J.C. Mitrius, D.J. Kahn, and D.C. U'Prichard. Dept. Pharmacol., Northwestern Univ. Sch. of Med., Chicago, Il. 60611.

α_2-receptors located at central and peripheral NE synapses have been characterized using both catecholamines (CA) and imidazolines (IM) as radioligands. Clonidine may act as a partial agonist while CAs are full agonists in inhibiting release of NE. However, in the CNS, CA and IM ligands label the same α_2-site in terms of high (nM) affinities for agonist competitors. To examine agonist vs. partial agonist interactions at α_2-receptors, we have compared the binding characteristics of (-)-^{3}H-epinephrine (EPI,40-50 Ci/mmol) and ^{3}H-p-aminoclonidine (PAC,40-60 Ci/mmol) in both neural (rat and bovine cortex) and non-neural (human platelets) tissue. ^{3}H-PAC and ^{3}H-EPI assays were performed as in brain, except that in platelet experiments, 2mM pyrocatechol was used. Platelet membranes were prepared with final resuspension in Tris 8.4-1.0 mM $MgCl_2$. Saturation of ^{3}H-PAC binding in cortex and platelets was single-order, while ^{3}H-EPI saturation was non-linear. Distinct components of ^{3}H-EPI binding in platelets had K_D values of about 2 nM and 20 nM. The total number of platelet α_2-receptor sites labeled by ^{3}H-EPI exceeded the number of ^{3}H-PAC sites. As in brain platelet ^{3}H-PAC and ^{3}H-EPI binding was stereospecific and consistent with the pharmacology of an α_2-receptor. Hill plots of both agonist and antagonist competitors against ^{3}H-PAC and ^{3}H-EPI gave n_H values of 1.0. No ^{3}H-Prazocin specific binding to α_1-receptors was observed in platelet membranes. However, ^{3}H-WB-4101, previously assumed to be an α_1-specific ligand, bound saturably and with high affinity to platelet membranes (K_D = 1.5 nM). Kinetic analysis of ^{3}H-PAC and ^{3}H-EPI binding to platelet membranes revealed rapid association and biphasic dissociation at 25° C. Unlike brain ^{3}H-PAC and ^{3}H-EPI binding in platelets was Mg^{2+}-dependent. In the absence of Mg^{2+}, the K_D of ^{3}H-PAC at platelet sites was 8.0 nM. With the addition of 1.0 or 5.0 mM $MgCl_2$, the K_D was reduced to 2.0 nM. In addition, Mg^{2+} was necessary for potent and complete guanine nucleotide inhibition of ^{3}H-PAC and ^{3}H-EPI specific binding. For both ligands, the nucleotide order of potency was similar to that found at brain α_2-receptors with GTP=GDP=Gpp(NH)p>ITP>GMP>CTP=ATP. However, GTP was a more potent inhibitor of ^{3}H-PAC (ED_{50}=0.2 µM) than of ^{3}H-EPI (ED_{50}=1.5 µM) binding. With 1.0 or 10 µM GTP present, previously non-cooperative interactions of agonist competitors and of ^{3}H-PAC itself at ^{3}H-PAC sites assumed marked cooperativity. The data suggest that interactions of agonist and partial agonist radioligands at the high-affinity conformation of brain and platelet α_2-receptors can be distinguished primarily by increased occupancy using a full agonist ligand. Thus efficacy may be related to extent of high-affinity binding.

288.4 CHARACTERIZATION OF α_2-RECEPTOR SITES IN NEUROBLASTOMA X GLIOMA CELLS. D.J. Kahn, J.C. Mitrius, and D.C. U'Prichard, Dept. of Pharmacol., Northwestern Univ. Sch. Med., Chicago, Il. 60611

Recently, Sabol and Nirenberg (J.Biol.Chem., 254:1913, 1979) demonstrated the presence of α-adrenergic receptors which are inversely coupled to adenylate cyclase (AC) on the hybrid neural cell line NG108-15(108CC15). The NG108-15 cell was used as a model for examining neural α-adrenergic receptor ligand interactions and the coupled biochemical response of AC inhibition. Binding isotherms of the agonist (-)-^{3}H-epinephrine (EPI) in NG108-15 membranes were non-linear as previously seen with brain ^{3}H-clonidine (CLO) binding. ^{3}H-EPI saturation constants were the same in NG108-15 and brain membranes. ^{3}H-p-aminoclonidine (PAC) shows a 3-5 fold lower affinity at a single site in NG108-15 membranes, compared to its one-site interaction in brain. ^{3}H-PAC labels fewer sites than ^{3}H-EPI (25-30,000 vs. 40,000 sites/cell, respectively). α-Receptor density on NG108-15 cells is thus comparable to that of opiate receptors. Pharmacological properties of NG108-15 ^{3}H-PAC and ^{3}H-EPI binding were similar to brain sites, with (-)-norepinephrine (NE, K_I 1.6 nM) and (-)-EPI (1.9 nM) affinities somewhat higher in the cells, while imidazoline and antagonist affinity constants were virtually identical. Binding studies confirmed the speculation that NG108-15 cells possess a pure α_2-receptor population: 40-fold stereoselectivity between (-)- and (+)-NE; NE and EPI were 25-fold more potent than isoproterenol in inhibiting ^{3}H-PAC binding; yohimbine was 10-fold more potent than WB-4101; and there was no detectable specific binding of the α_1 antagonists ^{3}H-prazocin or ^{3}H-WB-4101. Antagonist affinities were identical for inhibition of ^{3}H-PAC binding and AC inhibition. However, catecholamines (CA) were 2-3 orders of magnitude more potent in inhibiting ^{3}H-PAC binding than AC (K_D/K_I << 1). Thus both ligands label the receptor responsible for AC inhibition, but either coupling is inefficient, or ^{3}H-PAC and ^{3}H-EPI both label a high affinity state GTP selectively lowered NG108-15 ^{3}H-PAC binding with an ED_{50}=2µM. ATP was substantially less potent. As in the brain, high affinity NG108-15 ^{3}H-PAC binding and its regulation by GTP did not require Mg^{2+}. ^{3}H-EPI and ^{3}H-PAC thus appear to label analogous α_2-sites in NG108-15 cells and brain. GTP regulation and Mg^{2+} independency of binding in the two tissues are identical supporting the hypothesis that brain α_2-receptors are inversely coupled to AC as may also be the case with opiate receptors in both tissues. GTP/M^{2+} interactions are different for neural and platelet α_2-receptors, and NG108-15 cells provide an excellent model for examining α_2-coupling in neural tissue. Studies are in progress to correlate binding of various ^{3}H-ligands with AC and other membrane responses in naive cells and cells chronically treated with CA.

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288.5 β-ADRENERGIC RECEPTORS: FUNCTIONAL HETEROGENEITY INDUCED IN A HOMOGENEOUS POPULATION BY AGONIST, GTP, AND Mg^{2+}. William C. Broaddus,* Georges Vauquelin,* and Michael E. Maguire, Dept. Pharmacology, Sch. Medicine, Case Western Reserve Univ., Cleveland, OH 44106.

Pharmacological and biophysical studies have indicated previously that wild type mouse S49 cells contain a single class of β-adrenergic receptors (βARs) which are coupled to adenylate cyclase (AC). However, this homogeneous population is functionally heterogeneous. N-Ethylmaleimide (NEM) in the presence of agonist inactivates 65% of total βARs, while NEM alone or with antagonists has no effect on βAR density or affinity. The percentage of βARs inactivated is time- and NEM concentration-dependent, but always attains a maximum of 65%. We have found that only the βARs susceptible to agonist/NEM are coupled to AC, or show GTP and Mg^{2+} effects on agonist-specific receptor affinity. GTP plus Mg^{2+} but not Mg^{2+} alone protect against NEM inactivation. This functional heterogeneity is a common property of βARs since similar data is obtained in turkey and frog erythrocytes and human adipose tissue. Studies in mutant S49 cells and in sealed membrane vesicles of wild type S49 cells demonstrate the following. a) Agonist/NEM inactivation requires βAR interaction with the GTP/Mg^{2+} coupling protein (G/M) since cells containing βARs and cyclase catalytic unit but not G/M do not show agonist/NEM effects. b) Similar data show that GTP and Mg^{2+} act on βARs indirectly by interaction with G/M. c) The physical site of GTP and Mg^{2+} action is on the cytoplasmic face of the plasma membrane since in sealed membrane vesicles external GTP and Mg^{2+} do not alter agonist affinity for βARs nor protect against agonist/NEM inactivation unless the pore-forming agent alamethicin is added. d) βARs of the S49 cell are genetically homogeneous since the β_d mutant of S49 which has an 85% decrease in βAR density still exhibits homogeneous binding sites for βARs but heterogeneous functionality. Thus, βARs are, for structural or stoichiometric reasons, functionally heterogeneous even though molecularly homogeneous. It emphasizes the fact that receptor binding done in the absence of functional correlations, does not necessarily measure a physiologically relevant population of receptors.

288.6 PROPERTIES AND REGULATION OF β-ADRENERGIC RECEPTORS ON L6 MYOBLASTS. R.N. Pittman* and P.B. Molinoff (SPON: C.O. Rutledge). Dept. of Pharmacology, Univ. Colo. Health Sci. Ctr., Denver, CO 80262.

A binding assay has been developed for β-adrenergic receptors on L6 myoblast cells growing in monolayer cultures. Binding assays were performed on attached cells, cells in suspension following exposure to EDTA or trypsin and on membranes prepared from myoblasts. The binding of ^{125}I-IHYP was saturable, reversible, and stereoselective. The number of receptors per cell was a function of both plating density and time in culture. Receptors are of the β_2 subtype and there are approximately 14,000 receptors per cell at confluency. The density of receptors was reduced by 15-20% following disruption with a Polytron tissue homogenizer. The number of receptors per cell was the same on attached cells as on cells in suspension. However, when cells in suspension were allowed to attach, the apparent number of receptors per cell was greatly reduced within the first hour following plating. Receptors subsequently reappeared over the next 2-6 hours. No reduction in the density of β-adrenergic receptors occurred if attachment of cells was blocked by either mechanical shaking or by maintaining the cells in culture dishes treated with bovine serum albumin.

Insel and Stoolman (Mol. Pharmacol.,14:549,1978) and Terasaki and Brooker (J. Biol. Chem., 253:5418,1978) have reported that the affinities of agonists measured by inhibition of ^{125}I-IHYP or ^{3}H-DHA binding to β receptors on S49 and C6 cells are lower than are those determined with membranes prepared from the same cultures. The Kd values of antagonists were the same for intact cells and membranes. The Kd values for radioligand displacement by agonists in whole cells was approximately 2-3 orders of magnitude lower than the Kact for cyclic AMP accumulation. These phenomena are also observed in studies with L6 cells. The Kd for l-isoproterenol in L6 membranes was approximately 0.1-0.2 μM in the absence of GTP and 0.6 μM in the presence of GTP. The Kd for isoproterenol in L6 cells in suspension was 0.8-1.0 μM and for cells in monolayer culture about 50 uM. The Kact for isoproterenol stimulated cyclic AMP accumulation in monolayer cells was about 0.02 uM. Although the affinities of agonists were very low in studies with intact cells, antagonists had high affinities for the binding site. Viable L6 cells in suspension resemble membrane preparations with respect to agonist affinity; however, once cells reattach receptors again show low agonist affinity for displacement of radioligand. (Supported by USPHS NS 13289).

288.7 CHARACTERIZATION OF AGONIST BINDING TO β-ADRENERGIC RECEPTORS IN RAT LUNG MEMBRANES. Kim A. Heidenreich*, Gregory A. Weiland* and Perry B. Molinoff. Univ. Colo. Health Sci. Ctr., Dept. of Pharmacology, 4200 E. Ninth Ave., Denver, CO 80262.

Recent evidence suggests that the molecular interactions of agonists with β-adrenergic receptors differ from those of antagonists. There are differences in the thermodynamics of binding to the receptor and in the regulation of ligand binding to the receptor by ions and guanine nucleotides. Most of this evidence has come from indirect studies of agonist binding, that is, by observing agonist inhibition of radiolabelled antagonist binding to the receptor. We have examined agonist binding directly in rat lung membranes using radiolabelled hydroxybenzylisoproterenol (^{3}H-HBI). The K_D for dl-^{3}H-HBI derived from inhibition curves of ^{125}I-IHYP binding was 4.2 ± 1.0 nM (n=3) in the absence of guanine nucleotides and 57 ± 3.0 nM (n=3) in the presence of 100 μM Gpp(NH)p. Concominant with the decrease in affinity observed in the presence of 100 μM Gpp(NH)p was an increase in the Hill coefficient from 0.55 ± 0.017 to 0.88 ± 0.03. Specific binding of ^{3}H-HBI was stereoselective with l-propranolol and l-isoproterenol being 90-100 times more potent than their stereoenantiomers. Catecholamines inhibited ^{3}H-HBI binding with a potency order characteristic of a β_2-adrenergic receptor. Binding of ^{3}H-HBI (3 nM) to rat lung membranes reached equilibrium rapidly at 20° C with a t 1/2 of approximately 5 min. In the presence of 100 μM Gpp(NH)p, steady state binding was reduced by approximately 70%, and association was more rapid with a t 1/2 of <30 sec. Dissociation of ^{3}H-HBI from the receptor was very slow in the absence of guanine nucleotides (t 1/2 ~ 3 hr). As expected, in the presence of guanine nucleotides, dissociation was rapid and complete. For example, in the presence of a maximally effective concentration of Gpp(NH)p (100 μM) dissociation was biphasic with 80% of the binding dissociating in less than 30 sec and the remainder dissociating with a t 1/2 of about 5 min. Scatchard analysis of ^{3}H-HBI binding to rat lung membranes was carried out. In the absence of guanine nucleotides, Scatchard plots were curvilinear suggesting apparent high and low affinity components of binding. The total number of ^{3}H-HBI binding sites (581 ± 51 fmoles/mg protein, n=6) was similar to that measured with ^{125}I-IHYP (544 ± 91 fmoles/mg protein, n=6). In the presence of increasing concentrations of Gpp(NH)p, the affinity of ^{3}H-HBI was decreased and Scatchard plots became linear. Sodium chloride mimicked the effect of guanine nucleotides in lowering the affinity of the receptor for ^{3}H-HBI. Magnesium chloride had the opposite effect of promoting high affinity binding. These results are consistent with a two-step model for agonist binding to the β-adrenergic receptor. In this model, the initial step of agonist binding is the formation of a low affinity hormone-receptor complex which is followed by the formation of a high affinity ternary complex consisting of hormone, receptor and a guanine nucleotide binding site. The formation of the ternary complex appears to be regulated by the concentration of monovalent and divalent cations and by guanine nucleotides. Supported by the USPHS (NS 13289).

289.1 EVIDENCE THAT LYSOSOMES ARE INVOLVED IN THE INHIBITORY ACTION OF DOPAMINE ON PROLACTIN RELEASE. D.D. Nansel*, G.A. Gudelsky, and J.C. Porter. Depts. of Ob-Gyn and Physiology, Cecil H. and Ida Green Ctr. for Reprod. Biol. Sci., Univ. of Texas Southwestern Med. Sch., Dallas, TX 75235.

It is now well established that dopamine can act directly on the anterior pituitary gland to inhibit the release of prolactin. However, the intracellular mechanism(s) which mediate the inhibitory action of dopamine on prolactin secretion have not been ascertained. Since there is morphological evidence that lysosomes may have a role in the function of prolactin cells (Farquhar, M.G., In Adv Exp Med Biol, vol 80, 1977), the present study was undertaken in order to examine whether lysosomes are involved in the inhibitory action of dopamine on prolactin release. Incubation of anterior pituitary glands in the presence of dopamine resulted in an increase in the activity of the lysosomal enzyme, β-glucuronidase, in the tissue. This dopamine-induced stimulation of tissue β-glucuronidase activity was detectable after only 15 min of exposure of the gland to dopamine, and was dose-related between 10^{-9} and 10^{-5} M dopamine. Moreover, the dose-response relationship for the stimulatory effect of dopamine on anterior lobe β-glucuronidase activity was identical to the dose-response relationship for the inhibitory effect of dopamine on the release of prolactin from pituitary tissue into the medium. Preincubation of anterior pituitary tissue in the presence of the dopamine antagonist, cis-flupenthixol, an agent known to bind competitively to dopamine receptors, markedly attenuated both the stimulatory effect of dopamine on β-glucuronidase activity and the inhibitory effect of dopamine on prolactin release, suggestive of the view that the actions of dopamine on lysosomal enzyme activity and on prolactin release operate through a common cellular mechanism involving a dopamine receptor. In addition, preincubation of anterior pituitary tissue in the presence of either chloroquine or NH_4Cl, two agents that are known to interfere with lysosomal function, also markedly attenuated both the stimulatory effect of dopamine on β-glucuronidase activity as well as the inhibitory effect of dopamine on prolactin release. In view of these findings, we suggest that not only are the effects of dopamine on lysosomal enzyme activity in the anterior pituitary gland and on prolactin release closely related, but that the dopamine-induced stimulation of lysosomal enzyme activity is, in fact, an essential component of the mechanism by which dopamine inhibits the release of prolactin.

289.2 EFFECT OF ESTROGEN ON THE DOPAMINERGIC CONTROL OF PROLACTIN SECRETION. G.A. Gudelsky, D.D. Nansel*, and J.C. Porter. Depts. of Ob-Gyn and Physiology, Cecil H. and Ida Green Ctr. for Reprod. Biol. Sci., Univ. of Texas Southwestern Med. Sch., Dallas, TX 75235.

There is now considerable evidence in support of the hypothesis that dopamine released from tuberoinfundibular neurons into hypophysial portal blood inhibits the secretion of prolactin by a direct action on prolactin cells in the anterior pituitary gland. Estrogen, on the other hand, is known to stimulate the secretion of prolactin. In the present investigation, we have addressed the issue of whether this effect of estrogen is the result of an interference in the dopaminergic control of prolactin secretion. Treatment of ovariectomized female rats with estradiol benzoate (25 μg/kg, sc) daily for 5 days resulted in a marked elevation of the serum concentration of prolactin. However, the estrogen-induced increase in the serum prolactin concentration was apparently not the result of a suppressed release of dopamine, since dopamine concentrations in hypophysial portal plasma were, in fact, significantly higher in estrogen-treated rats than in vehicle-treated animals. On the other hand, when the sensitivity of the anterior pituitary gland to the inhibitory effect of dopamine on prolactin release was examined in vitro, it was found that dopamine was much less effective in inhibiting the release of prolactin from anterior lobes of estrogen-treated rats than from glands of vehicle-treated controls. We have shown previously that the prolactin cell has the capacity to internalize dopamine and incorporate it into prolactin secretory granules. When anterior lobes of estrogen- and oil-treated ovariectomized rats were incubated in the presence of dopamine (10^{-5} M) and then subjected to continuous sucrose density gradient fractionation procedures, it was found that the amount of dopamine that had become associated with prolactin secretory granules was reduced approximately 60% in anterior pituitary glands of estrogen-treated rats when compared to that in glands of vehicle-treated controls. In conclusion, it is unlikely that the effect of estrogen to elevate serum prolactin concentrations is due to a suppressed release of dopamine from tuberoinfundibular neurons into hypophysial portal blood but is due, rather, to a reduced sensitivity of the prolactin cell to the inhibitory action of dopamine. It is interesting to speculate that the ability of estrogen to antagonize the inhibitory effect of dopamine on prolactin secretion may be mediated through an estrogen-induced impairment in the capacity of the prolactin cell to internalize dopamine and incorporate it into secretory granules.

289.3 ACTH AND RELATED PEPTIDES IN HYPOPHYSES FROM NORMAL DOGS AND THOSE WITH CUSHING'S DISEASE. M. Peterson*, A. Liotta†, G. Colurso†, N. Halmi†, and D. Krieger† Divisions of Endocrinology, The Animal Medical Center, New York, New York, 10021*, and the Mount Sinai Medical Center, New York, New York, 10029†.

Among 11 hypophyses of dogs with Cushing's disease, 7 had pars distalis (PD) adenomas and 2 adenomas of the pars intermedia (PI). No discernable tumor was evident in the 2 others. Four of the PD tumors and both PI adenomas stained immunocytochemically for ACTH. The PI tumors also contained variable numbers of cells reactive for α-MSH. The occurrence of PI tumors secreting bioactive ACTH in dogs is of interest, since in the rat PI, ACTH (1-39), which is the biologically active form, is not released as such but is further processed to α-MSH and a C-terminal fragment, CLIP. The normal dog pituitary was therefore compared to that of the rat with immunocytochemical methods. Antibodies employed were against synthetic ACTH (1-39), β-lipotropin (β-LPH), synthetic human and porcine β-endorphin (β-END) and α-MSH. In both species, the corticotrophs of the PD stained with anti-ACTH and anti-β-LPH, but weakly or not at all with anti-β-END or anti-α-MSH. The PI of the rat contained a single cell type that reacted with all 4 antibodies. The predominant PI cell of the dog (called the A cell) stained well with anti-α-MSH, more weakly with anti-ACTH and anti-β-LPH, and not at all with anti-β-END. Scattered throughout the canine PI, and sometimes in clusters, were, however, cells that resembled PD corticotrophs in every respect (B cells): they stained strongly for ACTH and β-LPH and not for α-MSH. An ACTH bioassay using dispersed adrenocortical cells showed that the PI of dogs (unlike that of rats) contained high concentrations of ACTH, similar to those in the PD. (PD: 219 ± 29 ng/mg; total content 21.2 ± 3.2 μg. PI: 364 ± 180 ng/mg; total content 3.2 ± 1.5 μg.) This could be attributed to the presence of B cells in the canine PI. PI adenomas causing Cushing's disease in dogs could originate from the B cells or from A cells which have (at least in part) lost their ability to cleave ACTH to α-MSH and CLIP. The inability to detect immunoreactive β-endorphin in the dog PI may be secondary to a different structural form in this species.

289.4 GAP JUNCTION MORPHOMETRY IN THE RAT NEUROHYPOPHYSIS WITH DEHYDRATION. D. Friedenbach, C. Tweedle and G. Hatton. Depts. of Anatomy, Psychology and Zoology and the Neuroscience Program, Michigan State University, East Lansing, Michigan 48824.

Several parameters of gap junctions have been quantified during functional changes in the neurohypophysis of the rat. Three groups of adult male rats: control, 24h dehydrated (DEH) and 24h dehydrated + 24h rehydrated (REH), were vascularly perfused with buffered aldehydes either with or without $CaCl_2$. The neurohypophysis were routinely cryoprotected and freeze-fractured. Morphological parameters were quantified for all intracellular junctions where the contributing intercellular processes were identified. In addition a random sample of gap junctions was quantified for each treatment group with or without $CaCl_2$. Results were analyzed by ANOVA. Gap junctions for which the contributing elements were identified were all between pituicyte processes (n=19). Therefore, the random sampling of gap junctions in each treatment group was combined with the categorized junctions (n=79). Junction size (μm^2) is decreased 25% from controls in the DEH and REH groups. The presence of $CaCl_2$ increased junction area 60-65% within each treatment group but had no differential effect between control and treatment groups. The average area of tissue (replica) examined to find one gap junction was used as an index of gap junction frequency. Again, there were no significant differences between DEH and REH groups (46 and 40μm^2/junction). Each treatment group, however, had lower values than controls (64μm^2/junction). Therefore each treatment group had more gap junctions per unit area of tissue. $CaCl_2$ had no effect within each control or treatment group. When these data were expressed as μm^2 junction/μm^2 replica, there were no differences between control and either treatment group. $CaCl_2$ however increased the amount of junction per area within each group by an average of 84%.

In summary, following dehydration there are more and smaller junctions. This is also true following rehydration. It, therefore, is apparent that these pituicyte processes have not reestablished the same functional level as before despite a morphological reversion (Tweedle and Hatton, Soc. Neurosci. Abs., 1979). The addition of $CaCl_2$ to the fixative solution produced fewer and larger junctions but had no overall effect upon the changes in junctions with treatment except in magnitude. Work is currently under way on other types of intracellular junctions and junctions between other types of cellular processes.

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289.5 SPECIFIC IN VITRO UPTAKE OF ^{3}H-5HT IN THE PITUITARY OF THE RAT. M. A. Johns*, E. C. Azmitia, E. A. Zimmerman+ and D. T. Krieger. Depts. of Anat. and Endocrinol., Mount Sinai Med. Ctr., NY 10029 and Dept. of Neurol.+, Columbia Coll. of P & S, NY 10032.

There are conflicting reports over the presence and the nature of 5-HT and its metabolites in the anterior pituitary(AP). CNS serotonergic neurons, via their projections to the hypothalamus, have been implicated in the regulation of release of several pituitary hormones. To investigate the possibility of a direct 5-HT effect on pituitary cells two types of experiments were done:

(1) Determination of uptake of ^{3}H-5HT(10^{-8}-10^{-6}M) into AP of adult male and female rats in the presence and absence of excess (10^{-5}M) unlabelled 5-HT, NE, desipramine, fluoxetine(FLUOX) or metergoline (MET). Tissue was incubated (37°) with ^{3}H-5HT in Ringer's soln. with pargyline(10^{-4}M), dextrose(10^{-2}M) and ascorbic acid(10^{-3}M) for 10 min. The observed total uptake [218 ± 21 pmoles/gm at(5 x 10^{-8}M) ^{3}H-5HT] was reduced 62% by excess 5-HT and 50% by FLUOX(a 5-HT uptake inhibitor) whereas no effect was seen with MET(a putative specific 5-HT receptor blocker). Uptake was not affected significantly by NE or desipramine(a NE uptake inhibitor). These findings suggest the presence of a saturable, specific, non-receptor mediated uptake mechanism.

(2) Radioautography alone or combined with immunocytochemistry was performed to delineate the localization of ^{3}H-5HT uptake. Tissue was incubated with ^{3}H-5HT(10 min) fixed in Bouin's (½h), paraffin-embedded, sectioned (5µ) and processed sequentially for radioautography and immunocytochemistry. Deparaffinized sections were reacted by immunoperoxidase technique using primary antisera to hLH β, hTSH β, ACTH, ß-endorphin, hß-LPH, hGH, oPRL. Reaction products were formed with diaminobenzidine, and the sections were air-dried and processed for radioautography.

Radioautography showed three types of silver grain labeling patterns: 1) Dense aggregates of silver grains near blood vessels and between cells; 2) a random distribution of silver grains throughout the tissue; 3) a concentration of silver grains over certain pituitary cells. The type 1 pattern is similar to that seen when CNS serotonergic fibers are labeled by ^{3}H-5HT uptake, raising the possibility of 5-HT nerve terminals in the AP. Type 2 uptake was not blocked by excess 5-HT and probably reflects non-specific binding. The type 3 pattern was most common and the combined radioautography-immunocytochemistry procedure indicates that these are LH cells. These results suggest that 5-HT may have a role in gonadotropin regulation at the pituitary level.

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289.6 EFFECTS OF GABA ON THE ELECTRICAL PROPERTIES OF A CLONAL PITUITARY CELL LINE. J.M. Israel* and, B. Dufy*, (Spon : J.D. Vincent). U.176,INSERM,rue C.Saint-Saëns,33077 BORDEAUX (France).

The recent discovery that some anterior pituitary cells are excitable and are able to generate Ca^{2+}-dependent action potentials has led to the hypothesis that such an electrical activity may play a role in the stimulus-secretion coupling. In this study, we report the effects of GABA on the electrical properties of a Prolactin (PRL) secreting pituitary cells line (GH_3/B6). We have chosen to use GABA because its effects on post synaptic membrane properties have been extensively studied and it is also a potent inhibitor of PRL secretion. Stable intracellular recordings were obtained from 450 cells ; 160 cells were excitable (resting potential 46 ± 2 mV ; input resistance 425 ± 60 MΩ) ; 190 cells were spontaneously active. GABA directly applied to the cell membrane (10^{-4} M, 2 nl, 0.5 sec) provoked a biphasic change in the electrical properties of 108 cells recorded with 3M KCl micropipettes. The immediate effect consisted of an hyperpolarisation with an increase of input resistance and a reversal potential of 39 ± 2 mV. Spontaneous action potentials were suppressed by this hyperpolarisation. The effect lasted for 20 to 120 sec and was reversible. It was followed by a large and progressive decrease in the resistance with a slight depolarisation (2 - 5 mV). Simultaneously, the amplitude of the action potentials was dramatically reduced.The late effect was less consistent when using a 2M K-citrate electrode and it was never observed with a 0.6 M K_2SO_4 pipette. It may be concluded that this late effect of GABA is dependent on the intracellular fluid composition of the cell which can be altered according to the type of recording pipette used. Both immediate and secondary effects were blocked by application of picrotoxine (10^{-6} M) but were not affected by haloperidol which blocked the inhibitory effect of DA (Dufy, B. et al., Nature,282 :855, 1979). These observations show that the inhibitory effects of GABA and DA involve different receptor sites. The fact that GABA, which directly inhibits PRL secretion by pituitary cells, has also an inhibitory effect on action potentials is consistent with the concept of stimulus-secretion coupling. The mechanisms by which GABA acts on electrical properties of the endocrine cell membrane appear different from the conventional effects of GABA on post-synaptic ionic conductance in neurons.

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290.1 ADMINISTRATION OF PROPYLTHIOURACIL TO RATS FROM EARLY PREGNANCY THROUGH LACTATION: EFFECTS ON THE SUCKLING BEHAVIOR OF THE OFFSPRING. Y. Narayanan * and C.H. Narayanan (SPON: A. Jayaraman Rao). Dept. Anat., LSU Sch. Med., New Orleans, LA. 70119.

Female albino rats (Holtzman) were given propylthiouracil (PTU) in order to induce hypothyroidism. All treatments were begun from seven days of pregnancy and continued until 20 days after birth. Various methods of feeding and their effectiveness were tested. In one group, standard rat chow was coated with a suspension of PTU in sodium alignate to make up a 3% PTU diet. Other groups were fed with powdered rat chow containing 0.3% or 0.5% or 1% PTU. Mouth opening, hand and mouth coordinated activity of the fetuses, and the suckling behavior of the pups were studied. Control animals received standard rat chow and drinking water ad libitum.

In all cases, the experimental rats delivered after a delay ranging from about 24 hours to two days when compared with control animals raised under otherwise similar conditions. Fetuses and pups of all the experimental groups were without exception smaller in size. At birth, pups of control animals weighed on an average 8.10g, while pups of the experimental groups weighed 6.5g. At 13 days after birth, control pups weighed 30.30g, while experimental pups weighed 9.7g. Eyes in the experimental pups did not open until 18 days after birth. Mouth opening and hand-mouth synchronous activity in fetuses of the experimental series were drastically reduced in frequency and in some cases altogether absent. The pups of some experimental cases were unable to attach and suckle the nipples of their mother, while in others, suckling behavior was severely retarded. The neurological deficits produced by thyroid deficiency during development and their impact on the sequential development of non-neuronal elements such as the squamosomandibular joint are currently being examined.

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290.2 OPIOID CONTROL OF PLAY AND SOCIAL DOMINANCE. J.E. Jalowiec*, J. Panksepp, F. DeEskinazi, P. Bishop*. Dept. of Psychology, Bowling Green State Univ., Bowling Green, OH 43403.

Our previous work has indicated that brain opioids control social emotions. In the following studies we evaluated the effects of opiate agonists and antagonists on play behavior--a possible source process for development of social comptetence. Play was studied in 20-50 day old Long-Evans rats by measuring the frequency and duration of pinning behavior in pairs of socially isolated animals permitted 5 min periods of social interaction. Measures of dominance and submissive postures permitted continuous evaluation of the dominance status of animals. Naloxone (1-5 mg/kg s.c.) reduced pinning by 33-66%. Morphine at 1 mg/kg increased pinning by 80% while 5 mg/kg reduced pinning by 32%. If brain opioid activity mediates social affect, opiates may facilitate neural activity underlying "social strength" while opioid blockade may hinder processes which can increase the social status of animals. When one rat of each test pair was administered morphine and the other, naloxone (0.5 - 1.0 mg/kg), morphine animals invariably became dominant while naloxone treated ones became submissive. Stable dominance required several days to develop, but after 6 test sessions, morphine animals pinned naloxone animals twice as often as naloxone animals pinned morphine treated ones. Similar results were obtained when morphine or naloxone treated animals were pitted against saline treated ones. Drug reversal did not invariably reverse dominance, suggesting that social learning factors could counteract drug effects. Also, in animals permitted to establish their dominance relationships prior to drug treatment, naloxone tended to reduce and morphine to increase dominance, but the drugs did not consistently reverse established hierarchies. These results affirm the participation of opioid systems in organizing social behaviors. High opioid activity facilitates the development of social dominance while reduced opioid activity leads to more submissive behavior patterns in juvenile play.

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290.3 THE ONTOGENESIS OF CARDIAC AND BEHAVIORAL HYPERREACTIVITY IN SPONTANEOUSLY HYPERTENSIVE RATS. D. C. TUCKER and A. K. JOHNSON. Dept. of Psychology, Univ. of Iowa, Iowa City, Iowa 52242.

Substantial experimental evidence exists demonstrating that adult spontaneously hypertensive rats (SH) evidence both cardiovascular and behavioral hyperresponsivness to environmental stimuli (e.g. Mc Carty, R., Chiueh, C. & Kopin, I., Beh. Bio., 22:405, 1978). However, little is known of the course of early developmental events which are associated with this increased responsiveness. The ontogeny of autonomic control over heart rate (HR) in normotensive rats has also been well described (e.g. Adolph, E., Am. J. Physiol., 212:1095, 1965; Hofer, M. & Reiser, M., Psychosom. Med., 31:372, 1969). The rationale of our research program is to study the motor behavior and HR of SH rats and of normotensive rats from the Wistar Kyoto strain (WKY) prior to weaning in the hope of identifying critical periods or events that may influence the development of hypertension.

During a preliminary investigation, HR was sampled daily from implanted silver wire electrodes beginning at two days of age; divergence of HR between these litters of nondisturbed SH and WKY pups tested in their home cage was first seen at 16 days of age.

In the present study, 10 litters of SH and 10 litters of WKY 16 day old pups were studied under a sequence of four conditions. For each pup the testing session included 1-2 min. of baseline HR recorded in the home cage with minimal disturbance of the litter. The pup was then placed in a small open field apparatus which was maintained at nest temperature. After 2 min. a loud auditory stimulus was presented and the HR and behavior monitored for 30 sec. The pup was subsequently returned to the home cage and recording was continued for an additional 2 min. Throughout the recording period, behavior was coded as either still or active.

Sixteen day old SHR pups were observed to have higher HR than WKY of the same age both when still {$F(1,16)=7.9$, $p<.01$} and when active {$F(1.16)=94$, $p<.001$}. The magnitude of the HR differences between strains did not change with the testing situation. An estimation of activity level in the open field situation was made by counting the number of 5.5x8cm. grids the pup crossed during the test period. Although animals showed substantial locomotor activity, SH and WKY pups did not differ in the number of grids crossed.

The results of the present experiment define the period during which a marked change in cardiovascular function occurs in the SH. It is important to note that the hyperkinetic cardiovascular response is apparently distinct from increased locomotor activity. Future studies determining the controlling mechanisms associated with this increase of HR may suggest means through which interventions could be made during this potential critical period.

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290.4 ENHANCED LABELING OF ECF PROTEINS IN MOUSE BRAIN AFTER TRAINING. V. E. Shashoua and M. E. Moore*. Mailman Research Center, McLean Hospital, Harvard Med. Sch., Belmont, MA 02178.

In previous studies, we found that the labeling of three brain proteins is increased after goldfish acquired a new pattern of swimming behavior. Two of these proteins (β and γ Ependymins) were found to be rapidly labeled and secreted into the brain extracellular fluid (ECF). Antisera to the ependymins injected into the fourth ventricle of goldfish brain at critical periods after training [Shashoua and Moore (1978) Brain Res. 148:441-449] inhibited long-term retention of the behavior. Using these techniques as a model system, we now find that specific protein changes can also occur in mammalian brain after training. Thirsty mice (Balb c) were trained to find water in one arm of a T-maze according to the method of Naranjo and Greene [Expt. Neurol. (1980) in press]. At 1½ hours after completion of the training, each animal was injected with valine ^{3}H and labeled for one hour. An untrained control mouse (litter mate) received valine ^{14}C. After the labeling, the animals were anesthetized in ether, perfused with saline through the heart, and the brains from each pair (one experimental and one control) were pooled and extracted with 0.32 M sucrose to give the ECF fraction [Shashoua (1979) Brain Res. 166:349-358]. The extracted brains were then homogenized and separated by ultracentrifugation into the nuclear, synaptosomal, and cytoplasmic subcellular fractions. Each fraction, including the ECF fraction, containing ^{3}H labeled proteins from the trained mouse and ^{14}C labeled proteins from a control animal, was analyzed by SDS-polyacrylamide gel electrophoresis. Only the ECF fraction showed increased valine incorporation at two bands migrating at 32,000 and 27,000 daltons, respectively. The average increase for the two bands was 250% and 80%, respectively. Several types of control behavioral experiments showed no detectable protein changes by these double-labeling procedures. Thus pretrained mice which performed the task without acquisition of new information, thirsty animals and highly stressed mice showed no protein changes. The results suggest that it is essential to acquire new information before obtaining the concomitant increase in the labeling of the specific ECF proteins. These findings extend our previous studies in goldfish brain to mammalian brain and again focus on the brain extracellular fluid as an important channel of communication in the process of "fixing" information in the CNS.

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290.5 RABBIT VISUAL CORTEX AND THE EFFECTS OF DIFFERENT FLASH FREQUENCIES DURING STROBOSCOPIC REARING. H. E. Pearson, N. Berman, and E. H. Murphy. Anatomy Department, The Medical College of Pennsylvania, Philadelphia, PA 19129

The rabbit visual cortex appears resistant to most forms of visual deprivation, but we have shown that deprivation of the experience of visual motion during early postnatal life does result in alteration of the response characteristics of visual cortical cells (Pearson, Berman and Murphy, 1980). Such deprivation was achieved by raising the rabbits from birth in a stroboscopically illuminated environment, with a flash frequency of 4 per second (4 Hz rabbits). We have now extended the study to include a group of rabbits raised with a flash frequency of 8 per second (8 Hz rabbits).

Single unit recordings were made from primary visual cortex when the rabbits were at least 8 weeks of age. Responsive units were classified according to their receptive field properties and were compared with data obtained from normal rabbits. The greatest effect of the stroboscopic rearing, at both frequencies employed, was the reduction in the proportion of orientation selective cells which also show direction selectivity. In normal rabbits, 87% of orientation selective cells are also direction selective. This proportion was reduced to 45% in the 4 Hz rabbits and 37% in the 8 Hz rabbits. A significant decrease in the proportion of orientation selective cells was found with the 4 Hz rabbits but not with the 8 Hz rabbits. Orientation selective cells comprise 74% of responsive units in normal rabbits and 81% in 8 Hz rabbits, whereas in 4 Hz rabbits this was decreased to 59%. Therefore, the result of deprivation of visual motion during development is dependent on the flash frequency used. The lower frequency affects both orientation and direction selectivity, whereas the higher frequency affects only direction selectivity. Similar findings on the effects of different flash frequencies during stroboscopic rearing have been reported for the visual cortex of the cat (Cynader, Berman and Hein, 1973; Olson and Pettigrew, 1974; Cynader and Chernenko, 1976).

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290.6 BEHAVIORAL ESTIMATES OF VISUAL ACUITY IN CATS WITH EXPERIMENTAL AMBLYOPIA. G. D. Mower*, J.L. Burchfiel, K.S. Rockland, F.H. Duffy. Lab. of Developmental Neurophysiology, Children's Hosp. Med. Ctr. and Harvard Med. Sch., Boston, Mass. 02115.

Visual acuity of the normal and treated eyes of amblyopic cats was assessed in a discrimination task between square wave gratings (85% contrast) and a blank of matched mean luminance ($22 cd/m^2$). Three preparations were studied: monocular deprivation (N=2), surgical esotropia (N=2), and cats reared with a rotatable prism over one eye (N=2). Further studies are in progress.

The acuity of the normal eye was comparable in all preparations and was in the range we find for normal cats (3.5 - 5.0 cycles/degree). All preparations showed an acuity deficit with the treated eye and the magnitude of the deficit varied between preparations. Expressed as the ratio of normal eye acuity/treated eye acuity, the deficits were: monocular deprivation: 3.3, rotating prism: 2.8, esotropia: 2.3.

Comparisons were made between these acuity estimates and the physiological correlates of amblyopia measured in these and other animals with identical rearing histories. In all preparations, the deficits in spatial resolution of LGN X-cells driven by the treated eye were comparable, and indicated a reduction by a factor of 2 compared to the normal eye. Physiological deficits in visual cortex varied among the preparations. In terms of the percentage of cells responsive to the treated eye (both monocular and binocular cells), the results were: monocular deprivation: 30%, rotating prism: 40%, esotropia: 68%.

Thus, the magnitude of the deficits in LGN did not correlate with the magnitude of the behavioral deficit, and when cortical deficits were present, the behavioral deficit was greater than the LGN deficit would predict. Cortical deficits appear to add to LGN deficits in determining the behavioral acuity of cats with different forms of amblyopia.

290.7 FOUR HOURS OF ENRICHED EXPERIENCE ARE SUFFICIENT TO INCREASE CORTICAL WEIGHT OF RATS. P.A. Ferchmin and V.A. Eterović. Dept. of Biochem. Univ. Central del Caribe Sch. of Med. Cayey, P.R. 00633.

We have shown previously that a 4-day-exposure to environmental complexity (EC) is enough to increase brain weight of rats compared to littermates kept in impoverished conditions (IC) (Ferchmin, P.A., Eterović, V.A. and Caputto, R. Brain Research 20 (1970) 49-57). The purpose of the present study was to determine the minimum exposure time required to elicit detectable differences in cortical weight between EC and IC rats. Four groups of rats were exposed to EC for 4 days, either for 24 hours, 7 hours, 1 hour or 0 hours a day; they spent the remaining hours in IC (24hEC, 7hEC, 1hEC and IC groups). There were 20 animals in each condition. After sacrifice their brains were dissected into several regions and weighed. In accordance with previous results for this and other strains, occipital cortex was the brain region most affected by the environment: 1hEC > IC by 13.0%, $p<0.01$, 7hEC > IC by 10.7%, $p<0.05$ and 24hEC > IC by 7.6%, non significant. In the ratio of visual cortex over non-cortical brain, 1hEC > IC by 14.4%, $p<0.01$, 7hEC > IC by 11.9%, $p<0.01$ and 24hEC > IC by 9.3%, $p<0.05$. Although there is a trend of higher increases with shorter exposure times (1hEC > 7hEC > 24hEC), the three EC groups did not differ significantly. At variance with longer exposure times, total cortex was not significantly changed. It is possible that the occipital cortex is the initial focus of environmentally-stimulated trophic response, which then spread to other cortical regions.

This study demonstrates that a relatively gross anatomical change in occipital cortex can be induced by 4 hours of enriched experience. This period of time is comparable to the time necessary for the rat to master some common learning tasks. Therefore, the anatomical changes observed in EC vs IC rats could be related to brain adaptation for storage of the increased amount of information conveyed by the complex environment.

290.8 PLASTICITY, EXPERIENCE AND RESOURCE ALLOCATION IN MOTOR CORTEX AND HYPOTHALAMUS. D. N. Spinelli, Frances E. Jensen* and Gonzalo Viana Di Prisco*, Department of Computer and Information Science and Psychology, University of Massachusetts, Amherst, MA 01003.

Training normally reared kittens to avoid an "unsafe" visual stimulus by flexing one forearm has major effects on visual and somatic cortex adult organization. Here we show that two important interfaces to the world, motor cortex and the hypothalamus, are similarly affected. Punctate stimulation of the motor cortex reveals a four-fold increase in the area allocated to the control of movement of the trained forearm relative to the untrained one. Some of the motor responses with increased representation resembled elementary movements involved in correct responses during training. Cellular responses in the hypothalamus showed a shift toward cells with selectivity for the trained forearm; some of these cells showed the additional characteristic of selective responsivity to the visual stimuli used in training. It appears that the partitioning of the motor cortex and the repertoire of responses available to it are substantially influenced by early experience. The access that sensory stimuli have to the hypothalamus is also modified, possibly changing the way in which the adult will later cope with demanding tasks. The results dramatically demonstrate that simple early experience exerts widespread effect on structures which will later prove critical in setting the limits of individual potential.

291.1 ACTIVITY PATTERNS OF CROSS-REINNERVATED FLEXOR DIGITORUM LONGUS AND SOLEUS MUSCLES IN THE CAT DURING UNRESTRAINED MOVEMENT. M.J.O'Donovan*, M.J.Pinter*, R.P.Dum and R.E.Burke. (Spon. W.B. Marks) Lab. of Neural Control, NINCDS. NIH, Bethesda, MD 20205

As part of a study of "fast-slow" nerve cross union on the motor units of the largely fast twitch flexor digitorum longus (FDL) and pure slow twitch soleus (SOL; see Neurosci. Abstr. 5:765; 5:766, 1979), we examined EMG and mechanical activity in cross-reinnervated FDL and SOL muscles in 4 adult female cats. In 2 cats, FDL motoneurons innervated SOL muscle units (FDL→SOL); in 2 others, SOL motoneurons innervated FDL muscle units in one leg (SOL→FDL) while FDL was intentionally self-reinnervated (FDL→FDL) in the contralateral leg. Chronically implanted devices were used to measure FDL and SOL EMGs, forces and lengths (see Neurosci. Abstr. 5:380, 1979) 18 to 24 months after nerve cross-union (bipolar EMG electrodes were also implanted in several other muscles). After full recovery from aseptic surgery, data was recorded during treadmill locomotion, postural adjustments, and various reflexes (paw shaking, scratching and electrical stimulation of the sural nerve via implanted electrodes). At terminal experiments, force and length gauges were calibrated and sources of abnormal innervation determined. In 3/4 FDL→SOL muscles, innervation derived solely from FDL motor axons (pure cross-reinnervation). These muscles exhibited very low levels of EMG and force output during standing, and in locomotion displayed only short bursts of activity in the late stance and early flexion phases of stepping (typical of the normal FDL but very different from normal SOL, which has high activity levels in standing and throughout the stance phase of stepping). In the 4th FDL→SOL muscle, SOL axons had reinnervated parts of the SOL muscle (dual innervation) and its activity pattern was a mixture of the normal SOL and FDL patterns. In the reverse version of cross-innervation, the pure SOL→FDL muscle in 1 cat exhibited a high level of EMG activity during standing and throughout stance during locomotion; its peak force production (~600 g) was several times larger than the contralateral self-innervated (FDL→FDL) muscle. In the last cat, both SOL and FDL axons dually reinnervated the FDL muscle and it displayed activity throughout stance plus extra bursts at end stance and during swing (the latter similar to the contralateral FDL→FDL muscle). In all cases, activity during shake, scratch and sural-evoked reflexes were in exactly the patterns normally expected from the innervating motoneurons. Thus, the motor activities of cross-reinnervated muscles remain those characteristic of the normal innervating α motoneurons, resulting in alterations in muscle unit duty cycle that must be taken into account in the interpretation of such experiments.

291.2 THE EFFECTS OF AXOTOMY ON LOCAL RATES OF PROTEIN SYNTHESIS IN THE RAT SUPERIOR CERVICAL GANGLION. C.B. Smith*, P. Yarowsky, A.M. Crane* (SPON: L. Sokoloff). NIMH, Bethesda, MD 20205.

In a previous study (Agranoff et al., Trans. Am. Soc. Neurochem. 11:95, 1980) we observed that unilateral section of the hypoglossal nerve results in an increased rate of protein synthesis in the ipsilateral nucleus. In order to examine the influence of the afferent input on the response of the perikarya to injury of their axons, the rat superior cervical ganglion (SCG) was used as a model system because of the relative ease of manipulation of its input as well as its output. Four different experimental conditions were studied: 1). Axotomy, in which the external carotid nerve (ECN) was severed unilaterally at the level of the superior thyroid artery, 2). Deafferentation, in which the cervical sympathetic trunk (CST) was severed unilaterally about 1 cm from the caudal pole of the ganglion; 3). Axotomy/Deafferentation, in which both nerves were severed unilaterally; and, 4). Sham-operated controls.

Rats (290-355 g) were surgically prepared under halothane anesthesia. Three days later, the procedure for the determination of local rates of protein synthesis was carried out (Smith et al., Trans. Soc. Neurochem. 11:95, 1980). A pulse of the tracer, L-[1-^{14}C]leucine (100μCi/kg), was injected intravenously, and arterial plasma concentrations of labeled and unlabeled leucine were monitored during the entire 30 min of the experimental period. The SCG were then excised, frozen, and sectioned, and local tissue concentrations of ^{14}C were determined by quantitative autoradiography. Regional rates of leucine incorporation into protein were calculated on the basis of these measured variables.

It has been demonstrated (Bowers & Zigmond J. Comp. Neurol. 288:227, 1979) that the perikarya of the ECN reside only in the caudal region of the ganglion and that there are some perikarya in the caudal pole that send their axons down the CST. Axotomy alone resulted in an increased rate of protein synthesis in the caudal region compared to that of the control side. Deafferentation alone also caused an increase in this area, but it is uncertain whether this reflects an effect on the perikarya of the axons of the CST or ECN. In ganglia which were both axotomized and deafferented, however, the rate of protein synthesis in the caudal region was reduced to a level below that of the sham-operated animals.

In our previous study of the response of the hypoglossal nucleus to axotomy we also found an increased rate of glucose utilization in the axotomized nucleus as compared to the control. Similarly, in the present study we have determined by means of the deoxyglucose method that the local rates of glucose utilization are increased over control values in the caudal region of the SCG following either axotomy or deafferentation.

291.3 CHANGES IN GLUCOSE METABOLISM DURING HYPOGLOSSAL NEURON REGENERATION. Philip Singer and Sharon Mehler*. Research Service, Veterans Administration Medical Center and University of Kansas Medical Center, Kansas City, Missouri 64128 and Kansas City, Kansas 66103.

We have previously reported a marked increase in ^{14}C-2-deoxyglucose uptake (Singer and Mehler, Neurosci. Abstr. 5:683, 1979 and Exp. Neurol. in press) in the rat in hypoglossal nucleus beginning 24 hours after transection of the hypoglossal nerve. This suggests increased glucose use during motorneuron regeneration. Others have found a similar increase using the quantitative ^{14}C-2-dexyglucose method (Agranoff, Smith and Sokoloff, Trans. Am. Soc. Neurochem. 11:95, 1980). In order to better determine the pathways of this increased utilization we studied citric acid cycle, glycolytic, and pentose phosphate shunt enzymes with histochemical stains.

The left hypoglossal nerve of 150 gm male Sprague-Dawley rats was sectioned under nembutal 50 mg/kg IP and the animal allowed to recover. The animals were reanesthetized at 24, 48, 72 hrs and 7, 14, and 30 days and the brainstem removed and immediately frozen in Freon cooled to -70°C. 10 μ sections were made at -18°C and stained for succinic dehydrogenase (SDH), lactic dehydrogenase, (LDH), diphosphopyridine nucleotide (DPNH), triphosphopyridine nucleotide (TPNH) and glucose-6-phosphate dehydrogenase (G-6-PD). The operated and control nuclei were compared. Intracellular SDH and LDH decreased on the side of axotomy beginning at 24 hrs through 72 hrs but increased above control after this. However, staining of the neuropil for SDH and LDH on the side of axotomy decreased beginning at 72 hrs and remained decreased at 7, 14, and 30 days. Intracellular DPNH, TPNH and G-6-PD staining on the side of axotomy increased beginning at 48 to 72 hrs. It appears that the citric acid cycle, glycolysis and pentose phosphate pathways all ultimately participate in the increased glucose use after axotomy. However, because of the early decrease in intracellular staining of SDH and LDH, the early increase in glucose use is probably due to pentose phosphate enzymes and perhaps glycoprotein synthesis. Previous studies have shown both increases and decreases in citric acid cycle activity after axotomy. This probably reflects the bimodal early vs. late activity of this pathway when compared using different animal models and times after axotomy. The decreased SDH and LDH staining in the neuropil may reflect decreased action potential activity in dendrites after axotomy as opposed to synthetic activity in the cell body.

291.4 AN ULTRASTRUCTURAL STUDY OF RETROGRADE CHANGES IN HYPOGLOSSAL NEURONS OF CATS. J. L. Cova*, D. J. Allen and H. Aldskogius*. Departments of Anatomy, Medical College of Ohio, Toledo, OH 43699 and Karolinska Institutet, Stockholm, Sweden.

Adult cats under ketamine anesthesia were subjected to crush of the left hypoglossal nerve at the level of the posterior belly of the digastric muscle. Animals were sacrificed by intra-aortic perfusion with 4% neutral buffered paraformaldehyde followed by 2.5% neutral buffered glutaraldehyde at 1, 5, 10, 35 and 60 days after surgery. Two intact animals served as controls. Blocks of medulla containing the rostral 2 mm of the hypoglossal nucleus were embedded in epon-araldite. Thin sections were "stained" with lead citrate and uranyl acetate.

Preliminary results from animals killed at 5 and 10 days indicate that ultrastructural changes vary with the length of the postoperative survival period. At 5 days after axon crush, the nerve cell bodies of injuried neurons contained increased numbers of elongated, electron-dense mitochondria with reduced cross-sectional area. Occasionally these mitochondria assumed a lobulated configuration. Morphological changes in the RER were variable and ranged from partial to complete disaggregation. In certain instances, the disaggregated RER was confined to "islands" separated by neurofilaments and large numbers of mitochondria. Vesiculation and vacuolation of Golgi membranes were frequently observed. In many instances autophagic membranous foci of Golgi membranes were also present.

At 10 days after crush, the granular endoplasmic reticulum was markedly depleted, and when present consisted of broad, irregularly arranged cisterns containing an amorphous electron-opaque material. Rossette ribosomes were randomly distributed throughout the cytoplasm and within the abnormal cisternal arrays. The number of single ribonucleoprotein granules in the cytoplasm of reacting neurons appeared greater than those of intact neurons. The Golgi complexes appeared to be hypertrophic and exhibited a pattern of vesiculation and vacuolation similar to that observed 5 days after axon injury. These complexes frequently assumed a peripheral position in the cytoplasm. Large amounts of smudged electron-opaque material resembling lipofuscin was randomly dispersed throughout the cytoplasm at 5 and 10 days after surgery.

These results, when compared to published descriptions of morphological changes associated with axon reaction in neurons of different species and nerve cell populations, further emphasize the variability of the ultrastructural features of the retrograde response.

This investigation was supported by General Research Support Grant 2-1-4-94209, National Institutes of Health.

291.5 TASTE BUDS ON CAT CIRCUMVALLATE PAPILLAE REINNERVATED BY THE CAROTID SINUS NERVE. B. Dinger*, L.J. Stensaas, S.J. Fidone. Dept. Physiol., Sch. Med., Univ. Utah, Salt Lake City, UT 84112.

Mammalian taste buds disappear from the gustatory epithelium several days following deafferentation. Buds will reappear in their usual places subsequent to reinnervation of gustatory papillae by nerves known to contain gustatory fibers. Cross-reinnervation experiments by earlier investigators have failed to detect fibers with a gustatory potential in motor or general sensory nerves even though other work has shown that gustatory papillae transplanted to the anterior chamber of the eye form taste buds under the influence of either intrinsic trigeminal fibers or following innervation by similarly transplanted lumbar dorsal root ganglia. We have re-examined this question by testing the gustatory potential of arterial chemosensory and barosensory neurons in cross-reinnervation type experiments.

In cats, 2-19 months following cross-anastomoses of the carotid sinus nerve to the distal stump of the lingual branch of the IXth n. taste buds are present in their usual locations on the circumvallate papillae. These buds are normal in size and appearance when compared to tissue from reanastomosed control cats. The buds contain both light and dark cells organized into nuclear and apical regions with a taste pore and a taste pit. Light microscope autoradiography of this tissue several days following labeling of the petrosal ganglion shows that these buds are innervated by petrosal ganglion neurons. Whole nerve recordings made proximal to the anastomoses indicate that these receptors respond to the same gustatory stimuli as do normally innervated vallate buds.

We have investigated the possibility that the original gustatory fibers of the lingual n. have reinnervated the papillae in cross-anastomosed cats via several possible routes. The available neuroanatomical, histological and physiological data indicates that these taste buds are not innervated by such fibers.

We are currently testing the hypothesis that the fibers possessing a gustatory potential in these experiments normally serve an arterial chemosensory function in the carotid body. Supported by USPHS research grants NS 12636 and NS 07938.

291.6 NERVE REGENERATION THROUGH SEMIPERMEABLE TUBES. Betty Geren Uzman and Gloria M. Villegas*. V.A. Med. Center and U. Tenn. Health Sci. Ctr., Memphis, TN 38104 and Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela.

Mouse sciatic nerves known to regenerate through impermeable polyethylene tubes are more abundantly reconstructed across a 3 mm gap when regenerated through semipermeable acrylic copolymer tubes+. In the latter, regeneration is accomplished by the formation first, through the entire length of the tube, of a vascularized connective tissue cord, along the center of which course the earliest regenerating axons growing parallel to the length of the tube and enveloped in Schwann cells. At the periphery of the vascularized cord fibroblasts are arranged in circumferentially concentric layers between which, with time, fascicles of Schwann cell-enveloped axons accrue, increasing the diameter of the nerve fiber. Nerve outgrowth, radial enlargement and fiber maturation proceed proximodistally along the vascularized cord and from its center to the periphery. Central fibers reach the distal stump first and myelinate first. The regenerated nerve is gradually remodeled with the refasciculated appearance first obscured centrally, but retained for a longer time peripherally. Evaluation of the role of the vascularized cord, that very early spans the entire gap, in guiding and modeling the regenerated nerve-Schwann cell units will be reported separately from, and in combination with, that of the acrylic copolymer tube, after removal of one or the other, or both, at varying times after the initial nerve transection. Use of multiple tubes divides the regenerated segments across the gap. "Control"regeneration outside a tube results in a refasciculated structure composed of perineurial enveloped fascicles of 15 to 20 axons each, but the fascicles are not usually enclosed by a covering epineurium.

+ Amicon X-50 tubes.

291.7 FORMATION OF NEUROMA IN-CONTINUITY BY SENSORY FIBERS THAT FAIL TO REGENERATE AFTER SCIATIC NERVE CUT AND SUTURE. Z. Seltzer and M. Devor. Neurobiology Unit, Life Sciences Institute, Hebrew University of Jerusalem, Jerusalem, Israel.

Clinical studies have reported that sutured nerves are frequently a source of pain. To study possible causes, 2 normal adult rats served as controls and 5 adult rats were subjected to a transection of the sciatic nerve and to immediate end-to-end sutures of the epineural sheaths. Care was taken not to trap nerve fibers in the sutures. Four to five hundred and fifty days later, in acute electrophysiological experiments we recorded from single myelinated fibers in minute strands teased from L_{4-6} dorsal roots. We utilized collisions of action potentials evoked by electrical stimulation distal and proximal to the suture line, and in comparable sites in the normal rats. In the normal rats, all action potentials evoked by the distal stimulus collided with those evoked by the proximal stimulus. However, in each of the rats with sutured sciatic nerve we found that in sampled sciatic nerve fibers, a substantial number of fibers could not be evoked distal to the suture line (36.9% ± 21.6, mean and S.D.). Most conduction velocities of fibers that failed to cross the suture line were in the Aδ range. In contrast, the distribution of velocities of parent fibers that did cross, resembled fibers sampled from intact nerve. Moreover, the overall distribution of velocities of parent fibers sampled proximal to the suture line, regardless of whether they crossed the suture line or not, showed less Aαβ fibers than that of intact nerve. Therefore, we conclude that not all the fibers that failed to cross the suture line were Aδ fibers. Rather, a portion of these were Aαβ fibers, whose conduction velocity had been slowed down by the retrograde shrinkage process.

Since the distribution of the fibers that failed to cross the suture line is very similar to that of fibers in a nerve end neuroma and since in both lesion types rats exhibit very similar self-mutilation, which was suggested as the rats' attempt to alleviate its pain (Wall et al., Pain 7:109-113, 1979), we suggest, that fibers trapped proximal to a suture line compose a neuroma in-continuity, and may be one of the peripheral sources of pain in humans with sutured nerves.

292.1 DECREASED β RECEPTOR BINDING IN RAT BRAIN AFTER DAILY ADMINISTRATION OF PIPEROXANE. A. Swann* and S.J. Grant, Department of Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06510.

Certain psychiatric disorders, such as depression, may represent a failure of the brain to adapt to stress. Clinically effective antidepressant treatment may act by bringing about the required adaptation. A delayed decrease in β adrenergic receptor binding has been suggested as such an adaptation. One model for this effect is intermittent stimulation of the locus coeruleus (LC). In order to determine the relevance of this model, we examined the effect of intermittent LC stimulation with low doses of piperoxane on β-adrenergic receptor binding in the rat. Rats (11 in each group) received 1.0 mg/kg (I.P.) piperoxane or an equal volume of saline once daily for three weeks. This increased neuronal activity in the LC for about 30-60 min per day by blocking inhibitory α-2 adrenergic autoreceptors on LC neurons. Rats were killed 24-48 hours after the last injection. ^{3}H-hydroalprenolol (DHA) binding was measured in homogenate of cerebral cortex. Piperoxane reduced DHA binding from 6.26±0.96 (S.D.) to 4.75±0.89 pmol/g, ($p<.005$). These results showed that intermittent pharmacological stimulation of the LC produced persistent changes in β adrenergic receptor binding.

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292.2 DIFFERENTIAL REGULATION OF MUSCARINIC CHOLINERGIC RECEPTORS FOLLOWING CHRONIC CHOLINESTERASE INHIBITION. M.H. Smit*, F.J.Ehlert*, W.R.Roeske* and H.I. Yamamura. (SPON: W.D.Barber). Dept. of Pharmacology, Univ. of AZ HSC, Tucson, AZ 85724.

Rats injected with paraoxon, an irreversible cholinesterase inhibitor, display symptoms of excessive muscarinic activity which diminish with chronic administration. The role of receptor regulation in the acquisition of tolerance was investigated using specific muscarinic receptor affinity labels. The antagonist, [^{3}H] quinuclidinyl benzilate ([^{3}H]QNB), binds to a single population of sites having uniform affinity. The muscarinic agonist, [^{3}H]cis methyl dioxolane ([^{3}H]CD), binds to a heterogeneous population of sites. Three distinct affinities have been identified: superhigh (SH), high (H), and low (L). The pattern of receptor down regulation may help elucidate its mechanism and the physiological significance of these distinct populations of agonist binding sites. Male Sprague-Dawley rats (150-250g) were injected s.c. with 0.23 mg/kg twice daily for up to 2 weeks. Binding experiments were performed on washed membrane preparations using [^{3}H]-QNB and [^{3}H] CD. Following 2 weeks of paraoxon treatment, muscarinic receptors in the cerebral cortex were investigated. The following results were obtained by weighted nonlinear regression analysis.

	CONTROL	PARAOXON	%DECREASE
$[^3H]QNB$ B_{max} (fm/mg prot)	1360	766	44%
$[^3H]CD$			
B_{max} (SH) (fm/mg prot)	101.2	115.5	0%
B_{max} (H) (fm/mg prot)	531	337	38%
B_{max} (L) (fm/mg prot)	728	314	57%

It can be seen that there is a marked decrease in the total number of receptors identified by antagonist binding. Also there is a differential regulation of the various agonist binding sites. It is interesting to note that the order of regulation appears to be L>H>SH, with the SH not undergoing down regulation. This may offer some insight regarding the physiological significance of these sites. Following 2 day paraoxon treatment, there was no significant decrease in the number of antagonist binding sites. Agonist binding, however, measured at a concentration of 10nM [^{3}H]CD showed a significant decrease in binding. This may reflect an early desensitization process. The following models provide a conceptual explanation.

$$\text{(A) } H+R \underset{}{\overset{K_1}{\rightleftharpoons}} HR \qquad \text{(B) } H+R \overset{K_2}{\rightleftharpoons} HR \overset{K_3}{\rightleftharpoons} HR^*$$

A change in the process of receptor isomerization (K_3) will show up as a change in affinity of agonist binding (B), but not in antagonist binding (A). These data suggests that the process of receptor isomerization is changed.

292.3 CHRONIC ANTIDEPRESSANT TREATMENT ENHANCES α-ADRENERGIC RESPONSES IN BRAIN: A MICROIONTOPHORETIC STUDY. D. B. Menkes and G. K. Aghajanian. Departments of Pharmacology and Psychiatry, Yale School of Medicine, New Haven, CT 06510

Chronic treatment of animals with tricyclic antidepressants (TCAs) has been increasingly used to investigate biochemical and physiological changes in brain which may be related to the delayed therapeutic action of these drugs. Thus a number of biochemical studies have pointed to the production of β-adrenergic subsensitivity as a possible common mechanism of various antidepressant treatments. The present study was designed to determine the effect of chronic TCA treatment on adrenergic responsiveness in two brain areas, the dorsal lateral geniculate and the facial motor nucleus, which are characterized by postsynaptic (α_1) adrenoceptors. Adult male albino rats were pretreated once daily for 2-3 weeks with injections of various TCAs (imipramine, desipramine, chlorimipramine, amitriptyline [5 or 10 mg/kg] or iprindole [2.5 or 5 mg/kg]) or control drugs (saline, fluoxetine or chlorpromazine [10 mg/kg]). Single cell recordings were conducted 24 hours after the last injection in anesthetized (chloral hydrate, 400 mg/kg, i.p.) or unanesthetized (cerveau isole) preparations. In one study, carried out in the facial motor nucleus, single motoneurons were identified and sensitivity to iontophoretic norepinephrine (NE) and serotonin (5-HT) was determined as previously described (McCall and Aghajanian, Neurosci. 4:1501, 1979). Chronic TCA treatment was found to reduce (by at least 50%) the minimal iontophoretic dose of NE required to facilitate the activation of facial motoneurons. This change was not observed in animals receiving the control drugs. Enhanced responsiveness to 5-HT was also observed in chronic TCA animals, consistent with previous reports of heightened sensitivity to 5-HT in several forebrain areas (deMontigny and Aghajanian, Science 202:1303, 1978). Neurons in the lateral geniculate nucleus receive a prominent NE input and possess physiologically characterized α_1-adrenoceptors (Rogawski and Aghajanian, Brain Res. 182: 345, 1980). Accordingly, further experiments were designed to test whether chronic TCA treatment would enhance α-responsiveness in this forebrain system. As in the facial nucleus, 2-3 week treatment with a variety of TCAs produced at least a 2-fold reduction in the iontophoretic dose of NE needed to activate geniculate neurons. By contrast, treatment with saline or chlorpromazine failed to increase responses to NE. These results indicate that chronic TCA treatment can enhance single cell responses mediated by α_1-adrenoceptors in brain. It remains to be determined to what extent this effect contributes to the clinical response to antidepressant therapy.

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292.4 SEROTONIN RECEPTOR SUBTYPES AFTER CHRONIC TREATMENT WITH TRICYCLIC ANTIDEPRESSANT DRUGS. M. Ann Blackshear*, Larry R. Steranka and Elaine Sanders-Bush. Dept. Pharmacol., Vanderbilt Sch. of Med., Nashville, TN 37232

Saturation curves and Scatchard analyses of ^{3}H-LSD binding in cortex/hippocampus reveal only a single binding site with K_D and B_{max} value of 7.2 nM and 54.3 pmoles/gm tissue, respectively. However, in agreement with findings of Peroutka and Snyder (Mol. Pharmacol. 16, 687-699, 1979) displacement curves with spiroperidol and serotonin are clearly bisigmoidal suggesting two LSD binding sites. The site with high affinity for 5HT (K_D = 21 nM) is referred to as 5HT1; the other with low affinity for 5HT (K_D = 2000 nM) and high affinity for spiroperidol (K_D = 4 nM) is referred to as the 5HT2 site. Our studies suggest that these are distinct, non-interconvertible sites. Assay conditions have been adapted to measure these sites independently using 20 nM spiroperidol or 200 nM 5HT to saturate the 5HT2 and 5HT1 sites, respectively. Regional studies show that the hippocampus have a preponderance of 5HT1 sites (65-70%) whereas frontal cortex has nearly equal proportions of the two sites.

In agreement with other investigators, we have found no changes in the K_D or B_{max} for the 5HT1 site in the hippocampus or cortex after chronic treatment (2 or 3 weeks) with desmethylimipramine or chlorimipramine. However, in the frontal cortex of rats treated daily for 21 days with 10 mg/kg (i.p.) of chlorimipramine and assayed 48 hours after the last dose, a significant decrease in K_D value for the 5HT2 site is observed [5.75 ± 0.71 versus 3.85 ± 0.45 nM ($p = .05$), respectively, for control and drug-treated animals] with no change in B_{max} (24.2 ± 2.1 and 19.3 ± 2.0 for control and chlorimipramine treated animals). Moreover, small but insignificant decreases [K_D = 4.1 ± 0.76; B_{max} = 19.0 ± 2.1 ($p = .15$)] were observed in the frontal cortices of rats treated daily with 10 mg/kg of desmethylimipramine for 21 days and assayed 48 hours after the last dose. We therefore propose that changes in the 5HT2 site may relate to the reported postsynaptic 5HT receptor supersensitivity which develops on chronic administration of tricyclic antidepressants. (Supported by USPHS Grants MH-15452 and MH-26463 and the State of Tennessee).

292.5 EFFECT OF CHRONIC TRICYCLIC ANTIDEPRESSANT TREATMENT ON SEROTONERGIC AND HISTAMINERGIC RECEPTORS. S.W. Tang, P. Seeman and S. Kwan*. Psychopharmacology Unit, Clarke Institute of Psychiatry, 250 College Street, Toronto, Canada M5T 1R8.

Since certain tricyclic antidepressants block serotonin receptors and are also potent antihistamines, we tested the effect of long-term tricyclic antidepressant treatment on serotonin receptors in rat cerebral cortex (using ^{3}H-spiperone), on striatum dopamine receptors (using ^{3}H-spiperone), and on midbrain histamine receptors (using ^{3}H-mepyramine and ^{3}H-cimetidine). There are no previous reports on the effects of long-term antidepressant medication on histamine receptors, and previous findings on antidepressant-induced changes in serotonin receptors (using ^{3}H-serotonin) have differed. Rats received either amitriptyline (AMT) (10 mg/kg/day, i.p.), desmethylimipramine (DMI) (10 mg/kg/day, i.p.) or saline (1ml i.p./day) for 21 days.

Both AMT-and DMI-treated groups showed decreased specific ^{3}H-spiperone binding in the cerebral cortex. Scatchard analysis revealed that the decrease in binding was due to a reduction in maximal binding sites (B_{max}) and not to change in affinity. The absence of changes in specific ^{3}H-spiperone binding in the caudate nuclei, apart from showing that dopamine receptors do not change after chronic tricyclic treatment, also suggests that changes seen in the cerebral cortex were due to changes in serotonin receptors. ^{3}H-spiperone has been previously shown to label serotonin receptors in the rat frontal cortex.

^{3}H-cimetidine and ^{3}H-mepyramine have been shown to label histamine H_2-receptors and histamine H_1-receptors respectively. There were no changes observed in either specific ^{3}H-mepyramine or ^{3}H-cimetidine binding in the mid-brain, which suggests that histamine receptors in rats do not change under chronic exposure to tricyclic antidepressants (Supported by the Ontario Mental Health Foundation.)

292.6 DRUG-INDUCED CHANGES IN RAT PINEAL GLAND N-ACETYLTRANSFERASE ACTIVITY. Anthony Altar*, Robert L. Terry*, Gary L. Brammer*, and Loy D. Lytle. University of California, Department of Psychology, Laboratory of Psychopharmacology, Santa Barbara, California 93106.

Previous research indicates that the activity of the pineal gland enzyme, N-acetyltransferase (NAT) is regulated by the release of norepinephrine from afferent sympathetic nerves that innervate this organ. Increased release of norepinephrine from these sympathetic nerve terminals onto pineal gland *B*-adrenergic receptors during the onset of darkness or following stress or other manipulations produces increases in NAT activity via a cyclic AMP-dependent mechanism [J. Axelrod, Science 184: 1341 (1974)]. The present experiments were conducted to determine if several sympathomimetic amines including d-amphetamine sulfate and the monoamine oxidase inhibitor, pargyline HCl, increase pineal NAT activity in light-stimulated male and female rats.

Adult male or female Sprague-Dawley rats (approximately 250 g) were offered *ad libitum* access to food (Purina Rat Chow) and water during a 12:12 hr light-dark cycle (lights on at 0700 hr). At 1800 hr food was removed and animals were injected intraperitoneally with the 0.9% saline vehicle (1.0 mg/kg) or with 8.0 mg/kg of d-amphetamine sulfate, 8.0 mg/kg of l-isoproterenol HCl, or 40 mg/kg of pargyline HCl and were killed following 3 hr of continuous lighting. Additional amphetamine-treated male animals were killed 2 or 4 hr post-injection.

Each of the drugs tested produced significant elevations of NAT. The maximal amphetamine response occurred 3 hr following the injection. By 3 hr, preliminary results indicate that the amphetamine-induced increases in pineal NAT activity were elevated approximately 300% more in female animals compared to the males. Preliminary results also indicate that other drugs, such as pargyline, might produce larger increases in pineal NAT activity in females compared to males, thus ruling out a possible idiosyncratic effect of amphetamine. In previous research we have shown that amphetamine produces age- and sex-dependent changes in a variety of behavioral (locomotor activity and food consumption) and physiological (temperature regulation) responses in rodents [E. Meyer and L.D. Lytle, *Proc. Western Pharmacol. Soc. 21*:313 (1978)]. This drug produces larger changes for longer durations of time in the responses of adult female animals compared to males apparently as a result of slower rates of amphetamine metabolism and excretion by female animals compared to males. Although presently unproven, these findings indicate that a variety of psychoactive drugs may produce greater behavioral, physiological and neurochemical effects in females compared to males as a result of the relative inability of female animals to metabolize and excrete drugs.

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293.1 SHR RATS: ADRENERGIC HYPERRESPONSIVITY TO FOOTSHOCK STRESS BUT NOT TO COLD EXPOSURE. R. McCarty and C. C. Chiueh. Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22901 and Lab. of Neurosciences, NIA, Baltimore, MD 21224.

Recent studies from our laboratory suggest that the sympathetic-adrenal medullary system of spontaneously hypertensive (SHR) rats is hyperresponsive to several forms of stressful stimulation when compared to Wistar-Kyoto (WKY) normotensive controls (Behav. Biol. 23: 180, 1978; Physiol. Behav. 21: 951, 1978; Am. J. Physiol. 234: H690, 1978). The excessive release of catecholamines into the circulation in response to even mildly altering stimuli may contribute to the development and/or maintenance of high blood pressure in this animal model of essential hypertension. In the present study, we were interested in comparing the sympathetic-adrenal responses of SHR and WKY rats to two different challenges: cold exposure or inescapable footshock.

Chronic tail artery catheters were inserted into rats of each strain (4-10 months old) while under pentobarbital anesthesia. Two days after surgery, basal blood samples were obtained from rats while resting and undisturbed in their home cages. SHR and WKY rats were then exposed to one of two treatments: placement in a cold room (4°C) for 4 hr. or 5 min. of intermittent footshock (2.5 mA, 0.4 sec duration, every 5 sec). Blood samples were collected during both treatments and plasma levels of norepinephrine (NE) and epinephrine (EPI) were measured by a radioenzymatic assay.

No significant differences were obtained in basal plasma levels of NE or EPI for SHR and WKY rats. Exposure to cold for 20 and 240 min. resulted in significant increments in plasma levels of NE and EPI which were similar in SHR and WKY rats. In contrast, exposure to intermittent footshock resulted in significantly greater increments in plasma levels of NE and EPI in SHR rats than in WKY controls. Fifteen minutes after the termination of footshock, plasma levels of both catecholamines remained significantly above baseline in SHR rats but returned to baseline in WKY rats. Our results indicate that SHR rats are hyperadrenergic in their responses to some but not all forms of stressful stimulation.

293.2 LASTING EFFECTS OF REPETITIVE UNPREDICTABLY SCHEDULED RESTRAINT STRESS UPON VARIOUS STRESS MARKERS IN THE RAT. C. M. Quirce*, M. Odio* and R. P. Maickel, Lab. of Neurosci., Sch. of Pharmacy, Univ. of Costa Rica, San Jose, Costa Rica and Dept. of Pharmacol. and Toxicol., Sch. of Pharmacy and Pharmacal Sci., Purdue Univ., W. Lafayette, IN 47907.

Adult male rats were subjected to 25 four hr. sessions of physical restraint, carried out between 1300 and 1700 hrs. on randomly varied days over a total time span of 6 weeks. A previous report (Quirce and Odio, Soc. Neurosci. Abstr. 5: 456, 1979) indicated that such unpredictable chronic restraint caused elevations of plasma levels of corticosterone, free fatty acids, and glucose that were maintained for at least 48 hrs. after the last restraint session. In contrast, predictably scheduled sessions failed to produce such elevations. Further studies have indicated that the elevations of plasma corticosterone persist for at least 4 days after the last restraint; by the 6th post-stress day, these levels fall below control values. Free fatty acid levels remain elevated for at least 6 days post-stress, while plasma glucose levels decrease from elevated levels on day 2 post-stress to values below corresponding controls on 4 and 6 post-stress. These results suggest that repetitive exposure of rats to stressful stimuli on a non-predictable basis (a situation not dissimilar to the normal life situations of humans) produces a long-lasting perturbation of normal auto-regulatory mechanisms. This may provide a useful model for the study of stress-induced pathological states, since the stress-like responses are manifested, not only during the course of the application of stimuli, but persist for a significant period after the organism is no longer exposed to the stressful situations. (Supported in part by a grant from CONICIT of Costa Rica and by the Vicerrectoria de Investigacion of the University of Costa Rica.)

293.3 CENTRAL EFFECTS OF ECDYSTERONE CONTROLLING WANDERING BEHAVIOR IN THE CATERPILLAR, MANDUCA SEXTA. O.S.Dominick* and J.W.Truman (SPON: W.Calvin). Dept. of Zoology, Univ. of Washington, Seattle WA. 98195.

The prepupal wandering behavior of last (5th) instar larvae of the tobacco hornworm , Manduca sexta, is induced by a pulse of the steroid molting hormone, ecdysterone. Infusion of ecdysterone into intact larvae controls both initiation time and duration of the behavior in a dose dependent manner, and requires a latency of 10-20 hours as is typical of steroid hormone action. The ecdysterone induced effects include changes in neural function of a number of different parts of the CNS. In addition, all earlier instars which we have been able to test (2nd, 3rd,4th) can produce the behavior when the appropriate ecdysterone signal is precociously presented to them.

We can investigate ecdysterone induced changes in neural function directly by long term (24 hr) studies of the isolated (entire) CNS of Manduca larvae. Extracellular recordings are obtained from a compound nerve whose role in locomotion is known. Such recordings from the CNS isolated from larvae prior to edysterone release show consistently low levels of spontaneous bursting (8.8 ± 3.8bursts/15 min.), while the spontaneous activity of the CNS from wandering animals is much greater (42.9 ± 13.6 bursts/15 min.). Several independent lines of evidence indicate that these bursts represent locomotor behavior symptomatic of wandering: recordings from chronic preparations of whole larvae demonstrate that bursts from this nerve occur only in conjunction with observable locomotor events, and anteriorly directed phasing between ganglia is preserved.

The active bursting state characteristic of the isolated wandering CNS can be induced in vitro in "quiet" pre-ecdysterone nerve cords by prolonged incubation (15 hrs) in ecdysterone (1 ug/ml). Therefore, we feel that the behavioral action of this steroid is directly on the larval CNS. Several of the motoneurons which participate in the behavior, as well as the synaptic inputs to them have been identified and we are now studying these as possible sites for the alteration of neural function by a steroid hormone.

293.4 APPLICATION OF ANISOMYCIN TO THE LATERAL VENTROMEDIAL NUCLEUS BLOCKS THE ACTIVATION OF SEXUAL BEHAVIOR BY ESTRADIOL AND PROGESTERONE. T.C. Rainbow, P.G. Davis*, M. McGinnis*, and B.S. McEwen. The Rockefeller University, New York NY 10021.

We have found recently that peripheral administration of the protein synthesis inhibitor, anisomycin (Ani), inhibits the induction of sexual behavior in rats by estradiol (E_2) and progesterone (P) (Brain Res. 1980, in press). We report here that local application of Ani aimed to reach the lateral portion of the ventromedial hypothalamic nucleus (LVMN) is sufficient to block the activation of lordosis behavior by systemic gonadal steroids. Ovariectomized rats received Silastic implants of E_2 for 6h. They were tested for sexual behavior 24h after insertion of the Silastic capsules and 4-5h after systemic P injection. Removable 28 gauge cannulae were inserted bilaterally into the ventromedial hypothalamus during either E_2 or P exposure. When empty cannulae were placed in the vicinity of the LVMN, sexual behavior was uniformly high after treatment with E_2 and P (lordosis quotient (LQ) $\sim$80). However, there was a marked reduction in lordosis behavior when crystalline Ani was introduced into the LVMN during exposure to either E_2 or progesterone (LQ$\sim$20).

Rats treated intracranially with Ani showed high levels of sexual behavior when re-exposed to gonadal steroids one week later, indicating that the effects of Ani on behavior was reversible. Biochemical studies determined that 3H leucine incorporation into protein was inhibited in the hypothalamus after intracranial insertion of Ani. No inhibition was seen in the preoptic area, amygdala and pituitary in these animals. Similarly, we observed little spread of Ani outside the ventromedial hypothalamus when the area of inhibition was mapped with S^{35} methionine autoradiography. Our results support the idea that gonadal steroids induce lordosis behavior by acting on target cells in LVMN and suggest that E_2 and P may activate sexual behavior in part by inducing the synthesis of specific proteins in these neurons.

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293.5 CHANGES IN BRAIN SEROTONIN AND DOPAMINE RECEPTORS AFTER SLEEP
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It has been suggested that serotonergic and dopaminergic neurons may be involved in the sleep-wakefulness process. In the present study in vitro serotonin (5-HT) receptor binding was determined in cerebral cortex, upper brain stem and pons-medulla of adult male hamsters (body wt. 100-120 g) after sleep and wakefulness. The dopamine (DA) receptor binding was studied only in corpus striatum. Animals were decapitated following 50 min of non-rapid eye movement sleep (Macho & Sinha, Life Sci. 26, 291, 1980) or a comparable period of wakefulness. For specific binding of 5-HT receptor the membrane suspension (≃ 1 mg protein) was incubated in buffer (pH = 7.1) containing ^{3}H-5-HT (7 nM) in the presence or absence of unlabeled 5-HT (10 µM) at 37° C for 5 min. For specific binding of DA receptor the membrane suspension (≃ 250 µg protein) was incubated in buffer (pH = 7.1) containing ^{3}H-apomorphine (8 nM) in the presence or absence of unlabeled DA (10 µM) at 37°C for 10 min. Immediately after sleep the specific binding of 5-HT increased (46%) only in cerebral cortex with no significant changes in upper brain stem and pons-medulla. Unlike 5-HT binding, the specific binding of apomorphine decreased (36%) in corpus striatum. Scatchard analysis of 5-HT and DA receptor binding data indicate that after sleep the number of 5-HT binding sites increased (34%) without any significant change in the affinity of the receptor for the ligand. The affinity of the DA receptor to the ^{3}H-apomorphine decreased (123%) after sleep whereas a significant increase (75%) was found in the number of binding sites. [Supported by grants from NIH (NS13118) and AFSC (78-3532A)].

293.6 MIDBRAIN RETICULAR NEURONS DECREASING FIRING RATES DURING EEG DESYNCHRONIZATION AND AROUSAL.
Zeev Elazar, John P. Nelson, Robert W. McCarley and J. Allan Hobson. Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, Boston, MA 02115.

Since the classic studies of Moruzzi and Magoun, the mesencephalic reticular formation has been considered as the active neuronal substrate of EEG desynchronization and behavioral arousal. Thus it was surprising to Huttenlocher in 1961 to find cells which increased firing rate during synchronized sleep (S) compared to waking (W). Steriade's recent work has emphasized a subpopulation of neurons with the expected positive correlation between firing rate and EEG activation. We now report a confirmation and extension of Huttenlocher's observations emphasizing features not previously described for the interesting class of "S-on" cells.

The discharge pattern of neurons in the mesencephalic reticular formation (FTC) was studied in chronically prepared head-restrained cats free to make limb movements and postural adjustments. Extracellular action potentials from 40 neurons in 24 stereotaxically controlled penetrations of the midbrain were recorded continuously during sleep-waking cycles.

10 "S-on" cells were recorded. They discharged high amplitude spikes at low firing rates during W (median 0.64/sec, range 0.09-4.98/sec). Single sensory stimuli silenced or greatly slowed them for a few seconds. Spontaneous fluctuations between EEG desynchronization and synchronization during drowsy periods were correlated with slowing or speeding of the firing. No correlation was found between frequency of neuronal discharge and individual EEG spindles or delta waves but when sleep was established the firing rates increased gradually as the amplitude of slow waves increased in the EEG. During S firing rates were: median 1.69/sec, range 1.1-5.66/sec.

7 of the "S-on" cells were followed through desynchronized sleep (D). 2 cells showed long trains of very high frequency spikes (200-300/sec) alternating with periods of very slow rates. 2 cells decreased firing rates to the low waking level and other 3 cells had in D firing rates higher than in W. Interestingly, 4 of the 7 cells studied in D showed significant intensification of firing in the last seconds before and at D sleep onset in obvious correlation with the progressive loss of muscle tone.

These results suggest a possible reciprocal interaction between two classes of midbrain neurons in the regulation of EEG and an active role of midbrain neurons in the atonia of REM sleep.

293.7 RESPIRATORY RESPONSES TO ELEVATED CO_2 IN AWAKE AND SLEEPING CATS.
Theodore L. Baker, Kurt Sizer* and W. C. Dement, Sleep Research Center, Stanford University School of Medicine, Stanford, CA 94305

We measured ventilation in six adult cats during hyperoxic hypercapnia in waking, REM sleep and non-REM sleep (NREM) using a rebreathing method. Prelaryngeal airflow was recorded pneumotachographically via an endotracheal cannula inserted into a chronic tracheostomy. Tracheal end-tidal PCO_2 was measured with a Beckman infrared CO_2 analyzer. Data were collected and analyzed on-line with a PDP-11 computer on a breath-by-breath basis. Linear regression anaysis of either tidal volume (V_T) or instantaneous minute volume ($\dot{V}_E$) vs. end-tidal PCO_2 revealed a greater respiratory response to hypercapnia during waking than during sleep. CO_2 responses during NREM and REM were very similar, although the slope of the $\dot{V}_E$ vs.CO_2 line tended to be slightly greater during NREM. During all states, the increase in $\dot{V}_E$ during CO_2 rebreathing was due primarily to increased V_T. Computer plots of raw V_T data vs.time indicated a progressive and predictable breath-to-breath increase during waking and NREM. Breath-to breath variability of respiratory parameters was higher during REM sleep, but the ventilatory response to CO_2 was clearly intact. As during normocapnic REM sleep, increased respiratory variability during REM CO_2 rebreathe tests was associated with bursts of phasic events (eye movements, PGO waves, twitches). Brief respiratory irregularities, usually increased f, were superimposed upon a baseline of continuously increasing V_T. That is, f increased while V_T remained elevated at a level consistent with the ongoing CO_2 stimulus. Mean V_T during phasic REM episodes at higher PCO_2 levels was always greater than V_T at a lower CO_2. Increased f during phasic REM bursts typically resulted in a transient increase in mean $\dot{V}_E$. Progressively higher PCO_2 stimuli during REM CO_2 rebreathing tended to diminish the magnitude of the respiratory disturbances associated with phasic events. Arousal from REM rebreathing tests consistently occurred later and at higher PCO_2 levels, compared to NREM. We conclude that brainstem mechanism controlling V_T are responsive to elevated CO_2 during both stages of sleep. $^TCO_2$ sensitivity is increased even further during waking. We suggest that differences between REM and NREM CO_2 responses in the cat are largely qualitative and are attributable to phasic irregularities inherent to the REM state.

From Session 4: Symposium

MOLECULAR CHARACTERIZATION, RECONSTITUTION AND "TRANSPORT-SPECIFIC FRACTIONATION" OF THE SOLUBILIZED STX BINDING PROTEIN/ION GATE OF MAMMALIAN BRAIN. S.M. Goldin, V. Rhoden,* E.J. Hess*. Dept. of Pharmacology, Harvard Medical School, Boston, Mass. 02115.

The saxitoxin (STX) binding protein has been solubilized by sodium cholate, both from axolemma and from synaptosomal membranes of mammalian brain. Based on agarose gel filtration and on sedimentation properties in H_2O and D_2O sucrose gradients containing lipid and cholate, the solubilized particle has the following properties: Stokes radius, 120 Å; partial specific volume, 0.85 ml/g; mass, 1,020,000 daltons; f/fo, 1.6. No more than 570,000 daltons of this particle is protein. The solubilized STX binding protein was incorporated into homogeneous (∿550Å) unilamellar artificial phosphatidyl choline vesicles by removing the cholate using hollow fiber dialysis (Goldin, S.M. (1977) J. Biol. Chem. 252, 5630-5642).

Based on the expectation that the reconstituted STX binding protein contains functional monovalent cation gating activity ("action potential Na^+ gate") that can be activated by veratridine and inhibited by tetrodotoxin and STX, a strategy was devised for partial purification of the reconstituted Na^+ gate/STX binding protein by "transport-specific fractionation" (c.f. Papazian, D., Rahamimoff, R., and Goldin, S.M. (1979) PNAS 76, 3708-3712). This approach involves reconstitution of the transport system of interest into artificial vesicles before purification so as to insert only one or at most, a few membrane proteins into each artificial vesicle. The transport properties of the protein of interest are then used as a physical tool (e.g., transport-specific changes in vesicle density are created) to separate vesicles containing this transport system from the rest of the crude preparation and thus results in its purification.

When the entire vesicle population was preloaded with 0.4 M Cs^+, addition of veratridine allowed Cs^+ efflux from specifically those vesicles containing the ion gate; the concomitant reduction in intravesicular density permitted the ion gate/STX binding protein to be fractionated on density gradients. These observations demonstrate functional reconstitution and partial (30- to 50-fold) purification of the solubilized STX binding protein/putative action potential Na^+ gate of mammalian brain. Because reconstitution of transport activity is required for the viability of this approach, we know that what is being purified is an ion gating protein, and not just a toxin binding site. (Supported by NIH grant NS 15236, to S.M.G.).

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DUAL NEUROENDOCRINE CONTROL OF PITUITARY INTERMEDIATE LOBE FUNCTION IN THE FROG. M. Duff Davis* and Aubrey Gorbman* (SPON: R. Pinter). Dept. of Zoology, NJ-15, University of Washington, Seattle, Washington 98195.

The central lobe of the vertebrate pituitary, the pars intermedia, has recently been the subject of renewed interest since it was discovered that the highest concentration of endorphins is found here. It has been suggested that endorphin-like peptides are contained in a cell type previously believed only to synthesize melanophore stimulating hormone (MSH). Consequently, past and present studies on the CNS control of MSH secretion may also be applicable to the control of endorphin secretion. Although several types of neuronal populations exist in the pars intermedia, as yet, only an inhibitory control of MSH has been established through a catecholaminergic neurotransmitter. We present data here implicating the presence of both an excitatory and inhibitory neuronal input in the pars intermedia.

During the course of this study, six adult Mexican frogs, Rana berlandieri forreri, were used. The head of each was guillotined and the hypothalamo-hypophyseal complex removed to a nutrient bath and gassed with $95\%O_2$-$5\%CO_2$. Recording microelectrodes were positioned into the intermediate lobe to monitor endocrine cell membrane potentials. Platinum wire electrodes were used to stimulate a one millimeter area of median eminence. Biphasic 60 Hz, 100 microamp pulses were passed through these. When a cell exhibited spontaneous action potentials, the current was turned on. In about 75% of the 25 cells studied, a decrease in frequency of spikes was seen while 20% of them showed an increase. In the remaining 5%, no significant change in basal activity was found. Often, cells were observed to give phasic bursts of spikes with varying intervals of silence. The average latency between the onset of electrical stimulation and any change in spike rate was anywhere from 100 milliseconds to 10 seconds. The duration of this latency would indicate that not every cell in the pars intermedia is under synaptic contact with axonal endings. Instead, neurotransmitters may be released directly into intercellular spaces to perfuse the tissue. These observations indicate that the pars intermedia gland and its secretions are influenced by both inhibitory and excitatory hypothalamic control.

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INSULIN-LIKE FACTORS MAY SUBSTITUTE FOR SERUM IN THE MAINTENANCE OF CHICK SENSORY NEURONS IN VITRO. E.Y. Snyder* and S.U. Kim* (SPON: D. Pleasure) Lab. of Neuropathology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

The inability to culture neurons in the complete absence of complex, poorly defined biological additives (e.g. serum) has proven a major obstacle to a rigorous investigation of the nutritional and growth requirements of the developing nervous system. We have previously reported that insulin, when substituted for serum at supraphysiologic concentrations (50µg/ml), was both necessary[1,2] and sufficient[3,4] for the survival of 9-12 day embryonic chick dorsal root ganglia (DRG) neurons in vitro. (Indeed, in cell systems where a salubrious effect of insulin has been noted, it has usually been at supraphysiologic concentrations[5-8]).The present study was designed to explore the possibility that the actual requirement of sensory neurons may not be for insulin per se but rather for a closely related molecule for which insulin in high concentrations may serve as an analogue. A series of well-recognized growth factors were screened for their ability to support the survival in vitro of dissociated chick DRG neurons in serum-free media. Insulin-like growth factors, some at their biological concentrations (e.g. Insulin-like Growth Factor [IGF] (0.1mU/ml) and Multiplication Stimulating Activity [MSA] (50ng/ml)) were indeed found to be sufficient to promote survival. Non-insulin-like growth factors, even at high concentrations, were found to be inadequate (e.g. Fibroblast Growth Factor [FGF]), Epidermal Growth Factor[EGF], Nerve Growth Factor [NGF]).(A preparation of Somatomedin A+C, like insulin, was effective only at high concentrations (100mU/ml)). Insulin-like factors proved a sufficient substitute for serum in both NGF-free and in more conventional NGF-supplemented dissociated chick DRG culture systems. This data lends support to the notion that the occupation -- and occupation by whatever means -- of receptors sensitive to insulin-like activity (including, of course, by insulin itself) may prove a sine qua non for early neuronal survival in culture.

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LYSOSOMAL DISTRIBUTION IN NEURONS FROM THE CEREBRUM AND SPINAL CORD DURING EARLY VITAMIN E DEFICIENCY IN RATS: ELECTRON MICROSCOPIC STUDY. Arun S. Dabholkar. Dept. of Anatomy, Northwestern Univ. School of Med., Chicago, Ill. 60611 and Dept. of Anatomy, Kyoto Univ. School of Med., Kyoto, Japan.

Vitamin E is an antioxidant which is associated with cellular membrane and known to control the membrane stability and permeability.

The present study was designed to demonstrate appreciable change in lysosomal size and number in the early stage of vitamin E deficiency in nerve cells. Young male Wistar rats of 100 to 150 gms. of body weight were used for this experiment. They were fed vitamin E deficient diet (supplied by Eisai Co. Ltd., Tokyo) and control rats were kept on regular chow containing vitamin E. Thus, vitamin E deficient and control rats of 4 to 13 weeks were sacrificed. They were perfused and neurons from the cerebrum and spinal cord were studied electron microscopically to show the increase in lysosomes within 13 weeks of deficiency.

Lipid containing enlarged lysosomes as appeared could be termed as secondary lysosomes, chromolipoids or ceroids. It is known that a dietary antioxidant of vitamin E as alpha tocopherol plays significant role in the oxidative process by preventing oxidation of unsaturated lipids in cells. The enlarged structures are the result of copolymerization of unsaturated fats (lipid peroxidation) and proteins in depleting vitamin E from the cellular membranes.
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